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Psychological factors at learning that modify sleep-dependent consolidation of memory

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Index

Abbreviations

.ttf - True-Type Font

ANOVA - Analysis Of Variance

EEG - Electroencephalogram

MMFR - Modified Modified Free Recall

MTL - Medial Temporal Lobe

REM - Rapid Eye Movement

S1 - Stage 1 Sleep

S2 - Stage 2 Sleep

SEM - Standard Error Of The Mean

SPW - Sharp Wave Bursts

SRTT - Serial Reaction Time Tasks

SWS - Slow Wave Sleep

Introduction

The fact that sleep plays a vital role for the recovery of cognitive functioning is so evident that it does not need scientific prove to be accepted. Yet the precise process by which this is achieved, is a topic of increasing scientific interest. The public is probably more familiar with research that has concentrated on the possibility for information processing (learning) during sleep. Less known (but that is subject to change) is that sleep might play a crucial role in memory formation. Although this idea was already proposed in the 18th century and remained a topic of investigation until the early seventies of the past century, mostly due to mixed results (some showing a positive influence on retention some not) it slowly but steadily disappeared from the scientific arena. Due to new technological possibilities and the accompanied rise of (cognitive) neuroscience in the early nineties of the 20th century the possible of role of sleep in memory consolidation has experienced a revival. New sophisticated theories of the biological processes that underlie the consolidation of memory have been the result. Nevertheless psychological studies again have led to mixed results. The purpose of the work reported here is to provide a framework with which the effects of sleep on memory consolidation can be efficiently studied. Three studies are reported that together attempt do define the psychological conditions of learning under which the positive effect of sleep related memory consolidation can be identified at subsequent retrieval. First we compared two global memory systems i.e. explicit versus implicit memory. The results showed that only explicitly learned material seems to benefit from sleep. Henceforth we concentrated on explicit memory, and by manipulating encoding strength we aimed to re-examined the topic of an active versus a passive role of sleep in memory. This study clearly supported the view that consolidation is an active process and that its effect is most profound for material that is not too strongly encoded. Finally, based on the findings of the previous studies we sought to directly test the hypothesis that the underlying mechanism that supports memory consolidation is based on a replay of the newly acquired memory traces. In this study we tested memory for sequences and in particular how sleep affects the retrieval of a sequence in the forward and backward direction. The data confirmed the idea that the sleep related memory gain is greater for the weaker associations. Yet this effect was restricted to forward associations whereas it didn't seem to influence the backward order of retrieval. These data supported the hypothesis that replay supports the strengthening of memory.

Sleep stages

Unconsciousness during sleep is probably the core characteristic that separates sleep from wakefulness. Yet, although the organism is cut-off from the outside world or at least seems to be, the brain never really stops working. Depending on the brain activity as measured with an electroencephalogram (EEG) Rechtschaffen and Kales (1968) distinguished 5 different sleep stages in sleep architecture that cyclically succeed each other during the course of nocturnal sleep. The five stages have been labelled stages 1-4 and rapid eye movement sleep (REM). Stages 1-4 are also referred to as non-REM sleep and stages 3 and 4 together consist of slow wave sleep (SWS).

Stages in memory

Memory is not a unified cognition function, it can be subdivided in three stages i.e. encoding, consolidation and retrieval. Encoding refers to the acquisition phase were the new information is (temporarily) stored into neuronal networks. Retrieval refers to the behavioural utilisation of previously stored memory traces. Encoding does not result in instantaneous permanent memories. Memories are fragile in the beginning and remain vulnerable to disruption (from interference) for some time after encoding. Once new memory traces are encoded a period of post-processing starts which is called consolidation. Memory consolidation is conceptualized as a process triggered by a learning experience whereby the newly encoded representations transform into a robust and enduring form (Müller & Pilzecker, 1900;McGaugh, 2000). Consolidation is considered to counteract processes of forgetting and to make the memory representation less susceptible to interfering experiences. The consolidation process can also result in an improved performance on the learned behavior at a delayed retrieval testing, which occurs in the absence of any further overt practice of the learned behavior during the retention interval.

Just like memory is not a unitary stage, it is also not a unitary system (Squire, 1986;Squire *et al*, 2004). Instead different forms of memory have been distinguished. The most common dissociation consists that of declarative memory and procedural memory. Declarative memory consists of memories we can report about. It is thus, considered an explicit form of memory because it reflects conscious recollection (Squire & Zola, 1996). Those are further separated in semantic and episodic memory. Semantic memory refers to factual knowledge whereas episodic memory is considered memory for events with a clear memory for the context in which those events took place. Procedural memory on the other hand refers to types of memory that behaviorally manifest themselves without ones realization that memory is involved. These usually include (motor) skills, priming as well as conditioning, and are generally also encountered in the literature as unconscious or implicit forms of memory. Although this dissociation as proofed quite useful, it has also received a lot of criticism (e.g. Butler & Berry, 2001;Schendan *et al*, 2003) particularly because those memory systems often have been found to overlap. Anatomically, the medial temporal lobe (MTL), initially thought to be involved only in explicit memory has been shown to do so also in implicit memory (Rose *et al*, 2002;Stark & Okado, 2003). Functionally, declarative memory can also be guided by unconscious processes whereas implicit memory can also be guided by conscious processes (Butler & Berry, 2001).

Memory consolidation and Sleep

Substantial evidence has accumulated that the consolidation of newly acquired memories is supported by subsequent sleep (Jenkins & Dallenbach, 1924;Maquet, 2001;Walker & Stickgold, 2004;Gais & Born, 2004a). Different types of perceptual and motor skills have been found to be enhanced at retrieval testing when the training was followed by a period of sleep as compared to a period of wakefulness (Gais *et al*, 2000;Stickgold *et al*, 2000;Fischer *et al*, 2002;Walker *et al*, 2003). The findings of a gain in skill after a retention period of sleep have particularly contributed to the conceptualization of the consolidation process, and in conjunction with neuropsychological investigations of memory consolidation, support the notion that consolidation during sleep, involves an active kind of “system consolidation”. Systems consolidation denotes a process which, beyond strengthening the encoded neuronal trace and underlying synaptic connections, leads to a spreading of the original memory representation to new locations in the brain and thereby may be accompanied by structural changes to the representation itself (McClelland *et al*, 1995;Dudai, 2004).

Covert off-line replay of those newly acquired memory traces has been proposed as a key mechanism underlying the consolidation process. The process has been labeled covert because it occurs in the absence of any conscious recollection of those memory traces. In particular in declarative and spatial memory which both rely on the hippocampus, a reactivation of the same hippocampal neuronal circuits as during encoding, were observed during slow wave sleep after the learning period (Wilson & McNaughton, 1994;Louie & Wilson, 2001;Lee & Wilson, 2002;Ribeiro *et al*, 2004;Peigneux *et al*, 2004). Also, imaging studies have shown that at a delayed retrieval testing, memory representations have significantly changed in brain topography when subjects slept after learning (Orban *et al*, 2006;Maquet *et al*, 2003;Fischer *et al*, 2005;Walker *et al*, 2005;Takashima *et al*, 2006). These studies with regard to declarative memory are consistent with the notion of a two-stage memory system where memories are initially hold in hippocampal networks and are transformed into long-term memories by a spreading and gradual transfer of the representations to other presumably neocortical networks (McClelland *et al*, 1995;Buzsaki, 1996;Buzsaki, 1998;Sutherland & McNaughton, 2000;Gais & Born, 2004a;Dudai, 2004). This hipocampal-noecortical dialog is thought to take place primarily during slow wave sleep and is modulated via sharp wave bursts (SPW) (i.e. synchronous burst of neuronal activity in the CA3 region of the hippocampus). These again are superimposed upon ripple activity originating from the CA1 region of the hippocampus (Buzsaki, 1986) (i.e. high frequency local field potential oscillation (100-300 Hz)). In other words the role of the hippocampus in memory consolidation can be viewed as establishing a "template" that serves to bring together the neuronal representations that form a memory and implement those with previous memories stored in the neo-cortex.

The differential contribution of sleep-stages to memory consolidation

An ongoing debate concentrates around the question whether all sleep or only certain sleep stages contribute to memory consolidation. Traditionally the debate concentrated around SWS and REM sleep but more recently sleep stage-2 and particularly the role of spindles to memory is also being investigated (Born & Gais, 2003). In humans, sleep in the early part of the night, dominated by extensive epochs of SWS, has been found to enhance in particular declarative memories, (Fowler *et al*, 1973;Plihal & Born, 1997;Plihal & Born, 1999a). This effect has been related to higher proportion of SWS as compared to REM sleep during early sleep, as well as to accompanying low levels of cholinergic activity in the hippocampus (Hasselmo, 1999;Gais & Born, 2004b). On the other hand, for procedural forms of memory, a greater benefit has been found after periods of late nocturnal sleep (Plihal & Born, 1997;Plihal & Born, 1999a;Wagner *et al*, 2003). This late period of nocturnal sleep is characterized by high amounts of REM sleep, and contains little SWS. While not independent of SWS, procedural memory thus appears to be particularly strengthened by REM sleep related mechanisms (Stickgold *et al*, 2000;Gais *et al*, 2000;Fischer *et al*, 2002).

Hypotheses

The purpose of the three studys reported in this thesis is to discover under which conditions of learning sleep can actually benefit the subsequent consolidation of the newly established memories. In the first experiment using a task that allows the separation of the explicit and implicit component of memory. We used the process-dissociation procedure by Jacoby (1991) to compare the effects of sleep on estimates of explicit (recollection) and implicit (familiarity) memory formation on a word-list discrimination task. Subjects studied two lists of words before a 3-hour retention interval of sleep or wakefulness and recognition was tested afterwards. The retention intervals were positioned either in the early night where sleep is dominated by SWS, or in the late night where sleep is dominated by REM sleep. Sleep enhanced explicit recognition memory, as compared to wakefulness ($p<0.05$), whereas familiarity was not affected by sleep. Moreover, explicit recognition was particularly enhanced after sleep in the early night retention interval, and especially when the words were presented with the same contextual features as during learning, i.e. in the same font ($p<0.05$). The data indicate that in a task that allows separating the contribution of explicit and implicit memory, sleep particularly supports explicit memory formation. The mechanism of this effect appears to be linked to SWS.

Continuing, Study 2 focused exclusively on explicit memory and sought to clarify the nature of the sleep dependent memory improvement. Two mutually exclusive theories have been proposed, one proposed a passive (interference theory) and one an active (consolidation theory) role for sleep in memory formation. By building upon two previous studies (Ekstrand, 1967;Ekstrand *et al*, 1971) aimed at characterizing the role of interference for sleep-associated declarative memory consolidation. Protection from interference has often been proposed as an alternative mechanism to explain sleep-related memory improvements. Employing an “A-B, A-C” (word-pair lists) paradigm Experiment 2 shows that sleep provides recovery from retroactive interference induced at encoding, whereas no such recovery was seen at retrieval in different wake control conditions. In Experiment 3 employing non-interfering word-pair lists (“A-B, C-D”) sleep after learning, in comparison with waking, enhanced retention of both lists to a similar extent, however only when the encoding of lists was less intense (i.e., fewer and shorter presentations at learning). In combination, the results indicate that sleep actively enhances declarative memory consolidation with the benefit being greater for weaker associations regardless whether this is due to retroactive interference or weak encoding.

The third study further extends the findings from the second study, by attempting to find direct evidence for the proposed process by which sleep actively enhances memory. Replay of memory traces is thought to support their long term storage and to result in the transfer of those traces from the hippocampus where they are temporarily stored to the neo-cortex where they are being integrated with pre-existing cognitive schemas and memories. To examine this we investigated the effect of memory on sequences of events. Specifically were interested to see if sleep has a differential influence on the retrieval of the sequence in the forward or backward direction. Two groups of subjects (a sleep and a wake one) learned a list containing 32 triples of unrelated words. After two nights of which the first was spend awake by the wake group, retrieval was assed for the triples either in a forward or in a backward direction. Memory for the forward associations was generally better in both groups, yet this effect was differentially pronounced for the first word from each triple in the sleep group. The data show that sleep indeed supports consolidation of the temporal component of memory. This supports the idea that through replay, memory traces gain in strength and that the consolidation processes qualitatively alters the memory representations possibly inducing temporal direction in episodic memory.

Study 1: Sleep enhances explicit recollection in recognition memory

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Introduction

Recognition memory refers to a basic form of memory retrieval that has been widely used in experimental psychology. It is assumed that recognition performance is based on two different processes (Yonelinas, 2002;Rugg & Yonelinas, 2003). One refers to a conscious or explicit process of recollection, where the person initially searches for qualitative information about the context in which the event to be remembered took place. The other process refers to the experience of familiarity, induced automatically or implicitly at a re-occurrence of an event, and can emerge in the absence of any conscious knowledge about the context in which the event originally occurred. The two forms of memory involved in recognition can be separated and show different sensitivity to experimental manipulations. For example, elaborated encoding of stimuli and their context, facilitates explicit recollection but leaves judgments of familiarity at later recognition tasks rather unaffected (Yonelinas, 2002). Explicit recollection and familiarity-based implicit processes of recognition are probably supported by distinct neuronal systems. Studies in brain lesioned patients indicate that the hippocampus is critical for conscious recollection whereas this seems not to be the case for familiarity-based judgments (Manns & Squire, 2001;Yonelinas *et al*, 2002). Correspondingly, studies using functional magnetic resonance imaging have shown that the hippocampus is more active when recognition is based on recollection than on familiarity (Cansino *et al*, 2002;Dobbins *et al*, 2003). Here we were interested whether these two forms of recognition memory are differentially affected by sleep.

Sleep is considered to be a brain state optimizing the consolidation of memories (Stickgold *et al*, 2001;Maquet, 2001). Further, the consolidation of different types of memory seems to be tied to different sleep stages (Born & Gais, 2003). In humans, sleep in the early part of the night, dominated by extensive epochs of SWS, has been found to enhance in particular declarative memories, which refer to episodic and semantic memory and essentially rely on the hippocampal formation (Fowler *et al*, 1973;Plihal & Born, 1997;Plihal & Born, 1999a). This effect has been related to higher proportion of SWS as compared to REM sleep during early sleep, as well as to accompanying low levels of cholinergic activity in the hippocampus (Hasselmo, 1999;Gais & Born, 2004b). On the other hand, for non-declarative forms of memory, such as priming, which is considered an implicit form of memory and procedural memory, which refers to sensory motor skills and among others strongly rely on cortico-striatal circuitry, a greater benefit has been found after periods of late nocturnal sleep (Plihal & Born, 1997;Plihal & Born, 1999a;Wagner *et al*, 2003). This late period of nocturnal sleep is characterized by high amounts of REM sleep, and contains little SWS. While not independent of SWS, procedural memory thus appears to be particularly strengthened by REM sleep related mechanisms (Gais *et al*, 2000;Stickgold *et al*, 2000;Fischer *et al*, 2002).

So far, most of the studies examining the effects of sleep on memory consolidation in different memory systems used different tasks for this comparison. During the acquisition of task stimuli both explicit and implicit memories are developed in parallel (Tulving *et al*, 1999;Willingham & Goedert-Eschmann, 1999). In order to separate these two forms of memory at later retrieval testing, a number of recognition tasks have been developed that offer the opportunity to access the two memory systems in the framework of the same task. The process-dissociation procedure by Jacoby (1991) is one such approach that separates explicit recollection from implicit familiarity-based use of memory during recognition. The procedure provides a mathematical approach for the calculation of estimates of explicit and implicit memory in recognition tasks. The contribution of explicit recollection to recognition is estimated primarily on the basis of the subject’s responses indicating that he/she correctly identifies where and when a previously encountered stimulus occurred (e.g., a word is correctly classified as belonging to a certain study list). The use of familiarity-based implicit memory is estimated as the conditional probability of correctly recognizing an item as one that has been previously seen, given it was not recollected (see Methods for a detailed description). Both estimates of recollection and familiarity-based memory have been found to vary statistically independently under different experimental conditions (Yonelinas, 2002).

The purpose of the current study was to compare influences of early SWS-rich periods of retention sleep with late REM sleep-rich periods of retention sleep on estimates of explicit and implicit memory in a word recognition paradigm, using the process-dissociation procedure. Based on previous studies showing that declarative memory (hippocampus-dependent) improves in particular from SWS, we expected explicit recollection (also hippocampus-dependent) to benefit primarily from SWS as well. Moreover, explicit hippocampus-dependent memory was expected to benefit particularly when recollection is based on a more elaborate use of contextual features (i.e. when a word at recognition testing is presented in the same font as at acquisition, as compared with a presentation in a different font). On the other hand, familiarity-based implicit recognition being a non-declarative type of memory was expected to benefit in particular from REM-sleep rich periods of sleep. Participants had to learn two lists of words and they also had to memorize to which list each word belonged to. The words were presented in two different fonts, which changed in half of the words at recognition testing (contextual congruency manipulation). Learning was followed by a 3-h retention interval filled with either SWS-rich sleep or REM-rich sleep and recognition was tested 15 minutes after sleep. In a control group the 3-h retention intervals were spent awake. Saliva cortisol measured to control for possible confounding effects of glucocorticoid release on memory, was sampled before and after the retention intervals.

Methods

Participants

Twenty-four healthy, non-smoking, drug free subjects with no prior history of sleep disturbances participated in the main experiments and received a money reward for their participation (12 males; mean age 23.0 years, range 19-28 years). Subjects orally reported to habitually sleep 7-9 hours per night, and not to have had any major disruption of the sleep-wake cycle during the 6 weeks before experimentation. Subjects were acclimated to the experimental sleep condition by spending an adaptation night in the sleep laboratory, including the placement of electrodes. On the two experimental days the participants were instructed to get up at 7:00 h and not to take any naps during the day. They were instructed not to ingest alcohol or (after 15.00 h) caffeine containing drinks on these days. The study was approved by the local ethics committee. All participants gave written informed consent before participation.

Design and procedure

The experiment included two groups, a sleep group and a wake control group (6 men and 6 women in each group). Each group was examined in an early and a late night retention interval, with the order of the conditions balanced across subjects. The two experimental nights were separated by an interval of at least one week. In the early retention condition participants reported to the laboratory at 21:30 h. After electrodes were applied for standard polysomnography (only in the sleep group), participants performed on the recognition task from 22:15 -23:00 h (learning phase). In the sleep group the participants went to bed afterwards. Three hours after sleep onset, participants were awakened as soon as S1 or S2 sleep occurred. Awakening from SWS or REM sleep was avoided as this can decrease subsequent retrieval performance (Stones, 1977). Fifteen minutes after awakening, retrieval on the recognition task was tested. The participants in the wake group remained awake during the 3-hour retention interval between initial learning and retrieval testing. In this time they watched movies, played (computer) games or engaged in conversations with the experimenter.

In the late retention condition participants came to the lab at 22:30 h. After electrodes for sleep recordings were applied, subjects went to bed, and were awakened 3 hours after sleep onset as soon as sleep S1 or S2 occurred. Fifteen minutes later, the learning phase took place (2:15-3:00 h). Thereafter, participants in the sleep group went back to bed and slept for another 3 hours. Again, 15 minutes after awakening, retrieval was tested (6:15-700 h). In the same way as in the early retention condition the participants in the wake group stayed awake for 3 hours after learning.

After having performed the recognition task, participants rated their current feelings of activation, drowsiness, tiredness, motivation and concentration on 5-point rating scales. Saliva cortisol was sampled before and after the retention intervals. (Results from additional samples taken before and after testing did not add any relevant information, and hence are not reported here.) At the end of the whole experiment the participants were asked whether they had noticed the change in fonts which had taken place in some words during the recognition task.

Task materials

The words of the recognition task were selected from the Toronto word pool and translated to German. Of the 306 words selected, 18 words were used as buffer words, the remaining 288 were divided into six lists (each 48 words) balanced for imagery, frequency and concreteness. Three lists were used for each retention interval. Two served as study lists and one served as a novel one, the words of which were presented only during recognition. The words were presented using WespXP 1.98 (freeware from the Department of Psychology at the University of Amsterdam) on a 17 inch monitor, screen area was 1024 by 864 pixels using the 16-bit color mode, refresh rate was set on 75 Hz. Two true-type (.ttf) windows compatible fonts (font size 48) able to express special characters were used to present the words (i.e. “tsp tonight 1.ttf” and “tsp mcis 2.ttf”).

Recognition task

Two lists of words were presented at learning, each starting with 3 buffer words, followed by 48 study words. All words were presented randomly one at a time on a computer screen in front of the subject. The words were presented in white on a black background for 4.5 s with an interval of 1 s between each word. Half of the words were presented in one font and the other half in the other. The subjects were instructed to memorize the words and also to which of the two lists each word belonged to. They were told that some words would be harder to read than others (although this was not the case) by presenting them in different fonts.

During recognition testing after the retention interval, a list of words was presented which started with 9 buffer words (3 from each study list plus 3 new ones) followed by 144 test words. These test words included in random order all words from the two study lists and in addition 48 new words. Half of the words from each study list were presented in their original font ("congruent" word presentation), while the other half of the words were presented in the other font i.e. changed from "tsp mcis 2" to "tsp tonight 1" and vice versa ("incongruent" words). Subjects were asked to respond to each word with their right hand by pressing one of 4 buttons on a box within 3.5 s. They were instructed to press button "1" or button "2" (from left to right) if they remembered that the word belonged to either the first or the second list, respectively. Button "3" was to be pressed when the word was new to them and button "4" when the subject knew he/she had seen the word during acquisition but could not remember which list it belonged to. The 3.5-s response interval gave the subject ample time for the decisions, which is also supported by the fact that the recollection scores obtained here overall resembled those obtained under conditions of unlimited response time in previous studies (Yonelinas and Jacoby 1996).

To control for possible differences in encoding levels during the learning phase, the presentation of the study word list was preceded by a similar recognition task using 3-digit numbers as stimuli instead of words and, most important, with recognition tested immediately after acquisition. Here, each list consisted of only 8 numbers with 1 buffer number. In the recognition test 1 buffer number and 8 new numbers were added, and the font did not change in this task.

Data analysis

Estimates of recollection and familiarity were derived from scores of inclusion and exclusion, according to the process-dissociation procedure as has been described for the word-list discrimination task (Yonelinas & Jacoby, 1996). The inclusion score defines the amount of old words for which the subject correctly remembered the list they belonged to (buttons "1" and "2") plus the old words of which the subject knew he/she had seen them during acquisition but did not remember their list membership any more (button "4"). This results in: inclusion = (true positive list1) + (true positive list2) + (true positive button “4”). Exclusion is defined as the amount of old words that were falsely remembered to belong to a certain list, i.e. the words from list1 that were classified under list2 and vice versa (buttons “1” and “2” again) plus again the old words of which the subject knew he/she had seen them during acquisition but did not remember their list membership any more (button “4”), resulting respectively in: exclusion = (false positive list1) + (false positive list2) + (true positive button “4”). Inclusion and exclusion scores are then filled in the known formulas for recollection and familiarity as provided by Jacoby’s process-dissociation procedure being “recollection = inclusion – exclusion” and “familiarity = exclusion / (1 – recollection)”, respectively (Yonelinas & Jacoby, 1996). False alarms, delayed responses (>3.5 s), and buffer words were not included in the calculations.

The procedure used here differs from the original process-dissociation procedure used by (Yonelinas & Jacoby, 1996) in that it was based on 4 rather than 2 different button press responses requiring a slightly different behavioural strategy. However, the principles of the process dissociation in our procedure remained the same as in the original procedure. In the original procedure each of the two buttons are used to indicate a combination of two different decisions. Thus, one is used to indicate that a word is remembered to belong to one of the lists and *additionally* to indicate that a word is “known” when the list membership cannot be remembered. The other button is likewise used to indicate that a word is remembered to belong to the respective other list and *additionally* to indicate if a word is “new”. Because we wanted to avoid this complex double mapping of response decisions, participants had four buttons in our study, one for every type of response (i.e., two buttons to indicate a word’s list membership, and two for “know” and “new” responses, respectively). The calculation of the estimates for explicit recollection and implicit familiarity are not affected by this change, with the inclusion score as in the original procedure reflected by the sum of old words correctly remembered plus the correct “know” responses and the exclusion score reflected by the sum of false decisions regarding list membership plus the correct “know” decisions.

Statistical analysis was performed on the scores of recollection and familiarity-based recognition using analysis of variance (ANOVA) including a between subject factor “sleep/wake” and two repeated measures factors “night-half” (early vs. late night retention interval) and “context congruency” (congruent vs. incongruent font). Post hoc product moment correlations were calculated between recollection scores and corresponding familiarity scores to ascertain statistical independence of the two types of scores. Additionally, the percentages of correctly identified new words were analysed for the “sleep/wake” and “night-half” factors. A minimum of 33% (chance level) correctly identified “new” words was required for a subject to be included in the analysis. All subjects met this criterion. Pairwise comparisons were specified with *t*-tests. The significance level was set to $\alpha = 0.05$.

Standard polysomnographical recordings of sleep were scored offline according to the criteria by (Rechtschaffen & Kales, 1968). For each 30-sec epoch of recording the sleep stage was determined (W: wake, S1-S4: sleep stage 1-4, REM sleep). Sleep onset was defined by the occurrence of the first epoch of S1 sleep followed by an epoch of S2 sleep. Total sleep time and the percentages of each sleep stage were determined, with SWS being the sum of S3 and S4. Polysomnographic recordings from the late night of two subjects were incomplete due to technical failure, and were therefore not included in the sleep analysis. Saliva samples for the determination of cortisol were stored at -20˚C until assay using conventional radioimmunometric assay. Cortisol secretion during each retention interval was estimated by the mean value of the samples obtained immediately before and after the interval. The data from one subject were incomplete as a result of insufficient amount of saliva in some samples, therefore this analysis contained 11 subjects in the sleep group. Analysis of sleep and cortisol data also relied on ANOVA.

Results

Sleep and cortisol

Table 1 summarizes polysomnographic results for the early and late night retention intervals and associated concentrations of cortisol. Total sleep time as well as the percentages of wakefulness, stage 1 sleep (S1) and stage 2 sleep (S2) did not differ significantly from each other [$F(1,20) = 0.14, 2.52, 2.51, 1.44$, respectively, $P > 0.13$] Percentages of SWS and REM sleep exhibited the typical and highly robust differences, with the percentage of SWS being almost 4 times higher in the early than late night retention sleep, whereas the percentage of REM sleep was almost 3 times higher in the late night retention sleep [*F*(1,20) = 88.6, *P*<0.001 and *F*(1,20) = 45.69, *P*<0.001, respectively]. Sleep prior to learning in the late night (not included in Table 1) did not differ between the sleep and the wake group. The respective values for the sleep and wake group were: total sleep time, 196.21 ± 5.49 versus 195.91 ± 5.73 min, S1 - 6.96 ± 1.82 versus 6.30 ± 1.91 %, S2 - 41.81 ± 2.96 versus 45.28 ± 3.09 %, SWS - 37.78 ± 4.19 versus 34.59 ± 4.38 %, REM sleep - 10.18 ± 1.90 versus 10.67 ± 1.98 %, (*P* > 0.15, for all comparisons).

As expected, saliva cortisol values indicated significantly lower cortisol concentrations during the early than late night retention intervals [*F*(1,21) = 23.07, *P*<0.001] (Table 1), but the levels did not differ between the sleep and wake groups, [*P* > 0.12, for all comparisons].

Table 1. *Sleep and cortisol data*

	Early night		Late night		
Parameter	Mean	SEM	Mean	SEM	P<
Sleep time (min)	195.75	4.89	193.00	5.36	n.s.
Wake (%)	2.13	0.61	3.56	0.67	n.s.
S1 (%)	4.13	0.88	6.21	0.97	n.s.
S2 (%)	48.90	2.08	52.62	2.28	n.s.
SWS (%)	34.81	1.69	9.25	1.86	<.001
REM (%)	9.12	1.67	28.37	1.83	<.001
Sleep cortisol (µg/dl)	0.11	0.03	0.23	0.05	<.001
Wake cortisol (µg/dl)	0.08	0.03	0.36	0.06	<.001

S1, stage 1 sleep ; S2, stage 2 sleep ; SWS, slow wave sleep ; REM, rapid eye movement sleep. Cortisol values for the sleep and wake group for early and late night retention interval, estimated by average concentration in samples collected immediately before and after the retention interval. Right column: results from pairwise comparisons between the effects of early and late night. n.s., not significant.

Recognition performance

Immediate recognition testing on a number recognition task at learning, introduced as a control for performance differences during the learning phase, revealed an overall low performance, which however was clearly better than chance ($p<0.01$). There were no significant differences between the early and late night conditions as well as between the sleep and wake groups in this control task. Explicit recollection scores at this task were 0.22 ± 0.07 for the early sleep condition, 0.24 ± 0.07 for the early wake condition, 0.24 ± 0.06 for the late sleep condition, and 0.19 ± 0.06 for the late wake condition ($P>0.60$, for all comparisons). The respective values for familiarity-based judgements were 0.33 ± 0.04, 0.32 ± 0.05, 0.37 ± 0.03, and 0.35 ± 0.03 ($P>0.34$, for all comparisons).

Recognition testing after the retention interval on the word lists revealed distinct differences depending on the type of memory as well as on the type of retention interval (Table 2a). Explicit recollection was generally enhanced after retention intervals of sleep in comparison to wake intervals [main effect for sleep/wake manipulation, $F(1,22) = 4.33$, $P<0.05$]. The enhancing effect of sleep on explicit memory was particularly pronounced after early night retention sleep, and especially for the context congruent words (Figure 1a), as was revealed by the significant 3-way “sleep/wake” x “night-half” x “context congruency” interaction [$F(1,22) = 4.29$, $P=0.05$]. No other effect approached significance [$F(1,22) < 2.74$, $P>0.12$, for all comparisons]. The pattern of the 3-way interaction was also confirmed in a post-hoc analysis. First, one-way ANOVAs showed that the sleep and wake group differed primarily on recollection for congruent words in the early night [$F(1,11) = 7.50$, $P<0.05$]. In the late night this effect failed to reach the 5 % level of significance [$F(1,11) = 3.18$, $P<0.10$]. Additionally, for the incongruent words the difference between the sleep and wake group did not approach significance neither in the early retention interval [$F(1,11) = 0.95$, $P>0.34$] nor in the late night retention interval [$F(1,11) = 2.80$, $P>0.11$] . Furthermore, two-way ANOVAs performed separately for the sleep and wake group, showed a significant effect for the “night-half” x “context congruency” interaction [$F(1,11) = 5.09$, $P<0.05$] in the sleep group. The direction of this effect was further investigated by paired t-tests, which confirmed the enhanced explicit recollection of words presented in congruent context in the early night retention sleep over both (i) the same words in the late night retention sleep ($P<0.05$), and (ii) the words presented in incongruent context in the early night retention sleep ($P<0.05$, Figure 1a). The difference between words presented in congruent and incongruent context in the late night retention sleep or between the incongruent words in the early and late night retention sleep did not approach significance (*P*>0.59, for both comparisons). The analyses in the wake group showed no significant effects [*F*(1,11) < 0.54, *P*>0.48 for all comparisons].

Table 2

a. Early night retention interval

		Congruent words			Incongruent words		
		True pos	False pos	Know	True pos	False pos	Know
Responses	Sleep	0.71	0.10	0.04	0.64	0.12	0.03
	Wake	0.59	0.21	0.05	0.58	0.16	0.06
Recollection	Sleep	0.60 ± 0.06			0.52 ± 0.07		
	Wake	0.37 ± 0.06			0.41 ± 0.07		
Familiarity	Sleep	0.37 ± 0.03			0.32 ± 0.03		
	Wake	0.41 ± 0.03			0.36 ± 0.03		

b. Late night retention interval

		Congruent words			Incongruent words		
		True pos	False pos	Know	True pos	False pos	Know
Responses	Sleep	0.62	0.15	0.04	0.61	0.12	0.06
	Wake	0.51	0.16	0.07	0.51	0.14	0.08
Recollection	Sleep	0.47 ± 0.05			0.49 ± 0.05		
	Wake	0.35 ± 0.05			0.36 ± 0.05		
Familiarity	Sleep	0.36 ± 0.03			0.35 ± 0.04		
	Wake	0.34 ± 0.03			0.34 ± 0.04		

Mean ± SEM scores of explicit recollection and familiarity-based implicit memory in the early and late night retention interval. Respective upper lines indicate mean proportions of actual button press responses of true positive (correctly identified old words with correct list classification, false positives (correctly identified old words with wrong list classification), and “know” classifications (correctly recognized old words but without list membership classification, i.e., true positives button “4”).

For the familiarity-based recognition scores congruent words were slightly better remembered than the incongruent words [*F*(1,22) = 3.10, *P*<0.10] (Table 2 and Figure 1b). However, there was no general effect of sleep on familiarity-based recognition scores [*F*(1,22) = 0.13, *P*>0.70] and these scores also did not differ between early and late night retention intervals [*F*(1,22) > 2.07, *P*>0.17].

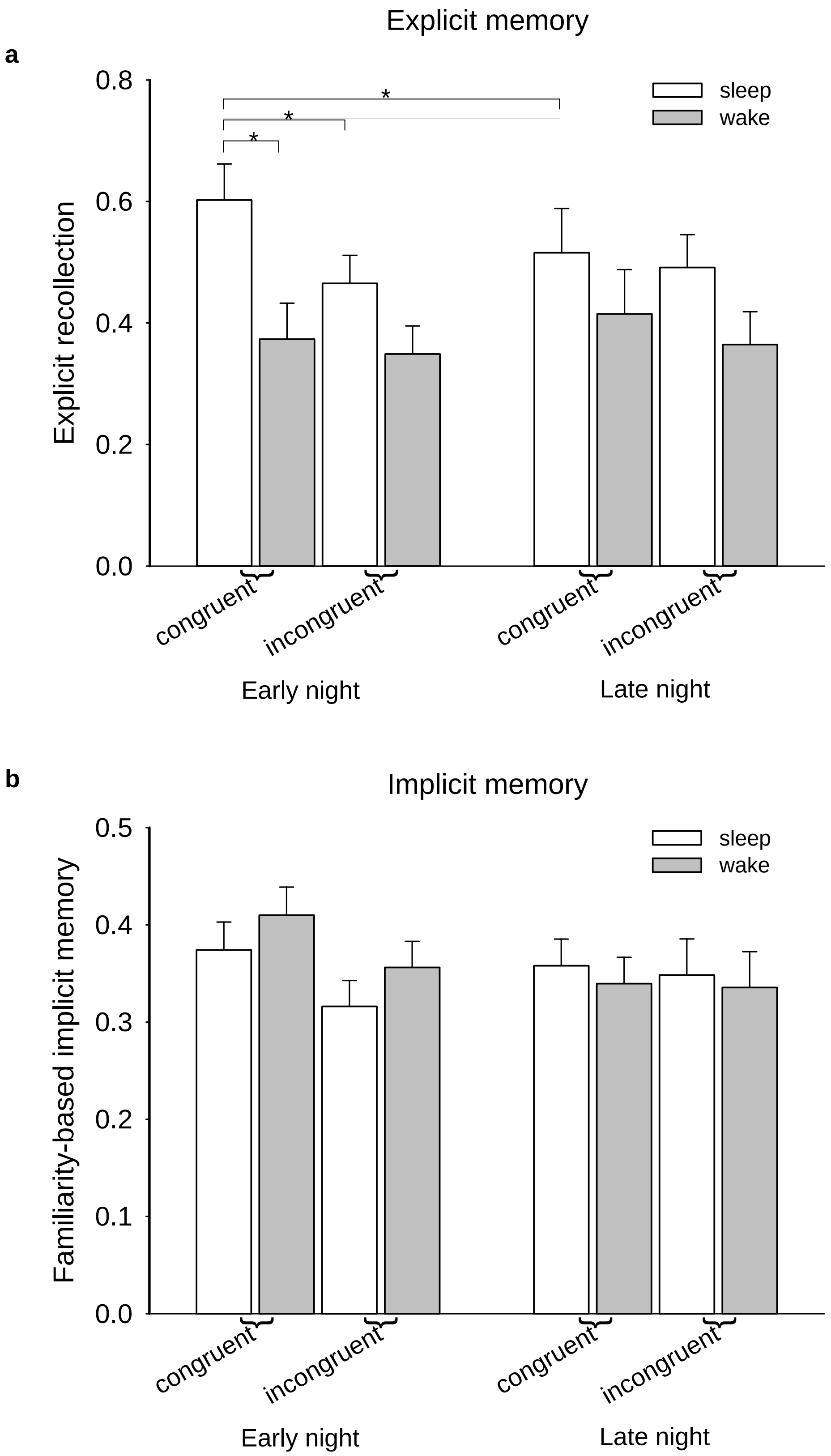
Explicit memory
a
0.8
0.6
0.4
0.2
0.0
Explicit recollection
*
*
*
sleep
wake
congruent
incongruent
congruent
incongruent
Early night
Late night
b
Implicit memory
0.5
0.4
0.3
0.2
0.1
0.0
Familiarity-based implicit memory
sleep
wake
congruent
incongruent
congruent
incongruent
Early night
Late night

Figure 1. Mean ± SEM scores for (a) explicit recollection and (b) familiarity-based implicit memory, as derived from the process-dissociation procedure for the sleep (white bars) and the wake group (grey bars) after early and late night retention intervals, separately for words presented in the same font as during acquisition (congruent context) as well as for words presented in a changed font (incongruent context). Note, sleep generally enhanced explicit recollection, and especially for the congruent words after early sleep. There were no significant effects of sleep on familiarity-based implicit memory scores. * $p<0.05$, for pairwise comparisons.

A supplemental control analysis showed no effect of the "sleep/wake" or the "night-half" manipulation on the ability to identify the new words. The percentages for correctly identified new words were well above chance and did not differ from each other (sleep group in the early night 78.68 ± 5.48 %, in the late night 78.20 ± 5.30 %, wake group in the early night 75.66 ± 5.48 %, in the late night 77.47 ± 5.30 %, $F(1,22) < 0.13$, $P>0.70$, for all comparisons). Moreover, control analyses did not indicate any significant correlations between scores of recollection and familiarity ($p>0.14$). The respective coefficients were in the sleep group $r= 0.06$ (early retention interval – congruent words), $r=-0.17$ (early retention interval – incongruent words), $r=-0.10$ (late retention interval – congruent words), and $r=0.01$ (late retention interval – incongruent words). In the wake group the respective coefficients were $r=-0.38$, -0.38, -0.40, and -0.46, respectively.

Questionnaires

After having completed the word recognition task at retrieval, subjects rated their current feelings of activation, drowsiness, tiredness, motivation, and concentration. In general, all variables, including tiredness (early sleep: 3.58 ± 0.28 vs. early wake: 3.92 ± 0.28, late sleep 3.50 ± 0.28 vs. late wake 4.00 ± 0.28) and subjective concentration (early sleep: 2.83 ± 0.27 vs. early wake: 2.42 ± 0.27, late sleep 2.75 ± 0.27 vs. late wake 2.67 ± 0.27), were closely comparable for both groups for both night intervals at retrieval testing ($P>$ 0.13, for all comparisons). None of the participants had noticed the change in fonts in the recognition task although some (2 in the sleep group and 1 in the wake group) said that they thought something was "strange" with some words.

Control experiments on retrieval function

In the light of evidence that performance on tasks relying on the frontal cortex are highly sensitive to an impairing influence of sleep deprivation (Harrison & Horne, 1998;Drummond *et al*, 2000), and since explicit recollection also involves this brain region (Fletcher & Henson, 2001), additional experiments in two separate groups of subjects (n= 22, age 20-35 yrs) examined whether a 3-hour period of early wakefulness induced any sleep deficit that would account for the inferior explicit recollection seen in this condition in the main study. A phonetic fluency task used to assess fluency of word retrieval from long-term memory (Aschenbrenner *et al*, 2000) required the subject to write down as many words as possible within two minutes starting with a certain letter (‘p’, ‘m’). The task was presented before and after 3-hour periods of sleep (n=12) and wakefulness (n=10) during the early night, scheduled in the same way as in the main experiments. Also, the digit span test (forward and backward) was presented to assess general prefrontal mediated working memory function. Retrieval fluency after the 3-hour periods did not differ between the sleep and wake groups [$F(1,20) < 1.40$, $P>0.25$, for all comparisons], and was on average even slightly higher in the wake group (Table 3). Also, digit span test performance was closely comparable between the groups [$F(1,20) < 1.60$, $P>0.22$, for relevant comparisons] and [$F(1,20) < 2.18$, $P>0.16$, for all comparisons] for both forward and backward respectively (Table 3).

Table 3

Effects of early sleep and wakefulness on retrieval function and working memory

	Fluency of word retrieval		Digit span (forward)		Digit span (backward)	
	Before	After 3 hours	Before	After 3 hours	Before	After 3 hours
Sleep	20.17 ± 1.54	19.83 ± 1.57	9.08 ± 0.70	9.92 ± 0.58	7.58 ± 0.48	8.25 ± 0.55
Wake	19.70 ± 1.68	22.60 ± 1.72	8.30 ± 0.77	8.50 ± 0.63	7.80 ± 0.52	7.50 ± 0.60

Mean ± SEM scores for fluency of word retrieval and digit span test performance (forward and backward)

Discussion Study 1

This study used the process-dissociation procedure (Jacoby, 1991) to separate effects of sleep on explicit and implicit memory consolidation. Compared to retention periods of wakefulness, sleep generally enhanced measures of explicit recollection. This effect was particularly pronounced during early sleep periods dominated by SWS, and when, at recognition testing, the words were presented in the same font as at learning before sleep. Interestingly, familiarity-based implicit memory did not benefit from sleep. There, only a small benefit was observed for contextual congruency (i.e. when the font of the words was kept the same at retrieval as at learning) which, however, was independent of sleep. As a whole, these results speak for a greater sensitivity of explicit than implicit memory formation to the enhancing effects of sleep that might be particularly linked to SWS.

The effects of sleep observed cannot be attributed to circadian variations, since changes in memory across periods of early and late sleep were compared with periods of wakefulness in the same phase of the circadian rhythm. This is also supported by the fact that saliva cortisol concentrations were closely comparable between the sleep and wake conditions and at the same time showed the normal circadian variation. Likewise, self ratings of fatigue and concentration did not differ between the groups at learning and retrieval testing. In addition, although at an overall low performance level, immediate recognition (of numbers) tested at learning did not differ neither between sleep and wake groups nor within each of these groups when tested in the evening or at night.

The results of our control experiment also argue against the view that the wake periods of about 3 hours during the early night induced any substantial effects arising from sleep deprivation. This experiment was conducted on the background of evidence that sleep deprivation (for 36 hours) has an impairing influence particularly on prefrontal mediated retrieval functions (Harrison & Horne, 1998;Drummond *et al*, 2000). Our results show that word retrieval from long-term memory as well as working memory functions remained unaffected by a 3-hour sleep deprivation in the early night as compared to early sleep. With regard to our finding of enhanced explicit recollection of context congruent words after early retention sleep, it should be emphasized that this effect was not only significant in comparison with early wakefulness, but also in comparison with late retention sleep (Figure 1).

A benefit of the process-dissociation procedure is that within the same task, the effects of sleep on explicit and implicit memory can be assessed simultaneously. This is important since earlier studies indicating a differential sensitivity to the effect of sleep and sleep stages depending on the type of memory system, relied mostly on completely different tasks to asses the respective memory systems. Using the same task to test explicit and implicit memory rules out that the differential effects of sleep observed here were due to some non-specific task characteristics not related to any of the two memory systems.

Our finding of a distinctly more pronounced improvement of explicit recollection after retention periods of early than late night sleep agree with a number of previous studies indicating a particular benefit of hippocampus-dependent declarative memory from just this early period of SWS-rich sleep (Born & Gais 2003). It has been proposed that the enhancing effect of early sleep on hippocampus-dependent memories relies on a reactivation of the newly acquired memory representations in hippocampal neuronal populations that occurs predominantly during SWS (Hasselmo & Wyble, 1997;Buzsaki, 1998;McNaughton *et al*, 2003). Such processes could explain a facilitated access at later explicit recollection of these memories. An involvement of the hippocampal formation in the sleep-associated memory process of interest is further supported by our finding that explicit memory enhancement during early sleep was most robust when the words were presented in the same context as during encoding. Several previous studies have consistently shown that one essential hippocampal function serves to bind encoded information with contextual cues, even in the absence of awareness for these contextual cues (Henke *et al*, 1999;Stark & Squire, 2001;Henke *et al*, 2003). Thus, a contextual dependence of the explicit memory enhancement during early sleep seems to be in agreement with the notion that this type of sleep particularly benefits hippocampus-dependent types of memory.

Contrary to our expectation, familiarity-based measurements of implicit memory were not enhanced by sleep, neither during the early part nor during the late part of the night. While this negative finding agrees with a recent study testing effects of early and late sleep on performance in a “remember/know” paradigm (Rauchs *et al*, 2004), it appears to contrast with a number of foregoing studies indicating that various forms of non-declarative memory, such as the procedural memory for skills (Plihal & Born, 1997) and the priming of words and faces (Plihal & Born, 1999a;Wagner *et al*, 2003) benefit in particular from REM sleep-rich periods of sleep. Those studies led us to suppose a generalized benefit for REM sleep-rich periods of sleep for non-declarative memories not depending on hippocampal function. However, it is not unlikely that familiarity, priming and procedural tasks, apart from relying on non-hippocampal brain regions, actually represent different types of memory (Wagner *et al*, 1998;Drummond *et al*, 2000;Stark & Squire, 2000;Rugg & Yonelinas, 2003) and thus may differ in other qualities that are crucial to their sensitivity to the enhancing effect of sleep.

In addition, there are hints that memory based on familiarity judgments decrease at a more rapid rate than recollection based memories (Yonelinas & Levy, 2002). It could be that in our experiment the time spent between encoding and retrieval was too long for any difference in familiarity-based judgments to remain detectable. However, this possibility seems unlikely on the background of evidence that when relatively long time intervals in the order of hours (and even days) are tested as in the present study, decline rates of familiarity were found not to be greater than those for measures of recollection (Hockley & Consoli, 1999).

The absence of effects of retention sleep on familiarity based recognition could have its origin also in more general conceptual implications of the process dissociation procedure that has not remained without criticism (Richardson-Klavehn *et al*, 2002;Mintzer *et al*, 2003). In particular, it is still under debate whether recollection and familiarity indeed are linked to two different memory systems, or if they refer to different retrieval mechanisms at recall to gain access to the same memory trace (Ratcliff *et al*, 1995). Along this line of reasoning the failure to find effects on familiarity-based implicit recognition scores here may just reflect a less efficient access to the memory trace of interest via this type of recognition judgement. Related to this, from the perspective of the processes-dissociation procedure the contextual effect that we observed in our study on explicit memory was also not expected. This is because manipulations of the perceptual features of stimuli in general are considered to affect only implicit forms of memory such as priming (Fleischman *et al*, 1997). Diverging from this view, our findings suggest that non-consciously attended information influenced explicit memory. This raises the problem of contamination, i.e., the possibility of partial overlaps between explicit and implicit memory (Butler & Berry, 2001), and thus implies a violation of the independence assumption of the process-dissociation procedure, which regards explicit recollection and familiarity-based implicit recognition as entirely independent from each other. Accordingly, the pattern of our results brings into question the notion that measures of implicit and explicit memory reflect memory systems as different and independent as assumed by the process-dissociation procedure, although (post hoc) linear correlation analyses of our data assured that scores of recollection and familiarity-based judgments were independent in a statistical sense. Adopting a perspective beyond the framework of the process-dissociation procedure, the present data would indicate that recognition of old words remains uninfluenced by sleep, but that after early sleep the subject's ability to identify the correct list for congruent words is enhanced. This view appears to be in line with findings from a recent study that early SWS-rich sleep enhances memory for temporal context information in an episodic memory task (Rauchs et al. 2004).

Our data of a selective enhancement of signs of explicit recollection by retention sleep are in line with previous reports of a preferential enhancement of explicitly guided memory during sleep (Robertson *et al*, 2004). Those studies were based on serial reaction time tasks (SRTT) and showed that offline improvement in task performance that occurred selectively across retention periods of sleep required that subjects were aware of the sequence of the task they trained before sleep. Recent experiments indicated that the hippocampus and closely connected temporal lobe structures can be involved in both explicit and implicit learning on the SRTT (Schendan *et al*, 2003). Thus, activation of prefrontal cortical circuitry including the dorsolateral and ventrolateral prefrontal cortex seems to be more relevant to the distinction between explicit and implicit processes on that task (Fletcher & Henson, 2001;McIntosh *et al*, 2003), which may apply to the recognition task used here as well. Notably, some evidence exists that slow oscillatory EEG activity dominating human SWS reflects processes of cortical reorganisation, especially in the prefrontal cortex, that could be linked to explicit processing (such as 'thinking') taking place during the wake phase (Steriade & Timofeev, 2003;Anderson & Horne, 2003a;Anderson & Horne, 2003b). From this perspective, use-dependent changes in prefrontal cortical circuitry during explicit processing in the wake phase could be a starting point for plastic changes underlying memory formation during SWS-rich sleep (Sejnowski & Destexhe, 2000;Huber *et al*, 2004;Molle *et al*, 2004).

Study 2: Sleep’s function in the spontaneous recovery and consolidation of memories

Submitted as:

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Introduction

While there is a wide agreement on the memory enhancing function of sleep, and despite evidence from studies of brain activity suggesting an active consolidation that reorganizes memory representations during sleep, the psychological characteristics of this consolidation are only poorly understood. Sleep has commonly been thought to benefit memory consolidation by protecting the newly encoded memory traces from more or less specific interference resulting from encoding of other events during the critical period of consolidation when the memory trace is still in a fragile state (Wixted, 2004). However, this view ascribes a merely permissive role to sleep in the strengthening of memories. Such a role is difficult to integrate with the gains in performance, as reported above, which have been observed after retention sleep, particularly those of explicit knowledge which suggest that sleep can actively promote a change in the structure of recently encoded memory representations (Wagner *et al*, 2004;Fischer *et al*, 2006). Structural changes in a memory representation implicate that the various associations making a representation are differently affected by the sleep-associated consolidation process. Particularly, in the case of two competing memories an active consolidation would be implicated if sleep rather than equally enhancing both memories, as predicted from a mere permissive role of sleep, strengthens these memories in a differential manner. In fact, one hypothesis that has been controversially discussed in this context states that sleep improves retention by releasing influences of interference, i.e., selectively strengthens memories that were competitively inhibited by others that were similar but more recently encoded (Ekstrand, 1977).

More specific Ekstrand and colleagues (Ekstrand, 1967;Ekstrand *et al*, 1971) examined this issue of competing memories using a declarative word-pair associate task. Two word lists (each consisting of 12 pairs of 3-letter words) were presented according to the classical "A-B, A-C" paradigm, in which subjects first learned to a criterion a list with a cue word ("A") each associated with a target word ("B") and, then, a second list where each original cue word ("A") was associated with a different target word ("C"). This type of learning typically shows decreased memory performance for the list learned first compared to the list learned second, although both lists where initially learned to the same degree. The decreased retention for the first learned list is ascribed to retroactive interference, i.e. forgetting as the result of learning new competitive information (Bower *et al*, 1994). In Ekstrand et al's studies memory was tested using the "modified modified free recall" (MMFR) procedure, which assess memory for both lists by presenting the cue word and asking for both associated targets (i.e., "B" and "C") as well as the order they were learned. Retrieval testing after 8-hr retention periods of nocturnal sleep and diurnal wakefulness indicated that sleep during the retention interval not only improved recall in general but had a particularly facilitating influence on the recall of the first learned (A-B) list. The finding of a greater benefit for the first (A-B) than the second (A-C) learned list after sleep in fact clearly supported the notion that sleep improves memory by facilitating spontaneous recovery from retroactive interference (Ekstrand, 1967). However, Ekstrand and co-workers challenged this interpretation by a second study (Ekstrand *et al*, 1971) examining recall of the same "A-B, A-C" paradigm, immediately, 20 min and 7-hours after learning. During the 7-hour retention interval subjects slept, although on all of these conditions sleep was systematically disrupted to introduce a deprivation of specific sleep stages. This study suggested that the spontaneous recovery from retroactive interference occurred already in the 20-min wake period after learning. Contrary to expectations there was no further improvement of first-list recall after the 7-hour sleep periods. This led the authors to conclude that the improved first-list recall found by Ekstrand (1967) after sleep was due to a spontaneous recovery from retroactive interference that had occurred already before sleep. Yet, it seems premature to abandon any contribution of sleep to the enhanced first-list recall based on this later work by Ekstrand et al. (1971), since this study – as judged from the present state of the art – lacks methodological scrutiny. Specifically the study did not include a condition of undisturbed retention sleep or a 7-hour retention interval of wakefulness. Experimental sleep disruptions as introduced during the 7-hour periods of retention sleep in that study can, per se, profoundly influence subsequent recall performance (Cipolli, 1995;Born & Gais, 2000). Also, the effect for the observation that recovery from retroactive interference occurred already 20 min after learning, in fact was not statistically significant.

Experiment 2: Re-examining the findings by Ekstrand 1967 and Ekstrand et al 1971

The methodological shortcomings in the studies mentioned above, in conjunction with hints at effects of sleep on interference during procedural memory formation (Walker *et al*, 2003), led us to replicate Ekstrand's first study from 1967, however, including some improvements regarding the procedure. Specifically, these changes should allow to directly compare effects of undisturbed sleep and wakefulness as well as effects of short 20-min retention intervals of wakefulness on recall in an "A-B, A-C" paradigm. Moreover, wake and sleep intervals covering the same nocturnal phase of the 24-h cycle should control for possible circadian confounds. To control for effects of fatigue (after prolonged periods of wake time) on recall performance, recall on the sleep and wake conditions was postponed until after a second post-learning night, in which subjects slept normally. Also several supplementary control conditions were introduced. To estimate the rate of forgetting occurring independent of retroactive interference, two additional groups of subjects (a sleep and a wake group) learned only one list (A-B). This type of control has been used in previous studies to obtain a measure of memory decay which could potentially mask spontaneous recovery (Postman *et al*, 1968;Postman *et al*, 1969;Wheeler, 1995). Another possible confound in such studies arises from the fact that learning in the evening before sleep provides the opportunity for subjects to spend some time thinking about the learned lists in the interval between the learning phase and falling asleep, thereby selectively improving memory in the sleep condition. To prevent such possibility of rehearsal, in an additional condition subjects performed distracting tasks after learning until falling asleep. Finally, in the study by (Ekstrand *et al*, 1971) subjects in the condition of delayed (by 20 min) recall were tested in the evening. However, retroactive interference in memory may well be subject to circadian influences. This led us to include two additional conditions where a delayed (by 20 min) recall testing took place either in the morning or in the evening.

Methods

Subjects

Ninety healthy, non-smoking, drug free, native German speaking subjects with no prior history of sleep disturbances and regular sleep-wake cycle, participated in the experiments and received a money reward for their participation (45 males, 45 female, mean age 24 years, range 19-34 years). The experiments were approved by the local ethics committee. All participants gave written informed consent before participation. All participants were tested individually. Prior to the actual testing sessions, subjects in the sleep condition had an adaptation night in the laboratory, which included the placement of electrodes. The participants were instructed to get up at 7:00 h and not to take any naps on the days of the experiment. They also had to abstain from taking any caffeine containing drinks after 15:00 h in the overnight conditions, and during the whole day on all other conditions. Alcohol was not permitted on the experimental days in any condition and throughout the whole experimental period.

Design and Procedure

The main experiment included four groups, the “sleep group” and three control groups, the “wake group”, the “immediate recall group” and the “delayed recall group” (each 10 subjects; 5 male, 5 female). The subjects of the sleep group reported to the laboratory around 21:30 h. Following the placement of electrodes and preparations for bedtime, the learning phase started at 22:00 h. The subject was seated in a room alone in front of a computer screen where word-pair associates from a list of 20 pairs were presented sequentially and repeatedly until the subject reached a criterion of 90 % correct responses. Depending on how many times the list of word-pairs had to be presented to reach the criterion, learning of a list took between 12 and 18 min. The subjects were then instructed not to rehearse the word-pair associates anymore. Subsequently, they played the computer game “Snood” which served as distractor task for 15 min. Afterwards the second list of word-pair associates was presented, and again the presentation was repeated until the learning criterion was reached, which took between 12 and 20 min. Then, just like after the first list of word-pair associates, subjects were asked not to further rehearse the word-pair associates. They were offered to go to the toilette, after which they immediately went to bed. Lights were turned off within 5-10 min after learning of the second list was completed. At 07:00 h the next morning subjects were awakened, and electrodes were removed. Before leaving the laboratory the subjects were told that they should follow their regular activities during this day, to go to bed around 23:00 h, and not to rehearse the word lists. Subjects reported back to the laboratory the next morning at 08:00 h for retrieval testing. Caffeine containing drinks were again not allowed on this morning. After having performed the retrieval test, the participants completed a 5-point self-rating questionnaire concerning feelings of activation, drowsiness, tiredness, motivation, attention, concentration and further psychological and physiological changes that may have occurred during the retention period and at retrieval testing. At the end of the session, by a standardized interview it was assured that the subjects had fully complied with the instructions, and the subjects also had to report the activities they had engaged during the day before. Since glucocorticoids are known to influence memory function (e.g., de Quervain *et al*, 2000;Maheu *et al*, 2004;Wagner *et al*, 2005) saliva cortisol was sampled, as an estimate of adrenocortical secretory activity, both before and after (i) learning of the word-pair associate lists, (ii) the retrieval test, as well as (iii) before leaving the laboratory after the first night.

The procedure for the subjects in the wake condition was the same except that after learning was completed the subjects remained awake throughout the night and the following day. They stayed in the laboratory until 07:00 h and afterwards went home. They were instructed not to go to bed before 23:00 h on this day. Throughout the experiment they wore an “activity watch” (Actiwatch®), which was used to assure that subjects had not brief minute periods of sleep during the experimental periods of wakefulness. Standard activities the subjects undertook during the night included watching movies, playing computer games, and going out for a walk in the experimenter's company. Reading was not allowed.

In the immediate and delayed recall conditions the procedure was the same as in the sleep group except that the subjects were tested throughout the day (between 10:00 and 23:00 h). In the immediate recall group the retrieval testing was performed right after the learning phase, i.e., after the participant had reached the learning criterion for the second list of word-pair associates. In the delayed recall group, the subjects first played Snood again for 20 min and then retrieval was tested.

A total of five supplementary conditions were introduced to control for different sources of confounds. Each of these conditions again included 10 subjects (5 women, 5 men). Two groups learned just 1 list before sleep and wake retention intervals, respectively ("sleep single list", "wake single list") followed by playing Snood, to estimate the rate of forgetting of the first A-B list independent of retroactive interference from learning of the A-C list. The actual lists used in these experiments were counterbalanced across subjects and conditions. Another group ("presleep-occupied") was tested under the same conditions as the sleep group of the main experiment with the exception that they were occupied with two distractor tasks in the interval between learning and sleep to prevent rehearsal of the word-pairs. Specifically, these subjects first played again Snood for 2 min after learning the A-C list and then went to bed where they were instructed to keep mental count of time and to press a hand-held button (with any finger they wished) every 30 felt seconds until they fell asleep (taken from Marshall *et al*, 1998). They should not keep themselves awake by performing the task. Finally, while in the delayed recall condition of the main experiment, subjects were tested throughout the day, two additional groups of subjects were run on this delayed (by 20 min) recall condition specifically in the morning (learning beginning at 7:30 h) and in the evening (beginning at 22:00 h), in order to explore possible confounds of circadian rhythm.

Memory Task and Materials

Two lists of word-pair associates were created each containing 20 pairs. The words were taken from lists used in previous studies on proactive and retroactive interference (Blank, 2002). The lists were constructed in such a way that maximum retroactive interference was obtained (Bower *et al*, 1994;Blank, 2002). This was done by choosing 20 nouns as "A" words, all from different semantic categories. Then, from another 20 different semantic categories, 20 pairs of words were selected that served as "B" and "C" words. However, each pair of "B" and "C" words was not connected to the same "A" word. Thus, two lists of word-pair associates were formed (A-B and A-C) each containing 20 pairs. Examples of A-B and A-C pairs are "Kirche-Pudel" and "Kirche-Brandy", "Märchen-Sherry" and "Märchen-Themse", "Stimmung-Segeln" and "Stimmung-Dackel", i.e. "Church-Poodle" and "Church-Brandy", "Fairy Tale-Sherry" and "Fairy Tale-Thames" (River), "Mood-Sail" and "Mood-Dachshund". Each word-pair was presented for 4 sec on a computer screen. When all the word-pairs from a list were presented, the "A" words were presented again, alone this time, for 3 sec. in which time the subjects had to name the "B" word that was associated with that particular "A" word. In the next 4.5 sec feedback was given by presenting the whole word-pair again. List presentation and the presentation of the "A" words alone were repeated until the subject made 2 mistakes or less, when responding to the "A" words. After 15 min the A-C associations were learned in an identical way. On the retrieval test the subjects were presented again with the "A" words and had to write down on a paper the two words that were associated with each "A" word, indicating also which word was first ("B") and which was second ("C") at learning. This type of memory testing is also referred to as the Modified Modified Free Recall test (MMFR) (Ekstrand, 1967;Ekstrand *et al*, 1971;Bower *et al*, 1994). Subjects had 20 sec to respond to each "A" word. Both during learning and retrieval all stimuli were presented in random order.

The words were presented using WespXP 1.98 (freeware from the Department of Psychology at the University of Amsterdam). Snood, which subjects played in the interval between learning the two lists, is a shareware game taken from www.snood.com. It is a fun game where the player has to solve puzzles. All subjects played the medium level. Actiwatches (Cambridge Neurotechnology, UK), used to monitor wakefulness in the wake group, are small, rugged, actigraphy-based data loggers that record a digitally integrated measure of gross motor activity and the subjects can wear them around their wrist. Salivary cortisol concentrations were determined by a conventional radioimmunometric assay. Sleep was assessed using standard polysomnography (Rechtschaffen & Kales, 1968)

Results

Recall performance scores were calculated as the percentage (with reference to the total number of list responses) of correctly remembered "B" and "C" words to the appropriate "A" word. The number of correctly recalled words for which the list was not correctly remembered was generally small (<1.5 in the sleep and wake conditions and 0 in the immediate and delayed recall conditions), and a separate evaluation of these cases did not add any further information. Therefore, this report is restricted to the recall scores that included the correct allocation of target words to the respective list. Recall scores of the main experiment were analyzed in a 4 x 2 repeated measurements ANOVA with the four groups sleep, wake, immediate and delayed recall as between-subjects variable and the factor list (A-B vs. A-C) as a within-subjects variable. Alpha was set to 0.05. Table 4A summarizes recall performance for each list in every group.

Table 4A

Main Experiment				
Learning (trials to criterion)	Sleep	Wake	Immediate Recall	Delayed Recall
A-B list	2.90 ± 0.34	2.90 ± 0.34	3.60 ± 0.34	3.10 ± 0.34
A-C list	3.10 ± 0.39	3.50 ± 0.39	3.40 ± 0.39	3.30 ± 0.39
Retrieval (% of total list)				
A-B list	73.00 ± 6.51	54.50 ± 4.80	78.00 ± 5.54	82.00 ± 4.23
A-C list	67.50 ± 4.90	70.50 ± 5.02	96.50 ± 1.98	96.50 ± 1.50
Difference (A-C minus A-B)	-5.50 ± 6.30	16.00 ± 5.42	18.50 ± 5.58	14.50 ± 4.74

Table 4B

Supplementary Controls					
	Presleep-	Single List		Delayed Recall	
Learning (trials to criterion)	occupied	Sleep	Wake	Morning	Evening
A-B list	2.50 ± 0.22	3.00 ± 0.26	3.50 ± 0.22	3.30 ± 0.26	3.30 ± 0.37
A-C list	2.60 ± 0.22			3.50 ± 0.31	3.30 ± 0.30
Retrieval (% of total list)					
A-B list	73.50 ± 5.53	87.00 ± 4.10	87.50 ± 4.10	76.00 ± 5.47	83.50 ± 3.80
A-C list	75.00 ± 5.96			93.50 ± 2.63	96.50 ± 1.30
Difference (A-C minus A-B)	1.50 ± 3.73			17.50 ± 5.07	13.00 ± 3.96

Learning (top) and Retrieval performance (bottom) in the Main experiment (A) and Supplementary control groups (B). The main experiment included a Sleep, Wake, Immediate Recall and Delayed Recall (by 20 min) group. The supplementary controls included a Presleep-occupied condition (subjects were occupied with a distractor task until sleep onset), a Single List Wake, a Single List Sleep condition (only one list was learned), and Delayed Recall Morning and Evening conditions (in which recall delayed by 20 min took place in the morning or evening to control circadian rhythm). For learning, the number of trials needed to reach the criterion when learning the A-B and A-C word-pair associates are indicated. For retrieval, the percentage (with reference to all 20 word-pairs in a list) of correctly recalled words as well as the difference scores between A-C and A-B associations are indicated. Data are means ± SEM.

At learning the number of trials to reach the criterion was well comparable between the four groups as there were no significant differences among them, $F(1,36)=0.36$, $\eta^2=0.03$, $F(1,36)=2.29$, $p=0.14$, $\eta^2=0.06$, $F(3,36)=1.52$, $p=0.23$, $\eta^2=0.11$, for main effects of group and list, and group x list interaction, respectively.

At retrieval testing, recall on the sleep condition in comparison with the wake condition was distinctly improved for the first A-B list. Recall on the second A-C list did not differ between the two conditions (Figure 2A). The 4 x 2 ANOVA revealed a significant main effect for group, $F(1,36)=12.60$, $\eta^2=0.51$, as well as for list, $F(1,36)=15.43$, $\eta^2=0.30$, and also a significant 2-way interaction between group and list, $F(3,36)=4.00$, $\eta^2=0.25$. Post-hoc analyses of homogeneous subsets (Waller-Duncan) revealed two subsets of significantly differing groups, one including the sleep and the wake groups and one including the immediate and delayed recall groups. Therefore, data from these subsets were further analyzed separately. In the comparison of the sleep and wake group, significance for the group x list interaction confirmed the preferential improvement in recall of the A-B list in the sleep group, $F(1,18)=6.70$, $\eta^2=0.27$. The main effects for both group, $F(1,18)=1.50$, $p=0.24$, $\eta^2=0.08$, and list, $F(1,18)=1.60$, $p=0.22$, $\eta^2=0.08$, were not significant. Post-hoc pair-wise comparisons performed separately on the retention rates for the A-B and A-C pairs further confirmed a superior recall of A-B associations in the sleep group compared to the wake group, $F(1,18)=5.24$, $p=0.03$, $\eta^2=0.23$, whereas the difference for the A-C associations was not significant, $F(1,18)=0.18$, $p=0.67$, $\eta^2=0.01$. Moreover, a separate comparison of the data from the wake group indicated a decreased recall of the A-B associations as compared to the A-C associations, $t=-2.95$, $p=0.02$. No such difference was observed in the sleep group, $t=0.87$, $p=0.40$.

Analysis of the data from the immediate and delayed recall groups of the main experiment revealed a main effect of list, $F(1,18)=20.31$, $\eta^2=0.53$, showing that, as expected, due to retroactive interference retention of the A-B associations was inferior to the retention of the A-C associations (Figure 2A). However, there was no difference in retrieval between the immediate and delayed recall groups, main effect for group, $F(1,18)=0.29$, $\eta^2=0.02$. The absence of a significant group x list interaction, $F(1,18)=0.30$, $\eta^2=0.02$, provided evidence that no spontaneous recovery of memory for the associations of the first list had occurred in the delayed recall condition. That memory for the A-B list recovered only after sleep was confirmed by an ANOVA comparing the difference scores in the retention of the A-C minus the A-B associations (Table 4A) across all four conditions (sleep, wake, immediate and delayed recall). This analysis revealed a significant main effect for group, $F(3,36)=4.00$, $\eta^2=0.25$. Post-hoc analyses for multiple comparisons (Turkey's HSD) and contrast analyses confirmed that the sleep group significantly differed from the other groups ($p<0.05$, for all comparisons), whereas the wake, immediate and delayed recall groups did not differ from each other ($p>0.95$, for all comparisons).

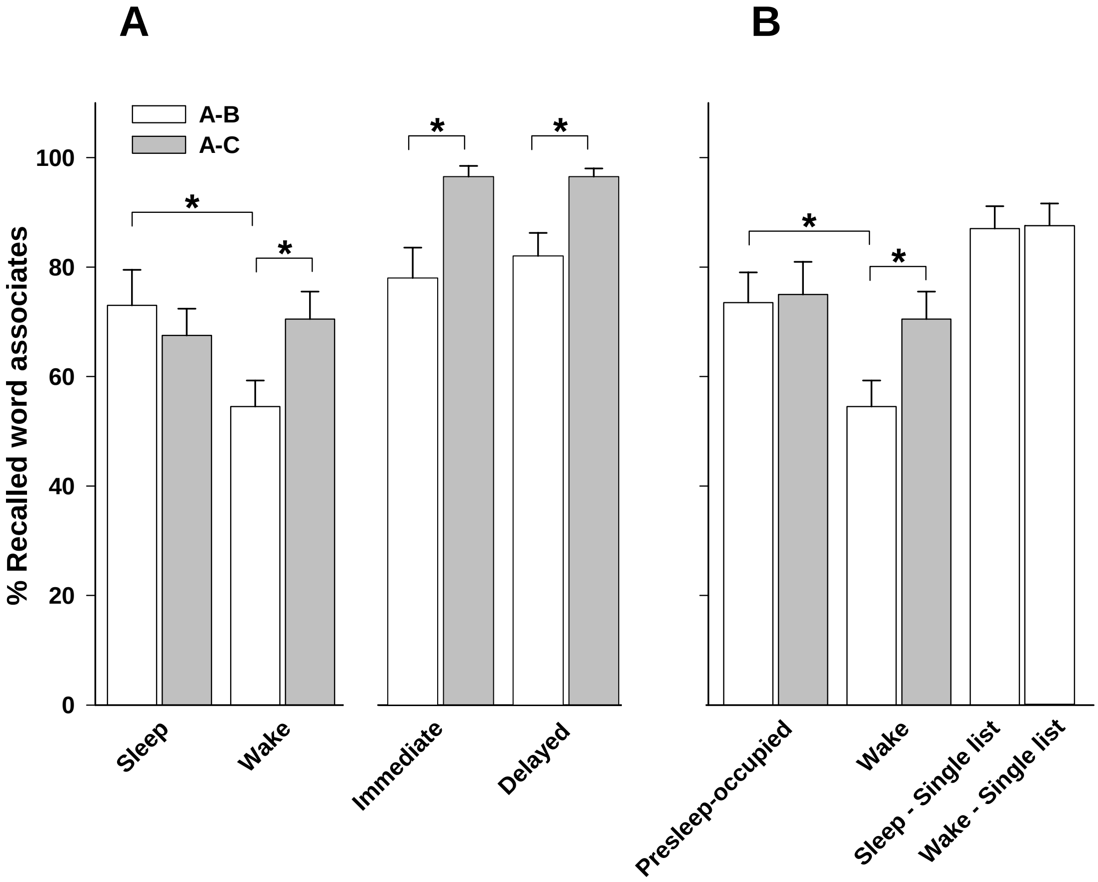

Figure 2. Recall performance in the main experiment (A) and supplementary control conditions (B). Means (± SEM) percentage of correctly recalled word associates from the first ("A-B") and the second ("A-C") list in an "A-B, A-C" paradigm. For the main experiment performance at retrieval testing is given for a Sleep condition (subjects had regular sleep on the first night after learning), a Wake condition (subjects remained awake on that first night), an Immediate recall condition (recall directly after learning), and a Delayed recall condition (recall 20 min after learning). The supplementary controls included a Presleep-occupied condition (subjects had regular sleep on the first night after learning but were occupied with a distractor task until sleep onset) and a Sleep and Wake condition where subjects had to learn only one list (Single List

Sleep, Single List Wake). Note, the Wake group of the main experiment is depicted twice to ease comparison with the Presleep-occupied condition. * $p<0.05$, for two-tailed pair-wise comparisons.

Results from the supplementary conditions introduced as additional controls are summarized in Table 4B. The two groups learning only one list, introduced to assess forgetting independent of retroactive interference, showed both high retention performance at retrieval testing which did not differ between sleep and wake conditions, $F(1,18)<0.01$, $\eta^2<0.001$, (Figure 2B). The data from the "presleep-occupied group" (which was occupied with distracting tasks throughout the interval between learning and onset of retention sleep) were first compared with those of the sleep group in the main experiment using a 2 x 2 repeated measures ANOVA. No effect in this analysis reached significance showing that the two groups were fully comparable, $F(1,18)=0.30$, $\eta^2=0.02$, $F(1,18)=0.29$, $\eta^2=0.02$, $F(1,18)=0.91$, $\eta^2=0.05$, for the main effects of group, list and the group x list interaction, respectively. Then, all analyses of the main experiment were repeated with the data from the presleep-occupied group replacing those of the original sleep group. Basically, these analyses replicated all effects of the original analysis. Importantly, it confirmed that the presleep-occupied group, like the sleep group in the main experiment, showed an improved recall selectively for the first A-B list, $F(1,18)=7.86$, $\eta^2=0.21$, for the "presleep-occupied/wake x list" interaction. The group main effect again failed to reach significance although a trend suggested that retention could be generally enhanced in the presleep-occupied group, $F(1,18)=2.98$, $p=0.10$, $\eta^2=0.14$. Post-hoc analyses confirmed the superior performance of the presleep-occupied group on the A-B list in comparison with the wake group. Moreover, the difference between the two groups for the A-B list was significant, $F(1,18)=6.73$, $p=0.02$, $\eta^2=0.27$, but not for the A-C list, $F(1,18)=0.33$, $p=0.57$, $\eta^2=0.02$. Finally, there was no significant difference between recall of the two lists in presleep-occupied group, $t=-0.40$, $p=0.70$, which supports the absence of interference in this condition.

The 2 (groups) x 2 (lists) ANOVA for the additional groups which were tested on delayed recall in the morning and in the evening as a further control of circadian effects, did not differ in their memory performance from each other (Table 4B, Figure 3). Thus, no hints for circadian variations were revealed, main effect for group, $F(1,18)=1.78$, *p*=0.20, η^2=0.09, and the group x list interaction, *F*(1,18)=0.49, η^2=0.03, were both not significant. Yet, the significant main effect for list confirmed the presence of substantial retroactive interference in both groups as the retention for the A-C list was superior to the A-B list, *F*(1,18)=22.49, η^2=0.56. Also, a combined analysis together with the data of the immediate and delayed-recall groups of the main experiment revealed only a significant main effect for list, *F*(1,36)=42.46, η^2=0.54, whereas the main effect for group, *F*(1,36)=0.75, η^2=0.06, and the two-way interaction, *F*(3,36)=0.28, η^2=0.02, were not significant. The number of trials the subjects needed to reach the criterion in the learning phase was very similar in the morning and evening, *F*(1,18)=0.31, η^2=0.01, *F*(1,18)=0.06, η^2=0.003, *F*(1,18)=0.31, η^2=0.01, main effects for list, group and interaction.

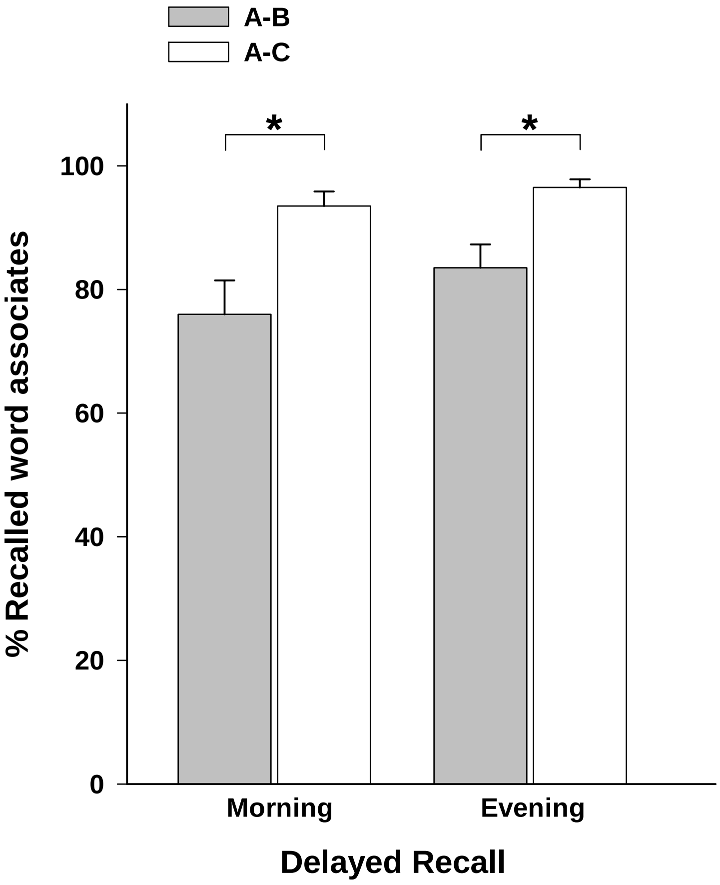

Figure 3. Performance for delayed recall testing. Means (± SEM) percentage of correctly recalled word associates form the first ("A-B") and the second ("A-C") list in an "A-B, A-C" paradigm. Performance at retrieval testing is given for two delayed recall conditions (recall 20 min after learning) one taking place in the morning and the other in the evening. * $p<0.05$, for two-tailed pair-wise comparisons.

Self-ratings after the end of the experiment did not provide any evidence for differences in feelings of activation, tiredness and concentration on the task at the time of retrieval testing between any of the sleep and wake groups or between the immediate and delayed recall groups, $F(1,46)<2.72$, $p<0.11$, $\eta^2<0.05$. When subjects judged how they had felt during the first night after learning, as expected the wake group reported to have been less activated, more tired and more demanded, $F(1,46)>14.33$, $\eta^2>0.24$.

Polysomnographical data scored visually off-line according to the criteria of Rechtschaffen and Kales (Rechtschaffen & Kales, 1968) showed that the subjects slept normally in the laboratory, with a mean amount of 427 (±13.35), 438 (±15.61) and 441 (±15.32) min of sleep in the sleep group in the presleep-occupied and the sleep single list group respectively. The percentages of sleep time in wakefulness, sleep stage 1 (S1), sleep stage 2 (S2), slow wave sleep (SWS) and rapid eye movement (REM) sleep were respectively: 0.57 ± 0.19, 9.57 ± 0.96, 47.68 ± 2.45, 22.53 ± 1.59, 17.72 ± 2.08 % for the sleep group, 0.68 ± 0.20, 7.85 ± 1.02, 47.87 ± 1.67, 24.56 ± 2.22, 18.16 ± 2.17 % for the presleep occupied group and 0.42 ± 0.18, 7.33 ± 0.85, 49.89 ± 2.78, 21.47 ± 1.68, 18.81 ± 2.57 % for the single list group. Mean salivary cortisol concentrations did not differ between the groups of the main experiment, neither at learning (collapsed across values before and after learning in µg/dl, sleep: 0.06 ± 0.01, wake: 0.13 ± 0.04, immediate recall: 0.22 ± 0.05, delayed recall: 0.23 ± 0.06) nor at retrieval testing (sleep: 0.61 ± 0.11, wake: 0.73 ± 0.11, immediate recall: 0.19 ± 0.03, delayed recall: 0.15 ± 0.03), $F(1,18)<1.38$, $p<0.26$, $\eta^2=0.07$. Also, sleep and wake groups did not differ in cortisol concentration in the morning after the night at the laboratory (sleep: 0.32 ± 0.07, wake: 0.43 ± 0.07), $F(1,18)=1.10$, $\eta^2=0.06$. In the sleep and wake condition, due to the circadian rhythm, cortisol concentrations were higher in the morning during recall testing than in the evening during the learning phase, $F(1,18)=47.85$, $\eta^2=0.73$. The same patterns were found for the subjects from the presleep-occupied, and the sleep and wake single list groups in the supplementary conditions. The difference in cortisol concentrations between the morning and evening delayed recall conditions was, as expected, significant, $F(1,18)=37.63$, $\eta^2=0.68$ (1.26 ± 0.19, 0.08 ± 0.01 mean values for the morning and evening groups respectively).

Discussion Experiment 2

Experiment 2 re-examined the issue of release from inhibition during sleep, using the same A-B, A-C paradigm as Ekstrand’s group. Our results show that memory performance for the first list of word-pair associates (A-B) was superior when learning was followed by nocturnal sleep than when learning was followed by waking. For the second list of word-pair associates (A-C) memory performance was not affected by sleep. The better memory performance on the first set of word-pair associates (A-B) in the sleep group confirms and extends the data reported by Ekstrand (1967), showing that sleep indeed counteracts retroactive interference as compared to wakefulness. Importantly, memory performance between the control groups of our study tested, respectively, immediately after learning and 20 minutes later, did not differ from each other, and all of these control conditions showed marked retroactive interference. This finding argues against the view proposed by Ekstrand et al, (1971) that recovery from retroactive interference, as observed by Ekstrand (1967), occurred spontaneously already during a 20-min waking period before sleep. In fact, since no substantial release from retroactive interference was observed in any of our wake control conditions, we conclude that this counteracting effect on retroactive interference is primarily sleep dependent.

This view is further supported by the results from several supplementary controls introduced in our experiment. Retention in the subjects learning only one list was distinctly better than retention of the first A-B list in the sleep and wake groups learning both lists. This pattern provides additional evidence for the presence of retroactive interference that is counteracted by sleep in the latter groups (Ekstrand, 1967). Recall performance on the single lists was indeed even higher than recall of the second A-C lists of the “A-B, A-C” task, and also did not differ between sleep and wake conditions which suggests that ceiling effects occurred that prevented differentially improving effects of sleep (see below). Results from the additional “presleep-occupied” group which was engaged in the performance of distractor tasks in the interval between learning and sleep indicated a pattern of retention entirely comparable with that of the sleep group of the main experiment, thus excluding that some unintentional rehearsal of the word-pairs in the periods before sleep onset confounded memory consolidation in this condition. Finally, there was no evidence that the absence of spontaneous recovery with delayed retrieval testing during wakefulness was in any way related to the time of testing during the day.

While in Ekstrand’s study (1967) sleep, by improving to a small but significant extent also memory for A-C associations, seemed to have a general facilitating effect over wakefulness, in our study the main effect of the sleep/wake factor did not reach significance since sleep failed to enhance retention of the A-C list. The difference could reflect circadian effects since in Ekstrand's study the wake retention interval was placed during daytime whereas in our study both sleep and wake retention intervals covered the night. However, several other explanations could account as well for the lack of a sleep-induced enhancement in retention of A-C association, which were examined in Experiment 3.

Experiment 3: Memory formation with reduced retroactive interference and weak encoding

Results of Experiment 2 indicate an enhancing effect of sleep for the first but not for the second list of an "A-B, A-C" paradigm. This could mean that the presumed consolidation process during sleep acts selectively to improve associations impaired by retroactive interference from subsequently encoded materials. This view appears to be attractive particularly in light of theoretical accounts assuming that forgetting is in principle due to interference rather than to a decay of memory traces (Jenkins & Dallenbach, 1924;McGeogh, 1933a;McGeogh, 1933b;Wixted, 2004). Thus, sleep's function would be to recover those memories that had been subjected to interference. A-C associations would not substantially benefit from sleep, in this view, because the degree of proactive interference diminishing these associations was too small. In fact, the rather short interval between A-B and A-C list learning used in our study is expected to induce only minor proactive interference (Underwood & Postman, 1960). Alternatively, however, the failure of sleep in Experiment 2 to enhance memory for the second list could reflect a ceiling effect in the sense that sleep simply does not enhance associations that are already strong at encoding. In fact, second list associations were distinctly stronger than first list associations even at recall testing after the wake retention interval. Such a ceiling effect could have prevented any further substantial improvement of second list associations by sleep, and could also explain why in the supplementary control conditions of Experiment 2 sleep did not improve retention when subjects had learned to criterion just one single list. A third possible though less likely interpretation of our data would assume that the order of lists determines the degree of memory benefit from sleep with the greater benefit for what is learned first.

To further explore these different interpretations regarding the absence of a sleep-associated improvement of the A-C associations, we conducted a second experiment in which the degree of retroactive interference was reduced between the lists by adopting an "A-B, C-D" paradigm. Previous studies showed that with this paradigm no retroactive interference is observed, and the two word-lists can thus be assumed to be independent from each other (Delprato & Garskof, 1969;Forrester, 1970). Additionally, to vary encoding strength a weaker encoding of the lists was induced by decreasing the criterion at initial learning from 90% to 60% correct answers.

Methods

Fifty subjects took part in the experiment (25 males, 25 females; mean age 23.4 years, range 19-31 years) conforming the criteria as described in Experiment 2. Design and procedure were the same as in Experiment 2 except that the delayed recall group was omitted. All together 5 groups of 10 subjects were tested (5 males, 5 females in each group). One sleep and one wake group were tested in both an intense and a weak encoding condition. In the fifth group immediate retrieval was tested (under intense encoding conditions) to assure that the A-B, C-D lists did indeed not induce retroactive interference.

The two lists of word-pair associates used for the task again contained 20 pairs each. The words were taken from the same database used in Experiment 2 (Blank, 2002). One list was directly obtained from Experiment 2 whereas the other one was new and contained 40 words from different semantic categories. Thus, all 80 words were unrelated. The setup of the memory task was also almost identical to that in Experiment 2, i.e. each word-pair was presented for 4 sec on a computer screen. When all word-pairs from a list were presented, the "A" words were presented again, alone this time, for 3 sec in which time the subjects had to name the corresponding "B" word. Then feedback was given by presenting the whole word-pair again. List presentation and the presentation of the "A" words alone were repeated until the learning criterion was reached, when responding to the "A" words. After 15 min the C-D associations were learned in an identical way. To vary the depth of encoding, at initial learning in the "intense encoding" condition the feedback presentation of the stimulus pairs was 4.5 sec and the presentation of the lists was repeated until the subjects made 2 mistakes or less, i.e., ≥90% correct responses (like in Experiment 2). In the "weak encoding" condition, the feedback was presented for only 1.5 sec, and list presentation was repeated until a criterion of 60 % correct responses was reached. On the retrieval test the subjects were now randomly presented with the "A" and "C" words for 10 sec, and had to write down on a paper the corresponding "B" or "D" word.

Results

As in Experiment 2 only the responses to the appropriate "A" or "C" words were included in the analysis and for all statistical analyses alpha was set to 0.05. The data of the immediate recall group were analyzed separately from those of the other groups with only the variable "list" (A-B vs. C-D). Retention data from the sleep and wake groups were analyzed in a global 2 x 2 x 2 analyses of variance (ANOVA) with the repeated measures factor "list" (A-B vs. C-D) and group factors representing, respectively, the "encoding intensity" (intense vs. weak) and the "sleep/wake" conditions. Subsequent analyses were performed separately for the conditions of intense and weak encoding. In order to compare retention between groups of intense and weak encoding (differing of course in their performance at initial learning) difference scores were calculated between the number of correct responses at retrieval testing and at the criterion trial during initial learning. Performance at learning was analyzed also separately for the conditions of intense and weak encoding.

At *learning*, as expected, the percentage of correctly recalled word-pairs at the criterion trial was well comparable between the sleep and wake groups (Table 5). Neither in the weak nor in the intense encoding condition did any effect reach significance, main effect for group, list and two-way interaction in the weak encoding condition, $F(1,18)=0.14$, $\eta^2=0.01$, $F(1,18)=2.06$, $p=0.17$, $\eta^2=0.10$, $F(1,18)=2.80$, $p=0.11$, $\eta^2=0.13$, respectively and in the intense encoding condition, $F(1,18)=0.40$, $\eta^2=0.02$, $F(1,18)=0.09$, $\eta^2=0.01$, and $F(1,18)=2.32$, $p=0.15$, $\eta^2=0.11$. Also the number of trials to reach the criterion was closely comparable between the sleep and wake groups (Table 5), as the main effects for group and the group x list interaction, $F(1,18)<0.15$, $\eta^2<0.01$, in the weak condition and $F(1,18)<0.05$, $\eta^2<0.003$, in the intense and did not reach significance. In the intense encoding condition subjects needed slightly less trials to reach the criterion for the second list of word-pairs, $F(1,18)=10.0$, $\eta^2=0.36$, main effect for list, and this held true also for the immediate recall group, $t=2.24$, (Table 5), but not in the weak encoding condition, $F(1,18)<0.01$, $\eta^2<0.001$, main effect for list.

The analysis of the *retention* scores (Table 5) confirmed that at retrieval testing the word-pairs were generally remembered better after intense encoding than after weak encoding, $F(1,36)=4.52$, $\eta^2=0.11$, for main effect of learning intensity. No other main effect reached significance, $F(1,36)=0.15$, $\eta^2=0.004$, for list, $F(1,36)=3.20$, $p=0.08$, $\eta^2=0.08$ for sleep/wake. However, the difference in retention between intensely and weakly encoded word-pairs was less clear for the sleep groups, which showed a distinctly improved retention for the weakly encoded word-pairs. This pattern was confirmed by significance for the "sleep/wake x learning intensity" interaction, $F(1,36)=4.17$, $\eta^2=0.11$, (Figure 4). No other interaction in this analysis was significant, $F(1,36)=0.27$, $\eta^2=0.01$, for list x learning intensity, $F(1,36)=0.27$, $\eta^2=0.01$, for list x sleep/wake. The improved retention of weakly encoded word-pairs in the sleep group was further confirmed in separate analyses of the intense and weak encoding conditions. For weakly encoded word-pairs retention was better in the sleep than in the wake group, $F(1,18)=7,33$, $\eta^2=0.29$, main effect for group. This improving effect of sleep on memory after weak encoding was equally revealed in both lists. Both the main effect of list and the group x list interaction did not reach significance, $F(1,18)<0.01$, $\eta^2<0.001$. For intensive encoding no effect reached significance, $F(1,18)=0.03$, $\eta^2=0.002$, for sleep/wake main effect, $F(1,18)<0.39$, $\eta^2<0.02$, for both the list main effect and group x list interaction. Comparison of retention scores in the immediate recall group revealed also no significant difference in memory between the two lists, $t=-0.89$, thus confirming the absence of substantial interference between the lists. Finally to further explore the hypothesis of a ceiling effect preventing a sleep associated improvement of memory for the A-C list in Experiment 2 we compared the data from the sleep group of Experiment 2 with those of the sleep group in the intense encoding condition. The 2 x 2 repeated measures analysis revealed a significant effect for group, $F(1,18)=7.38$, $\eta^2=0.29$, whereas the main effect for list as well as the 2-way interaction did not reach significance, $F(1,18)<0.74$, $\eta^2<0.04$.

Table 5

	Intense Encoding			Weak Encoding	
	Sleep	Wake	Immediate	Sleep	Wake
Learning					
A-B list (trials)	3.10 ± 0.32	3.00 ± 0.32	3.10 ± 0.32	2.1 ± 0.22	2.1 ± 0.22
C-D list (trails)	2.60 ± 0.25	2.50 ± 0.25	2.60 ± 0.25	2.2 ± 0.25	2.2 ± 0.25
A-B list (%)	95.00 ± 1.05	97.00 ± 0.82	94.00 ± 1.25	80.00 ± 3.42	82.00 ± 3.00
C-D list (%)	96.00 ± 1.00	95.50 ± 1.17	95.50 ± 1.17	86.50 ± 3.73	81.50 ± 2.36
Retention					
A-B list (%)	84.00 ± 3.48	88.50 ± 4.72	95.50 ± 1.17	68.50 ± 5.87	59.50 ± 5.35
C-D list (%)	85.00 ± 2.11	83.50 ± 3.73	97.00 ± 1.33	75.50 ± 4.31	59.00 ± 6.01
A-B (Retr – Learn)	-11.00 ± 3.64	-8.50 ± 4.54	1.50 ± 1.50	-11.50 ± 3.34	-22.50 ± 3.59
C-D (Retr – Learn)	-11.00 ± 2.08	-12.00 ± 3.51	1.50 ± 1.30	-11.00 ± 2.45	-22.50 ± 4.23

Learning (top) and Retention performance (bottom) for Sleep and Wake groups under conditions of Intense and Weak Encoding of A-B and C-D word-pair associates. For intense encoding Immediate recall was tested additionally to control for emergence of retroactive interference. For learning, the number of trials needed to reach the criterion when learning the A-B and C-D associations is indicated. Retention is first indicated (top two lines) as the percentage of correctly recalled words at retrieval testing separately for each list, and then (bottom two lines) by the difference between correctly recalled words at retrieval minus those correctly recalled at learning. The total of 20 word-pairs in a list is set to 100 %. Data are means ± SEM.

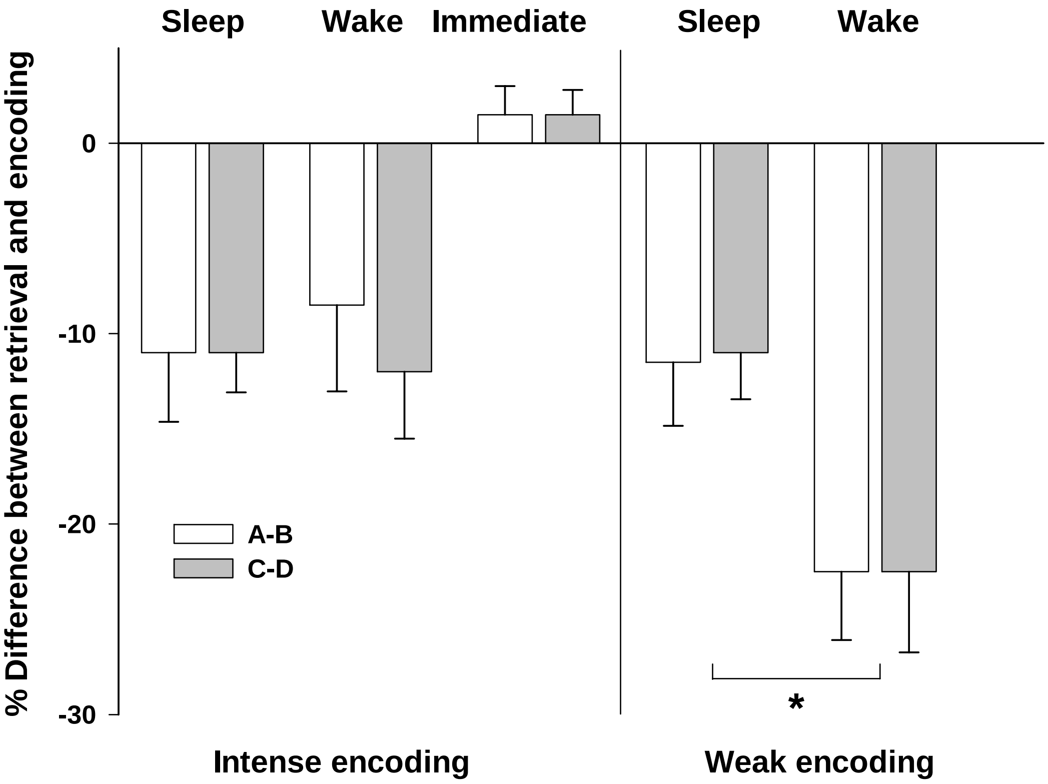

Figure 4. Retention performance indicated by the difference of correctly recalled words at retrieval minus those correctly recalled during learning. Differences refer to percentages with the total of 20 word-pairs in a list set to 100 %. Means (± SEM) retention scores for the first ("A-B") and the second ("C-D") list in an "A-B, C-D" paradigm are indicated for a Sleep condition (subjects had regular sleep on the first night after learning), a Wake condition (subjects remained awake on that first night), both with Intense and Weak encoding at initial learning. In addition, retention (of intensely encoded associations) is indicated for an Immediate recall condition (recall directly after learning). * $p<0.05$, for two-tailed pairwise comparisons.

Self-ratings at the end of this experiment did not provide any evidence for differences in feelings of activation, tiredness and concentration at the time of retrieval testing between the sleep and wake groups in this experiment, $F(1,38)<0.25$, $\eta^2<0.01$. When subjects judged how they had felt during the first night after learning, as expected the wake group reported to have been more tired and demanded by the experiment,

$F(1,38)>25.47$, $\eta^2>0.40$. Polysomnographical data confirmed normal sleep in the subjects of the sleep groups. Sleep time averaged 455 ± 14.83 min. The percentages of wakefulness, S1, S2, SWS and REM sleep were 0.53 ± 0.17, 7.38 ± 1.43, 49.52 ± 2.33, 22.09 ± 1.73, 19.06 ± 2.07 %, respectively. Mean salivary cortisol concentrations were analyzed in a 2 (weak/intense) x 2 (sleep/wake) x 2 (learning/retrieval) ANOVA. As expected only the main effect for the factor learning/retrieval reached significance due to the circadian variation, $F(1,36)=130.23$, $\eta^2=0.81$, all other comparisons were not significant, $F(1,36)<0.18$, $\eta^2<0.03$, (concentrations in µg/dl at learning, for weak encoding sleep: 0.14 ± 0.03, wake: 0.11 ± 0.02, at retrieval testing, sleep: 1.05 ± 0.21, wake: 1.20 ± 0.20, for intense encoding sleep: 0.08 ± 0.03, wake: 0.08 ± 0.03, at retrieval testing, sleep: 1.19 ± 0.18, wake: 1.37 ± 0.18). Also, the sleep and wake groups did not differ in cortisol concentration in the morning after the night at the laboratory, $F(1,36)<2.18$, $p<0.12$, $\eta^2<0.07$, (weak encoding sleep: 0.47 ± 0.07, wake: 0.28 ± 0.10, intense encoding sleep: 0.50 ± 0.07, wake: 0.53 ± 0.07).

Discussion Experiment 3

This second experiment explored why in Experiment 2 sleep only enhanced memory for the first list of word-pair associates in the “A-B, A-C” paradigm. An “A-B, C-D” rather than “A-B, A-C” paradigm was adopted here in order to reduce retroactive interference, which allowed to decide whether sleep-associated memory consolidation would selectively act on memories impaired due to retroactive inhibition or would improve memory also independent from interference. The conditions of intense and weak learning were introduced to test if the lacking sleep-associated improvement in recall of the second, A-C list associations reflected a ceiling effect, such that sleep in comparisons with wakefulness does not further enhance strength of associations that have been already intensely encoded. Results confirmed the efficacy of our experimental variations. Retrieval performance of the immediate recall group was closely comparable for both lists indicating that memory for the A-B and C-D lists indeed did not interfere. Moreover, as expected, retention was generally better after intense than weak encoding of the associations. The main result of the experiment was that in the absence of retroactive interference between the two lists, sleep enhances memory for both lists to an equal extent. However, the memory enhancing effect is observed only with a weaker encoding of the associations. This pattern clearly speaks for a ceiling effect that occurs with intense encoding and prevented in Experiment 2 a sleep-dependent improvement of retention for the second list (i.e., A-C) associations. It is to note, however, that this ceiling effect of sleep associated memory consolidation refers to the specific conditions present in Experiment 2, since in Experiment 3 retention of both lists was better than in Experiment 2 indicating that in principle superior performance would have been possible also in the sleep condition of this first experiment. It is likely that interference generated in Experiment 2 was associated with some degree of general response competition that adversely affected also A-C list performance (Postman et al. 1969).

Because the lists in this experiment did not induce interference, the enhanced recall of both lists after retention sleep cannot be explained in terms of an alleviating influence of sleep on retroactive interference. Moreover, the benefit from sleep being comparable for the first and second list excludes that the order of encoding plays a relevant role for the extent of sleep-associated consolidation. The most parsimonious interpretation of the data, in fact, appears to be that sleep enhances the associative strength of declarative memories, with this effect being stronger for weaker associations regardless of whether the weakness of the associations is due to a weaker encoding or interference. The notion that weakly learned associations benefit more from a period of retention sleep than strong and over-learned materials has been discussed previously (e.g., Empson & Clarke, 1970;Ekstrand, 1977;Tilley & Empson, 1978;Cipolli, 1995), although to our knowledge a direct test of this notion has not been made for declarative memory. Additionally, our findings tie in with observations that sleep enhances memory for more complex materials involving multiple but relatively weak associations to a greater extent than retention of simpler tasks, although this view has been elaborated mainly with procedural tasks (Scrima, 1982;Smith, 2001;Kuriyama *et al*, 2004).

General Discussion Experiments 2&3

Forgetting can be caused by interference, and persistent memories may develop on the basis of mechanisms alleviating memory traces from the retroactively interfering influences of more recently encoded materials. Here, we reassessed the role of retroactive interference in sleep-associated memory formation in a declarative word-pair associates task. Building on two previous studies by Ekstrand and colleagues (Ekstrand, 1967;Ekstrand *et al*, 1971) we sought to clarify whether the enhancing influence of sleep on these memories results from a facilitating influence on the recovery of the memories from retroactive interference.

Our first experiment confirmed this view showing that sleep in fact nullifies retroactive interference accumulating during the learning of conflicting information as is the case with the “A-B, A-C” paradigm. This finding is in line with the results of Ekstrand’s first study (1967) which likewise indicated a stronger enhancement of retention by sleep for the A-B than A-C associations. Introducing several controls, our study distinctly expands support for this function of sleep. A contamination of the recovery from retroactive interference observed after retention sleep by circadian oscillators can be excluded since the critical processes of encoding, retention, and retrieval took place in both the sleep and wake conditions at identical phases of the circadian rhythm. The two groups were comparable with respect to the trials needed to reach the criterion for each list of word-pair associates at encoding. Also, concentrations (in saliva) of the stress hormone cortisol known to impair memory retrieval (de Quervain *et al*, 2000) did not differ between the sleep and wake conditions during encoding or retrieval. Retrieval was tested two days after learning allowing the subjects of the wake group to have a night of recovery sleep. This procedure safely excludes the possibility that fatigue from the first night of sleep deprivation could account for the observed sleep effect. Self-ratings performed at the time of retrieval testing confirmed that vigilance and feelings of tiredness were comparable in both conditions. On the other hand, postponing the retrieval test until after the second night following learning induced a generally greater loss of memory reflecting the unspecific decay of traces over time. Also, assuming that memory consolidation persists for several nocturnal sleep periods after the learning experience, sleep on the second night could add to the consolidation process. In this case, the recovery sleep which we allowed in the wake condition might have acted to diminish the critical difference in retrieval performance between the sleep and wake conditions of our experiments.

Despite positive evidence from his first study, Ekstrand et al. (1971) questioned that sleep was in fact the cause for the recovery from retroactive interference observed at retrieval after retention sleep. Based on a non-significant effect (*p*=0.2) towards spontaneous recovery after a 20-min retention period of wakefulness, the authors argued that "...the recovery found by Ekstrand (1967) probably had occurred before his Ss actually fell asleep" (Ekstrand et al. 1971, p. 142). Here, we found no evidence that a 20-min retention period of wakefulness induces any substantial recovery from retroactive interference which can be taken as a clue further confirming the null hypothesis in this context. Nevertheless some differences in the design between our Experiment 2 and Ekstrand et al's experiments should be discussed to clarify what made perhaps the authors of those experiments arrive at so different conclusions. First, in our study the interval between learning the two lists of word-pairs was 15 min during which the subjects performed a distractor task, whereas in the experiments by Ekstrand et al. (1971) the interval consisted of just the time needed to read the task instructions (about 2 min). While one might suppose that the longer interval in our study decreased retroactive interference, previous experiments Blank (2002) using the same 15-min time interval between the learning of the two lists demonstrated most robust interference effects. In fact, our data from all three "delayed" control groups of Experiment 2 confirmed substantial retroactive interference effects of a size similar to that observed by Blank (2002) and also similar to that reported by Ekstrand et al. (1971) with a shorter interval, thus excluding the interval length between learning of the A-B and A-C associations as a possible modulator of the outcome in these studies. These conditions additionally provided evidence that emergence of spontaneous recovery is not essentially controlled by circadian rhythms, because the relatively impaired recall of A-B associations 20 min after interpolated learning did not differ between subjects tested in the morning or evening hours. Postman et al. (1969) suggested that mainly the degree of initial learning predicts whether spontaneous recovery will occur, stating that the better the learning of the A-B associations the more likely they are to recover after interference, but that the rate at which this takes place is slowed down as a function of the amount of interference induced. In our study we aimed to induce maximum interference, which on this background might explain why we failed to observe spontaneous recovery after short intervals. This is also in agreement with other studies that have successfully demonstrated spontaneous recovery after short retention intervals (for review see Brown, 1976;Wheeler, 1995). Yet, our study extends this notion by adding that time alone is not sufficient, instead sleep might be even more important as the subjects remaining awake after learning did not exhibit any signs of recovery.

Another factor that might have influenced memory consolidation and the occurrence of spontaneous recovery during the 20-min delay is the subject's general activation during the delay. In our study, subjects in this interval were busy with the distractor task, whereas in Ekstrand et al.'s (1971) experiments the subjects rested in a lying position in a darkened room with only the instruction not to fall asleep (which however was not actually monitored by polysomnography). Gottselig et al. (2004) indicated that in comparison with busy waking, a period of restful waking provides also benefits to memory formation on an auditory discrimination task, although these benefits were on average less pronounced than the effects of sleep. Thus, it appears that even during quiet wakefulness some conditions are established that, although to a lesser extent than during sleep, promote processes of memory consolidation. Such conditions amongst others include at the psychological level a reduced non-specific retroactive interference from sensory stimuli and at the neurophysiological level a generally reduced cholinergic brain activity (Rasch *et al*, 2006;Wixted, 2004;Gais & Born, 2004b;Foster & Wilson, 2006).

Experiment 2 failed to replicate Ekstrand's (1967) observation that second list associations showed a less but still significant benefit from retention sleep. Results of Experiment 3 indicate that this failure most likely reflects a sleep specific ceiling effect. Comparing the effects of sleep and waking on the retention of non-interfering (A-B, C-D) lists after intense and weak encoding, Experiment 3 revealed an improvement of memory after sleep which was equal in size for both lists. However, the sleep-dependent benefit was observed only after weak encoding of the lists, and did not reach significance when the subjects had learned the associations almost perfectly (i.e., to a criterion of ≥90 % correct responses). Obviously, more weakly encoded associations in the declarative memory system show a greater consolidation benefit from sleep than strongly encoded associations suggesting that sleep-dependent consolidation can reach an asymptotic level in parallel with the prior learning curve. This may also explain why some previous studies failed to reveal a sleep-induced improvement in retention after certain learning tasks (Smith, 2001;Paller & Voss, 2004;Rauchs *et al*, 2005). Using non-interfering word lists Experiment 3 at the same time excluded that recovery from interference is the only way in which sleep enhances declarative memory retention. In fact, retroactive interference could be considered just another way to weaken associations encoded shortly before, thereby boosting the memory enhancing effect of sleep. Yet, the superior memory performance in the intense encoding condition in Experiment 3, as compared to the sleep group in Experiment 2, suggests that in this latter group some specific factor negatively influenced retention. Most likely this is caused by the simultaneous activation of competing associations (Postman *et al*, 1969). According to this notion retention of both the original (A-B) and the interpolated (A-C) lists is inhibited, in particularly *after* retroactive interference that favors the most recently learned list, has started to dissipate, a condition that is clearly met only in this group of Experiment 2. Additionally, this interpretation also explains the substantially better retention in both the single list groups as well as the immediate and delayed retrieval groups.

Our data support the view that sleep enhances an active consolidation process that leads to a differential weighting of associative connections, since the strengthening effect of sleep was greater for the associations that were weaker at encoding whether due to retroactive interference or weaker learning. This outcome cannot be explained by a merely permissive function of sleep, which predicts an equal benefit for both competing memory traces during sleep. Thus, the present data concur with neurophysiological studies of brain activity underlying sleep dependent memory formation pointing to a reprocessing of newly acquired memory representations during sleep, which in the case of hippocampus dependent declarative memories appears to take place preferentially during slow wave sleep (Wilson & McNaughton, 1994;Plihal & Born, 1997;Plihal & Born, 1999b;Peigneux *et al*, 2004;Drosopoulos *et al*, 2005). It has been argued that these neuronal signs of reprocessing during slow wave sleep merely reflect residual activity in neuron assemblies used previously for encoding, whereas the enhancing effect of sleep on memory results from a general down scaling of synaptic efficiency (Tononi & Cirelli, 2006). However, that view predicts that downscaling erases weak synaptic connections below a certain threshold whereas strong connections survive, which contradicts the present findings of a greater sleep-dependent gain for the weaker rather than stronger associations. A growing number of neuro-imaging studies indicating that post-learning sleep induces specific topographical changes in the brain's representation of a memory at delayed retrieval, likewise speak for an active consolidation process that reorganizes certain aspects of the representation (Fischer *et al*, 2005;Walker *et al*, 2005;Takashima *et al*, 2006). Notably, these studies have provided first evidence that sleep-dependent changes in the neuronal organization of hippocampus-dependent memories can occur also in the absence of overt behavioral changes (Orban *et al*, 2006) which leaves the possibility that in our experiments post-learning sleep compared with wakefulness may have induced some representational changes even in those conditions (e.g., single list learning in Experiment 2 and intense encoding in Experiment 3) where a sleep associated improvement in recall was not observed. Neurophysiological investigations have also identified some prerequisites enabling hippocampal reprocessing of neuronal representations and hippocampo-neocortical information transfer presumed to underlie consolidation of declarative memories during slow wave sleep, such as a low activity of the neurotransmitter acetylcholine and a synchronizing impact of slow oscillations on hippocampal activity during this time (Rasch *et al*, 2006;e.g., Buzsaki, 1998;Hasselmo, 1999;Sirota *et al*, 2003;Marshall *et al*, 2004;Gais & Born, 2004b;Moelle *et al*, 2006). On the background of these converging hints from studies of brain activity, it is tempting to speculate that a hippocampo-neocortical transfer of newly encoded information provides a frame also for the present finding that sleep preferentially consolidates weaker associations in the declarative memory system, once they have been explicitly learned. At learning the strength of newly encoded associations, as varied here by experimental manipulations of retroactive interference and encoding intensity, is likely linked to a joint activation of prefrontal and hippocampal neuron networks (e.g., Otten *et al*, 2001;Yancey & Phelps, 2001;Moelle *et al*, 2006). In contrast, reprocessing of hippocampal memories during slow wave sleep takes place while prefrontal activity is distinctly diminished and thus, being no longer prefrontally controlled, it might unselectively cover strong and weak associations (Buzsaki, 1996;Maquet, 2000). In this case output signals from hippocampal reprocessing of memories during sleep would not systematically differ in amplitude between weak and strong associations leading to a relatively enhanced signal transfer for the weaker associations. This leveling of output signals could also benefit the discovery of shared structure in representations in the hippocampo-neocortical interaction (McClelland *et al*, 1995).

Finally, it should be noted that a sleep-associated consolidation relying on the active reprocessing of memory representations, while excluding a merely permissive role of sleep for memory formation, is not incompatible with the notion that sleep enhances consolidation by protecting from interference, if it is assumed that the respective brain structures cannot engage simultaneously in encoding and reprocessing of representations. This assumption is indeed supported by computational as well as experimental work indicating that processes active during sleep switch the brain from a mode of encoding to a mode of memory consolidation (Rasch *et al*, 2006;e.g., Hasselmo, 1999).

Beyond the possible role of sleep for recovery from retroactive interference, the present data may add to the conceptualization of the interference phenomenon itself. The response set suppression hypothesis proposed by Postman (1969) states that unlearning of the first list of targets, rather than resulting from interference between individual cue-target pairs, reflects the diminished availability of the whole set of first list targets due to the learning of a new set of targets. However, this view relies critically on findings indicating similar amounts of first list recovery in an ”A-B, A-C“ paradigm and an ”A-B, C-D“ paradigm. Hence it seems difficult to apply this view on the present data where sleep preferentially recovered first list targets only under ”A-B, A-C“ conditions, whereas the sleep dependent gain in memory was closely comparable for both lists in the A-B,C-D condition. Alternatively, Anderson (2003) proposed that interference rather than representing a learning phenomenon occurs primarily at retrieval as a result of an inability to suppress a dominant memory that interferes with a weaker one during recall. This view well fits the observation that memory performance in the sleep group of Experiment 2 was generally lower than that of the sleep group in the intense encoding condition in Experiment 3, which is likely due to interference that memory suffered in the sleep group of Experiment 2 in a post-encoding stage. Still this conceptualization does not appear adequate here since in Experiment 2 maximum interference was generated in the learning phase, while at retrieval testing the subjects were stimulated to recall both, i.e., B and C targets, to avoid any overt response competition. Moreover, the main experimental manipulation targeted sleep in the first night after learning, implicating that changes in response competition at retrieval are secondary to effects of post-learning sleep on consolidation processes. In conclusion, the present data of Experiment 2 appear to be most consistent with the classical two-factor theory of interference (Melton & Irwin, 1940), assuming that unlearning represents the weakening of cue-target association due to inhibitory control in a similar way as extinction in classical conditioning. While this theory makes no prediction regarding the occurrence of spontaneous recovery, the present data may partly fill this gap inasmuch they indicate that spontaneous recovery is facilitated by sleep and a putative reprocessing of memories during sleep that to a certain extent occurs also during wakefulness (Peigneux *et al*, 2006;Foster & Wilson, 2006).

Our findings stand in contrast with results of a study investigating the role of interference for the sleep-dependent consolidation of procedural motor memories (Walker et al, 2003). Training subjects on two similar finger tapping sequences, those authors observed no signs of predominant retroactive interference at an immediate retrieval testing (5 min after training), but relatively worse performance for the first sequence compared to the second sequence after a night of retention sleep. This pattern suggesting that the sleep-dependent consolidation induces rather than alleviates retroactive interference is opposite to the pattern observed here for declarative memory. At a first glance, it may appear that these divergent outcomes are compatible if it is assumed that in our experiments the improving effect of sleep on retention of first list (A-B) associations does not reflect recovery from retroactive interference, but builds mainly upon an incomplete consolidation of the A-B associations, because subjects after learning of the A-B list started to play Snood which may sap some resources needed for consolidation. However, this view is not supported in particular by results of the additional “presleep-occupied” control group introduced in Experiment 2. This group displayed likewise a selective sleep-dependent improvement of the A-B word associations, although in this group (playing Snood after learning of A-B *and* A-C associations) the extent of consolidation is assumed to be equal for both lists. Accordingly, the divergent effects of sleep on interference observed here for a declarative task and by Walker et al. (2003) for a procedural task remain difficult to integrate and, presently, can at best be ascribed to the basically different neuroantamical structures and mechanisms underlying these two memory systems which, in addition, may competitively interact during memory formation (Poldrack & Packard, 2003). However, it should be noted that there are also findings indicating that sleep provides recovery from retroactive interference in the procedural memory system as well. Fenn, Nusbaum, & Margoliash (2003) trained subjects to discriminate computer generated speech sounds. Although immediate performance increased as a result of training, performance declined over the course of the subsequent waking hours presumably due to non-specific interference. Nevertheless, perceptual performance was restored again after the following night of sleep. A control group showed that this effect was solely dependent on the presence of sleep, and independent of the time interval between initial training and sleep. Employing a motor sequence tapping task, Kuriyama et al. (2004) showed that the benefit from sleep was greatest for the most difficult sequence trained before retention sleep. Complexity was manipulated by having the subjects learn 5- and 9-element sequences by using fingers either from one or both hands. Both speed and accuracy improved after sleep but the largest benefit was observed in the most difficult condition. The authors further analyzed the reaction times for every transition to identify easy and more difficult transitions. Not unexpectedly the difficult transitions benefited remarkably more from sleep than the easy ones. Complexity has been likewise discussed as a possible factor influencing sleep-associated consolidation in declarative tasks (for a review, Smith, 2001). Assuming that more difficult responses in such tasks are those where the response is selected with greatest interference from competing response tendencies, this view seems to be well in line with our notion that, for explicitly learned materials, the strengthening influence of sleep is stronger for weak than strong associations, independent of whether low associative strength results from interference or weaker encoding.

Study 3: Sleep "renders" memory in a forward direction.

Submitted as:
Drosopoulos, S., Vahlenkamp, E., Wagner, U., & Born, J. Sleep "renders" memory in a forward direction.

Introduction

"Time" is such an essential component in the organization of memory that without being able to retrieve the sequential order that events are to be performed in, behavior would be severely impaired to an extent where even tying ones shoelaces would probably seem like an impossible task. Although the exact mechanism of how events are stored in time is not yet fully understood, in cognitive neuroscience, serial memory has been captured according to two distinct types of models. One type relies on associative processes between items (for review see Kahana, 2002). Here, an association is formed between an item of a serial list and its preceding as well as its succeeding item. These models typically work best when applied to pairs of items but their usage has also been tested on longer serial lists, although often less successful (Kahana & Caplan, 2002). In order to better predict empirical data a second type of models have been proposed that rely on positional coding. Such models typically state that during encoding of a serial list every item is encoded together with the time context of its occurrence. During retrieval not only the item but also its original encoding time context needs to be retrieved (Brown *et al*, 2000). The demonstration of (i) certain robust properties of memory for sequences and (ii) dissociations between memory for pairs and longer sequences, have shed light on how temporal memory is organized. Memory for sequences has traditionally been tested using lists of words, letters or digits were subjects are either requested to name as many items as possible belonging to a certain list (free recall) or are requested to recall a series of items in their original presentation order or in backward order (serial recall). In both cases accuracy measurements or reaction times can be obtained.

A crucial difference between memory for pairs (two items, A and B) and longer lists (A, B, C or more) is that memory for pairs exhibits symmetry in retrieval, meaning that the probability to retrieve B given A equals the probability to retrieve A given B (Asch & Ebenholtz, 1962;Kahana, 2002). In longer lists memory usually favors the forward direction of retrieval over the backward direction (Li & Lewandowsky, 1995;Kahana, 1996;Kahana & Caplan, 2002;Thomas *et al*, 2003). Kahana (1996) was the first to experimentally show that during free recall of a list of words, after a certain item was retrieved the item retrieved next was much more likely to be from a nearby serial position and that it was more likely to be one succeeding the retrieved word than to be one preceding it in the serial order. Memory is better for both directions when probed with two successive words than when probed with only one (Kahana & Caplan, 2002). Another consistent finding distinguishing forward from backward retrieval is that in forward retrieval accuracy but also reaction times peak at the first item and both linearly decrease over the next items and finally reach their lowest point after approximately 5 items (Li & Lewandowsky, 1995;Kahana, 1996;Kahana & Caplan, 2002;Thomas *et al*, 2003). In backward recall on the other hand the highest accuracy rates are obtained for the first and the last items of a sequence revealing a U shaped function whereas reaction times to those items typically show an inversed U function with the slowest reaction times in the middle of the sequence (Thomas *et al*, 2003). Thus, in forward recall a primacy effect is exhibited whereas for backward recall both primacy and recency effects can be observed. Associational models typically view this findings as an epiphenomenon, explaining this asymmetry in terms of rehearsal differences of the order of presentation that alter the availability of each association making the retrieval of the forward association easier (Asch & Ebenholtz, 1962;Waugh, 1970). Positional order models yet assume that forward and backward recall are driven by different retrieval strategies. Several explanations have been given to explain these findings. One common assumption in most models of serial memory is that retrieval can be separated in two stages, i.e. the preparation stage and the search stage (Anderson J.R. *et al*, 1998). During the preparation stage (which mostly affects the first item to be retrieved from a serial list) the whole list is processed and prepared for retrieval. This explains the longer reaction times for the first position in forward retrieval. Due to the recency effect though, in backward retrieval memory performance is much less affected by this stage (Murdock, 1995). At the subsequent positions the search stage prevails and depending on how a model handles transitions between items different predictions can be made concerning the course of retrieval. As retrieval progresses memory tends to worsen due to decay or interference from every additional item. On the other hand, a reduction of cognitive load also occurs, that counteracts the worsening of memory since every retrieved item leaves one less as a possible alternative for the next position. In backward retrieval things are a little more complicated; two additional processes have been proposed to explain the generally longer reaction times and lower accuracy rates. On one hand it has been suggested that backward retrieval requires a reversal of the original sequenced that is then searched in a forward direction until the desired item is reached (Thomas *et al*, 2003). Another view poses that backward retrieval engages of additional visuo-spatial information (Li & Lewandowsky, 1995).

Likewise, the consolidation of newly encoded memories has long been thought to be regulated during subsequent nocturnal sleep (Maquet, 2001;Walker & Stickgold, 2004;Gais & Born, 2004a). Off-line replay of those newly acquired memory traces has been proposed as a key mechanism underlying the consolidation process. In particular in declarative and spatial memory which both rely on the hippocampus, a reactivation of the same hippocampal neuronal circuits as during encoding, were observed during slow wave sleep after the learning period (Wilson & McNaughton, 1994;Louie & Wilson, 2001;Lee & Wilson, 2002;Ribeiro *et al*, 2004;Peigneux *et al*, 2004). Also, imaging studies have shown that at a delayed retrieval testing, memory representations have significantly changed in brain topography when subjects slept after learning (Orban *et al*, 2006;Maquet *et al*, 2003;Fischer *et al*, 2005;Walker *et al*, 2005;Takashima *et al*, 2006). Together these data are support the notion of a two-stage memory system where memories are initially hold in hippocampal networks and are transformed into long-term memories by a spreading and gradual transfer of the representations to other presumably neocortical networks (McClelland *et al*, 1995;Sutherland & McNaughton, 2000;Dudai, 2004;Gais & Born, 2004a). In this experiment we assed whether the consolidation of newly encoded sequential information during sleep might promote the induction of a forward temporal direction in memory, thus revealing a property of consolidation which beyond the quantitative strengthening of memory might also qualitatively alter their representations by selectively strengthen associations that promote an unidirectional retrieval of a sequence of events. To test this prediction we adapted a probed recall task for triple word lists used by Kahana and Caplan (2002). The choice to use triples was based on previous findings showing that memory for triples resembles memory for longer lists (Kahana & Caplan, 2002). We hypothesize that unless forward and backward retrieval rely on the same mechanism, retention will be better in the forward direction (since replay occurs in a forward direction as well). Also, since the retrieval of the second and on positions in a serial list depend foremost on the search stage we expect a differential effect of sleep on the position of each item ion the serial order. Subjects were presented with triples of unrelated words (A, B, C) they had to memorize as belonging together. After three successive presentations, base line learning was determined by assessing memory for half of the triples. This was done by asking the question "which word came next?" and then presenting the first word of each triple. The subject was requested to type the respective word (i.e. the B-word, further referred to as 1st position). Feedback was given regardless of the answer by presenting the correct word. The question was presented again after which the subject had to type in the correct word (i.e. C-word, from now on referred to as 2nd position). Finally, feedback was given by showing the correct word. Memory for all the triples was tested again after two nights, the first of which the subjects in the wake group remained awake. The same procedure was used for this memory test as for the base line determination but this time, in half of the triples (balanced over those which were used for base line determination and those that were tested for the first time) memory was probed in a backward direction by asking the question "which word came before?" and presenting the last word of each triple. The subjects were then requested to type in the respective word (i.e. B-word, from now on referred to as 1st position). The question appeared again and then the second word of the triple to which the subjects had to respond again by typing in the requested word (i.e. A-word, referred to further on as 2nd position). Additionally, this time no feedback was given. For specific procedures and task material see the methods section.

Materials and Methods

Subjects

Twenty-eight healthy, non-smoking, drug free, native German speaking subjects with no prior history of sleep disturbances and regular sleep-wake cycle, participated in the experiments and received a money reward for their participation (14 males, 14 female, mean age 24 years, range 19-34 years). The data from two females were excluded from the analysis, those were: one from the sleep who had not reached the cut off score of 50% at learning and one from the wake group who did not manage to comply with the instructions to remain awake during the day. The experiments were approved by the local ethics committee. All participants gave written informed consent before participation. All participants were tested individually. Prior to the actual testing sessions, subjects in the sleep condition had an adaptation night in the laboratory, which included the placement of electrodes. The participants were instructed to get up at 7:00 h and not to take any naps on the days of the experiment. They also had to abstain from taking any caffeine containing drinks after 15:00 h in the overnight conditions, and during the whole day on all other conditions. Alcohol was not permitted on the experimental days in any condition and throughout the whole experimental period.

Design and Procedure

The subjects of the sleep group reported to the laboratory around 21:30 h. Following the placement of electrodes and preparations for bedtime, the learning phase started at 22:00 h. First the subjects were subjected to the word fluency task for two minutes (Aschenbrenner *et al*, 2000). They were provided with a sheet of paper where they had to write down as many words as they could, starting either with the letter M or P, the order of which was balanced across subjects. Thereafter, the subjects were seated in a room in front of a computer screen where the 32 word-triples were presented to them. They were told to memorize the three words of each triple as belonging together. After three successive presentations memory was probed for half of the triples in order to estimate base line learning. The learning and base line testing phase took approximately 40 min. The subjects were then instructed not to rehearse the words anymore. The participants completed a 5-point scale, self-rating questionnaire concerning feelings of activation, drowsiness, tiredness, motivation, attention, concentration and other psychological and physiological characteristics that might be differently affected by the retention period. They were offered to go to the toilette, after which they immediately went to bed. Lights were turned off within 10 min after learning was completed. At 07:00 h the next morning subjects were awakened, and electrodes were removed. Before leaving the laboratory the subjects were told that they should follow their regular activities during this day, to go to bed around 23:00 h, and not to rehearse the word lists. Subjects reported back to the laboratory the next morning at 08:00 h for retrieval testing. Caffeine containing drinks were again not allowed on this morning. Before retrieval testing the word fluency task was administered again for two minutes. After having performed the retrieval test, the participants completed again the 5-scale, self-rating questionnaire as they had after the learning session. Also they had to rate on a 5 point scale the quality of their sleep the night before. At the end of the session, by a standardized interview it was assured that the subjects had fully complied with the instructions, and the subjects also had to report the activities they had engaged during the day before. Since glucocorticoids are known to influence memory function (de Quervain *et al*, 2000;Maheu *et al*, 2004;Wagner *et al*, 2005) saliva cortisol was sampled, as an estimate of adrenocortical secretory activity, both before and after (i) learning of the words, (ii) the retrieval test, as well as (iii) before leaving the laboratory after the first night.

The procedure for the subjects in the wake condition was the same except that after learning was completed the subjects remained awake throughout the night and the following day. They stayed in the laboratory until 07:00 h and afterwards went home. They were instructed not to go to bed before 23:00 h on this day. Throughout the experiment they wore an “activity watch” (Actiwatch®), which was used to assure that subjects had not brief minute periods of sleep during the experimental periods of wakefulness. Standard activities the subjects undertook during the night included watching movies, playing computer games, and going out for a walk in the experimenter’s company. Reading was not allowed.

Memory Task and Materials

The words were taken from lists used in previous experiments in our laboratory. All words were unrelated and the triples were randomly generated. All words contained 6-8 letters and had no more than three syllables. One half of the triples were used for base line determination after learning and the other half were used in the retrieval test only, this was done in a balanced direction. Each of these two halves was again spited in two and in 8 of the triples memory was probed in a forward direction whereas in the other 8 in a backward. This resulted in 4 versions of the retrieval task. Each word appeared in the middle of the screen for 4 sec. Between every string of triples a fixation cross was presented for 4 sec. The blocks were presented 3 times in total. Immediately after presentation is completed, base line learning was assessed. First, for 1.5 sec the question appeared "which word came next?" The first word of a triple was presented and remained on the screen for 15 sec in which time the subjects could type their answer which also appeared in lower left corner of the screen. If they were done before 15sec had passed, they could terminate the trial by pressing "Enter". Feedback was provided irrespective of the answer by presenting the correct answer for 1.5 sec. The question appeared again for 1.5 sec and then the second word of that triple was presented in the same procedure as for the first word. Again afterwards feedback was provided. A fixation cross appeared on the screen for 4 sec. indicating the transition to a new triple. The retention test was built up the same way as the base line determination task only here all triples were tested. During backward probing the question asked was "which word came before?". In this task there was no feedback provided.

Data analysis

At base line determination a cut-off score of at least 50% correct answers was maintained in order to assure that subjects had indeed followed the instructions and learned sufficiently. Retention data were calculated only for those triples that were not used for base line determination because in the triples used for base line determination the forward probing of memory might have influenced further consolidation. Also those triples can be considered over learned which also affects subsequent consolidation (Drosopoulos *et al*, 2006). Retention percentages were obtained by calculating the words for each position that the subjects still could retrieve compared to their score at base line learning. The calculation of the backward retention data was done based on the original positions during base line determination. This means that retention for the 1st position was calculated based on the score for the 1st position at base line learning and idem ditto for the 2nd position. Although this might seem contra intuitive, this approach was chosen because the effect of position was stronger than that of direction.

Results

Analysis of retrieval.

Only the triples that were not already tested after learning for base line determination were included in the analysis. The main finding of this study is that subjects in the sleep group were better in the retention of the forward associations than the subjects in the wake group and that this effect was distinctly evident for the 1st position (B-words). Data from the retrieval testing were analyzed in a 2x2x2 repeated measurements analysis with the between subjects factor sleep/wake and the within subjects factors forward/backward retrieval and 1st/2nd position in the memory test. For means with ± SEM. of retrieval percentages refer to Table 6. The analysis revealed that in general memory retention was better for the words retrieved in forward than in backward direction [$F(1,24)=12.26$, $p=0.002$] and also for the words in the 2nd as compared to the 1st position [$F(1,24)=5.03$, $p=0.034$]. Yet, the main finding showed that sleep enhanced retention over wakefulness specifically in forward retrieval for the 1st position [significant three-way interaction $F(1,24)=11.78$, $p=0.002$], see Figure 5. This effect was further confirmed in subsequent post-hoc analysis of pair-wise comparisons, showing that the sleep and the wake group only differed in the percentage retrieved words in forward retrieval for the 1st position ($p=0.013$). Additionally, with respect to differences within the groups, the sleep group was distinctly better in the forward as compared to the backward association ($p=0.007$), but this effect was stronger for words in the 1st than in the 2nd position ($p=0.008$). The wake group on the other hand showed better retention for words in the 2nd position irrespective of direction ($p=0.007$). Here forward retrieval was slightly better compared to the backward direction of retrieval but this effect was not significant ($p=0.12$), nor was there any evidence for an interaction between direction and position in this group (*p*=0.11).

Table 6

	Sleep group		Wake group	
Base line (% words remembered)	1st position	2nd position	1st position	2nd position
Forward retrieval	79.81 ± 5.93	89.42 ± 6.46	76.92 ± 3.96	86.54 ± 3.87
Backward retrieval	81.73 ± 7.16	89.42 ± 3.42	75.96 ± 5.00	87.50 ± 5.48
Retrieval (% with reference to base line)				
Forward retrieval	73.76 ± 6.55	65.56 ± 7.07	48.99 ± 6.55	68.30 ± 7.07
Backward retrieval	39.79 ± 8.35	55.65 ± 8.13	45.27 ± 8.35	50.40 ± 8.13

Base line learning (top) and Retrieval performance (bottom) in a Sleep and Wake group of subjects. For Base line learning, the percentage of correctly retrieved words for the 1st and 2nd position in Forward and Backward memory probing is given. Note that for base line learning the distinction between Forward and Backward retrieval refers to how the words were probed at Retrieval testing since for base line learning all words were probed in a Forward direction. For Retrieval, the percentage (with reference to the respective base line learning category) of correctly recalled words is given for the 1st and 2nd position in the Forward and Backward probing. Data are means ± SEM.

Baseline determination.

Data from the recall data immediately after learning was accomplished, were analyzed in a 2x2x2 repeated measurements analysis with the between subjects factor sleep/wake and the within subjects factors forward/backward retrieval and 1st/2nd position in the memory test. Although all words were tested in a forward direction, factor direction was still included (based on the direction the words were tested during retrieval after the retention period) to assure comparability of these subgroups at encoding. The analysis did not reveal any differences between the groups (*p*>0.60, for all comparisons, see Table 6), this way assuring that the groups were fully comparable at encoding. Here only the factor position was significant showing that more words in the 2nd position were retrieved as compared to the 1st [$F(1,24)=19.47$, $p<0.001$].

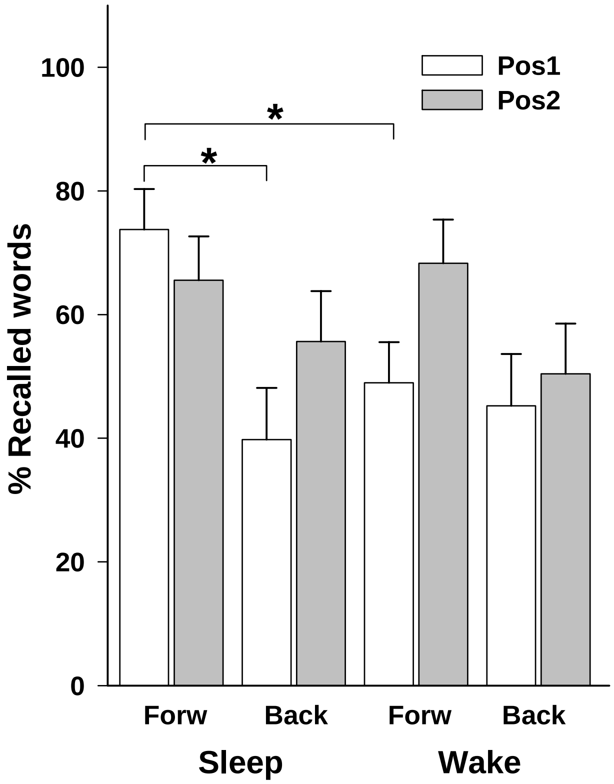

Figure 1. Percentages of retrieved words with reference to base line learning in the sleep and wake group for forward and backward retrieval, separated for the 1st (i.e. the 1st word asked to be retrieved, this is always the B-word of each triple) and the 2nd position (i.e. the 2nd word asked to be retrieved, this is the C-word of each triple in forward retrieval or the A-word of the triple when backward retrieval was probed). The figure summarizes the main finding of this study that subjects in the sleep group were better in the retention of the forward associations than the subjects in the wake group and that this effect was distinctly evident for the 1st position. Note also the pronounced improvement in forward associations in the sleep group again especially for the 1st position and the better retention for the 2nd position in the wake group. * $p<0.05$, for two-tailed pair-wise comparisons.

General cognitive functioning and self ratings.

Cognitive functioning between the groups was similar between groups as no differences were observed between the groups on a word fluency task (Aschenbrenner *et al*, 2000) where subjects had to write down as many words as they could in two minutes time starting with either the letter “M” or “P”, neither before learning (21.07 ± 1.16 and 19.85 ± 0.93 for the sleep and wake group respectively) or before retrieval testing (22.07 ± 1.10 and 19.40 ± 1.29 for the sleep and wake group respectively), ($p>0.16$ for all comparisons). Subjects also did not differ with respect to their rating of the sleep quality of the recovery night ($p>0.99$), both groups rated the quality of their sleep 3.83 ± 0.21 on a five point scale with 1 being very poor quality and 5 very high. Self-ratings after the learning phase and the end of the experiment showed that the wake group felt more awake, less tired and less sleepy after learning, whereas after the retrieval test the opposite pattern was observed ($p<0.05$, for all comparisons). This pattern though most likely reflects anticipatory differences to the experimental condition. To confirm this, first correlations were calculated among these six questions Tiredness after learning was the only question the significantly correlated (either positively or negatively with the other questions ($p<0.05$, for all comparisons). This factor then was correlated to the retrieval percentages in the sleep and wake group (1-tailed). No correlation was significant ($p>0.14$ and $p>0.25$, for all comparisons in the sleep and wake group respectively). Finally, the factor tiredness after learning was introduced as an extra covariate to the original 2x2x2 repeated measures analyses on the retrieval data. The factor was not found significant alone or in interaction with any of the other variables ($p>0.26$ for all analyses), in contrary the main finding of the study remained unaffected by this addition, ($p<0.05$, for the three-way interaction).

Physiological measurements.

Polysomnographical data scored visually off-line according to the criteria of Rechtschaffen and Kales (1968) showed that the subjects in the sleep condition slept normally in the laboratory, with a mean amount of 427 (±13.35), min of sleep. The percentages of sleep time in wakefulness, sleep stage 1 (S1), sleep stage 2 (S2), slow wave sleep (SWS) and rapid eye movement (REM) sleep were respectively: 0.57 ± 0.19, 9.57 ± 0.96, 47.68 ± 2.45, 22.53 ± 1.59, 17.72 ± 2.08 %. Mean salivary cortisol concentrations did not differ between the groups of the main experiment, neither at learning (collapsed across values before and after learning in µg/dl, sleep: 0.06 ± 0.01, wake: 0.13 ± 0.04, nor at retrieval testing (sleep: 0.61 ± 0.11, wake: 0.73 ± 0.11, (*p*>0.58 for all comparisons). Also, sleep and wake groups did not differ in cortisol concentration in the morning after the night at the laboratory (sleep: 0.32 ± 0.07, wake: 0.43 ± 0.07, *p*=0.26). In the sleep and wake condition, due to the circadian rhythm, cortisol concentrations were higher in the morning during recall testing than in the evening during the learning phase,(*p*<0.001).

Discussion Study 3

In this study we tested memory for triples of words probed either in a forward or in a backward direction after a retention interval where subjects either slept on two consecutive nights or spend the first night awake. The main finding of this study is that sleep as compared to wakefulness enhanced memory for the forward retrieval of memory but only for the 1st and most difficult position. The subjects were comparable at encoding since at base line determination after learning was completed the sleep and wake group were indistinguishable. Also since subjects had to achieve a score of at least 50% correct responses at base line learning, the possibility that differences in the strength of encoding, shown in previous studies to modify the need for consolidation, can be ruled out as an explanation for the obtained results as well. Although self ratings about subjective tiredness, activation and sleepiness differed between the groups in a condition-confirmatory way (i.e. the wake group reported to be more activated and less sleepy at encoding but more tired and less activated in the morning after retrieval testing), those differences most likely reflect condition anticipatory instead of true differences between the groups. This was supported by additional analyses of the correlations between the scores on these questions and the retrieval scores which were not significant, as well as an additional analysis including the factor tiredness after learning (best predictor of all the other factors as well) as a covariate in the original analysis. This analysis too did not reveal a role in the retrieval of the groups. Also, the control measurements taken in our study to assure comparability of the sleep and the wake group, showed that the main finding cannot be attributed to other unspecific variables as fatigue, related to the experimental manipulation. Cognitive performance on a word fluency task both before encoding and retrieval testing showed that the groups were fully comparable with respect to general memory functioning. At the same time circadian variations that could differentially affect memory performance can also be excluded as both groups were tested under similar time points in the circadian phase. While, the wake group was sleep deprived for one night, by postponing retrieval testing until after an additional night of recovery sleep, tiredness and fatigue can be excluded as variables that could explain the observed differences in retention. Also, concentrations of the stress hormone cortisol (in saliva) known to affect memory (de Quervain *et al*, 2000;Wagner *et al*, 2005) retrieval did not differ between the sleep and wake groups during encoding or retrieval.

The finding that sleep specifically enhanced memory for the 1st position but only in the forward direction of retrieval is of particular interest because as already mentioned in the introduction the retrieval of this position is considered to be the most hard since it is depends much more on the preparation stage of sequence retrieval. Hence, the effect of sleep on consolidation here can be viewed as a process that renders the information, making the sequence readily available for retrieval. Due to the long retention interval this effect cannot be explained as an typical rehearsal depended primacy effect (Tan & Ward, 2000). The absence of a sleep depended enhancement of the 2nd position is compatible with previous findings showing that sleep enhances memory for weaker associations (Drosopoulos *et al*, 2006). It is possible that memory for the 2nd position was much easier than for the 1st position. This is because here only the search stage is involved in retrieval, plus there is only one possibility left for this position. Additionally, even though in our study we did not directly probe the 2nd position with two probes, since this position was probed immediately after the 1st, it is very likely that the subjects actually benefited from this in similar manner as in Kahana and Caplan (2002). The finding that memory for the backward direction not only did not profit from sleep but that the accuracy rates for the 1st position are lower than for the 2nd position, which normally profit from the recency effect and compensate for the delay caused by the preparation stage, show that a rendering of the sequence occurs only for the forward direction. Thus, our data support the view that forward and backward retrieval represent different retrieval processes and that consistent with previous findings (Drosopoulos *et al*, 2006) sleep seems to reorganize the weights of the associations in a way that will benefit memory retrieval the most. In this manner the present findings also add to our understanding of the chronological organization of long-term episodic memories, which although depend on additional mechanisms (Friedman, 1993) shows similarities with memory for sequences as well.

Our data are in agreement with neurophysiological studies showing that replay of newly acquired memory representations during sleep promotes their consolidation (Fischer *et al*, 2005;Walker *et al*, 2005;Takashima *et al*, 2006). Findings in animals and humans testing declarative and spatial memory relying on hippocampal function, as does temporal memory (Nadasdy, 2000;Fujii *et al*, 2004;Morrone *et al*, 2005;Howard *et al*, 2005;Dragoi & Buzsaki, 2006), have shown that single neuron or neuronal networks are reactivated during sleep in a similar way as during encoding, a process taking place primarily during SWS (Bland, 1986;Skaggs & McNaughton, 1996;Lee & Wilson, 2002;Moelle *et al*, 2006) although REM sleep has also been reported (Louie & Wilson, 2001). Thus, this finding suggests that the replay of memory during sleep apart from strengthening those traces could be the key mechanism that explains how temporal order is embedded and maintained in the trace of an episodic memory. This is also in agreement with the view of Waugh (1970) who viewed the asymmetry in retrieval direction of serial lists as an epiphenomenon resulting from rehearsal of the sequences. An intriguing finding on animal exploratory spatial behavior is that apparently, replay of the novel experiences, already takes place in the wake animal immediately after the behavior was exhibited, but that in contrast to the patterns observed during sleep this reply is in a backward direction (Foster & Wilson, 2006). This finding is particularly puzzling since one would expect that in this case the backward direction of retrieval should not be inferior to the forward direction especially not in the wake group. Although the reason this is not the case needs further investigation it could be that this backward replay immediately after arriving at a certain place simply reflects the need for an animal to also be able to find its way back. Hence, this reversed replay in spatial navigation might trigger a process relevant for that specific behavior that is not relevant for other forms of sequential or episodic memory.

Our finding have also implications for computational models that try to explain serial memory, obviously off-line replay should be implemented in such models promoting the asymmetry in direction of retrieval. Although some models have been conceived that do rely on replay of (hippocampal) memory traces to explain memory consolidation (Morita, 1996;Kali & Dayan, 2004;Johnson & Redish, 2005;Leibold & Kempter, 2006) their outcomes have not been tested with regard to backward retrieval.

Recently it has also been argued that signs of reprocessing during sleep merely reflect residual activity in neuron assemblies used previously for encoding, whereas the enhancing effect of sleep on memory results from a general down scaling of synaptic efficiency (Tononi & Cirelli, 2006). That view predicts that downscaling erases weak synaptic connections below a certain threshold whereas strong connections survive. This contradicts the present and other findings of a greater sleep-dependent gain for the weaker rather than stronger associations. Although this view is consistent with our finding that backward associations did not profit from sleep, the sleep and wake group did not differ from each other in the backward associations and even though the sleep group was worse than the wake group in the 1st position at backward retrieval this difference did not reach significance. Thus, in the light of our data an erasure of the weak associations as a result of consolidation is not fully supported.

In conclusion our findings support the notion that sleep promotes the consolidation of newly learned sequences especially by rendering the sequence and making it readily available for memory, thus particularly speeding up the preparation stage of memory. Additionally, the data support the notion that sleep beyond its mere contribution to the storage of memory, actively and differentially alters the memory representations as this effect was only observed for forward direction of retrieval.

Conclusions and General Discussion

The purpose of the three experiments reported in this thesis is to discover under which conditions of learning sleep can actually benefit the subsequent consolidation of the newly established memories. Study 1 used the process-dissociation procedure (Jacoby, 1991) to separate effects of sleep on explicit and implicit memory consolidation. Compared to retention periods of wakefulness, sleep generally enhanced measures of explicit recollection. This effect was particularly pronounced during early sleep periods dominated by SWS, and when, at recognition testing, the words were presented in the same font as at learning before sleep. Interestingly, familiarity-based implicit memory did not benefit from sleep. There, only a small benefit was observed for contextual congruency (i.e. when the font of the words was kept the same at retrieval as at learning) which, however, was independent of sleep. As a whole, these results speak for a greater sensitivity of explicit than implicit memory formation to the enhancing effects of sleep that might be particularly linked to SWS. In Experiment 2 we re-examined the issue of release from inhibition during sleep, using the same A-B, A-C paradigm as Ekstrand’s group. Our results show that memory performance for the first list of word-pair associates (A-B) was superior when learning was followed by nocturnal sleep than when learning was followed by waking. For the second list of word-pair associates (A-C) memory performance was not affected by sleep. Importantly, memory performance between the control groups of our study tested, respectively, immediately after learning and 20 minutes later, did not differ from each other, and all of these control conditions showed marked retroactive interference. Experiment 3 explored why in Experiment 2 sleep only enhanced memory for the first list of word-pair associates in the “A-B, A-C” paradigm. An “A-B, C-D” rather than “A-B, A-C” paradigm was adopted here in order to reduce retroactive interference, which allowed to decide whether sleep-associated memory consolidation would selectively act on memories impaired due to retroactive inhibition or would improve memory also independent from interference. The conditions of intense and weak learning were introduced to test if the lacking sleep-associated improvement in recall of the second, A-C list associations reflected a ceiling effect, such that sleep in comparisons with wakefulness does not further enhance strength of associations that have been already intensely encoded. Results confirmed the efficacy of our experimental variations. Retrieval performance of the immediate recall group was closely comparable for both lists indicating that memory for the A-B and C-D lists indeed did not interfere. Moreover, as expected, retention was generally better after intense than weak encoding of the associations. The main result of the experiment was that in the absence of retroactive interference between the two lists, sleep enhances memory for both lists to an equal extent. However, the memory enhancing effect is observed only with a weaker encoding of the associations. Study 3 tested memory for triples of words probed either in a forward or in a backward direction after a retention interval where subjects either slept on two consecutive nights or spend the first night awake. The main finding of this study is that sleep as compared to wakefulness enhanced memory for the forward retrieval of memory but only for the 1st and most difficult position.

Our finding of a distinctly more pronounced improvement of explicit recollection after retention periods of early than late night sleep agree with a number of previous studies indicating a particular benefit of hippocampus-dependent declarative memory from just this early period of SWS-rich sleep (Born & Gais 2003). It has been proposed that the enhancing effect of early sleep on hippocampus-dependent memories relies on a reactivation of the newly acquired memory representations in hippocampal neuronal populations that occurs predominantly during SWS (Hasselmo & Wyble, 1997;Buzsaki, 1998;McNaughton *et al*, 2003). Such processes could explain a facilitated access at later explicit recollection of these memories. An involvement of the hippocampal formation in the sleep-associated memory process of interest is further supported by our finding that explicit memory enhancement during early sleep was most robust when the words were presented in the same context as during encoding. Several previous studies have consistently shown that one essential hippocampal function serves to bind encoded information with contextual cues, even in the absence of awareness for these contextual cues (Henke *et al*, 1999;Stark & Squire, 2001;Henke *et al*, 2003). Thus, a contextual dependence of the explicit memory enhancement during early sleep seems to be in agreement with the notion that this type of sleep particularly benefits hippocampus-dependent types of memory.

Contrary to our expectation, familiarity-based measurements of implicit memory were not enhanced by sleep, neither during the early part nor during the late part of the night. While this negative finding agrees with a recent study testing effects of early and late sleep on performance in a “remember/know” paradigm (Rauchs *et al*, 2004), it appears to contrast with a number of foregoing studies indicating that various forms of non-declarative memory, such as the procedural memory for skills (Plihal & Born, 1997) and the priming of words and faces (Plihal & Born, 1999a;Wagner *et al*, 2003) benefit in particular from REM sleep-rich periods of sleep. Those studies led us to suppose a generalized benefit for REM sleep-rich periods of sleep for non-declarative memories not depending on hippocampal function. However, it is not unlikely that familiarity, priming and procedural tasks, apart from relying on non-hippocampal brain regions, actually represent different types of memory (Wagner *et al*, 1998;Drummond *et al*, 2000;Stark & Squire, 2000;Rugg & Yonelinas, 2003) and thus may differ in other qualities that are crucial to their sensitivity to the enhancing effect of sleep.

Our data of a selective enhancement of signs of explicit recollection by retention sleep are in line with previous reports of a preferential enhancement of explicitly guided memory during sleep (Robertson *et al*, 2004). Those studies were based on serial reaction time tasks (SRTT) and showed that offline improvement in task performance that occurred selectively across retention periods of sleep required that subjects were aware of the sequence of the task they trained before sleep. Recent experiments indicated that the hippocampus and closely connected temporal lobe structures can be involved in both explicit and implicit learning on the SRTT (Schendan *et al*, 2003). Thus, activation of prefrontal cortical circuitry including the dorsolateral and ventrolateral prefrontal cortex seems to be more relevant to the distinction between explicit and implicit processes on that task (Fletcher & Henson, 2001;McIntosh *et al*, 2003), which may apply to the recognition task used here as well. Notably, some evidence exists that slow oscillatory EEG activity dominating human SWS reflects processes of cortical reorganisation, especially in the prefrontal cortex, that could be linked to explicit processing (such as 'thinking') taking place during the wake phase (Steriade & Timofeev, 2003;Anderson & Horne, 2003a;Anderson & Horne, 2003b). From this perspective, use-dependent changes in prefrontal cortical circuitry during explicit processing in the wake phase could be a starting point for plastic changes underlying memory formation during SWS-rich sleep (Sejnowski & Destexhe, 2000;Huber *et al*, 2004;Molle *et al*, 2004).

Our data support the view that sleep enhances an active consolidation process that leads to a differential weighting of associative connections, since the strengthening effect of sleep was greater for the associations that were weaker at encoding whether due to retroactive interference or weaker learning. This outcome cannot be explained by a merely permissive function of sleep, which predicts an equal benefit for both competing memory traces during sleep. A growing number of neuro-imaging studies indicating that post-learning sleep induces specific topographical changes in the brain's representation of a memory at delayed retrieval, likewise speak for an active consolidation process that reorganizes certain aspects of the representation (Fischer *et al*, 2005;Walker *et al*, 2005;Takashima *et al*, 2006). Notably, these studies have provided first evidence that sleep-dependent changes in the neuronal organization of hippocampus-dependent memories can occur also in the absence of overt behavioral changes (Orban *et al*, 2006) which leaves the possibility that in our experiments post-learning sleep compared with wakefulness may have induced some representational changes even in those conditions (e.g., single list learning in Experiment 2 and intense encoding in Experiment 3) where a sleep associated improvement in recall was not observed. In contrast, reprocessing of hippocampal memories during slow wave sleep takes place while prefrontal activity is distinctly diminished and thus, being no longer prefrontally controlled, it might unselectively cover strong and weak associations (Buzsaki, 1996;Maquet, 2000). In this case output signals from hippocampal reprocessing of memories during sleep would not systematically differ in amplitude between weak and strong associations leading to a relatively enhanced signal transfer for the weaker associations. This leveling of output signals could also benefit the discovery of shared structure in representations in the hippocampo-neocortical interaction (McClelland *et al*, 1995).

It should be noted that a sleep-associated consolidation relying on the active reprocessing of memory representations, while excluding a merely permissive role of sleep for memory formation, is not incompatible with the notion that sleep enhances consolidation by protecting from interference, if it is assumed that the respective brain structures cannot engage simultaneously in encoding and reprocessing of representations.

This assumption is indeed supported by computational as well as experimental work indicating that processes active during sleep switch the brain from a mode of encoding to a mode of memory consolidation (Rasch *et al*, 2006;e.g., Hasselmo, 1999).

The finding from the third study that sleep specifically enhanced memory for the 1st position but only in the forward direction of retrieval is of particular interest because the retrieval of this position is considered to be the most difficult since it is depends much more on the preparation stage of sequence retrieval. Hence, the effect of sleep on consolidation here can be viewed as a process that renders the information, making the sequence readily available for retrieval. The absence of a sleep depended enhancement of the 2nd position is compatible with previous findings showing that sleep enhances memory for weaker associations (Drosopoulos *et al*, 2006). The finding that memory for the backward direction not only did not profit from sleep but that the accuracy rates for the 1st position are lower than for the 2nd position, which normally profit from the recency effect and compensate for the delay caused by the preparation stage, show that a rendering of the sequence occurs only for the forward direction. Thus, our data support the view that forward and backward retrieval represent different retrieval processes and that consistent with previous findings (Drosopoulos *et al*, 2006) sleep seems to reorganize the weights of the associations in a way that will benefit memory retrieval the most. In this manner the present findings also add to our understanding of the chronological organization of long-term episodic memories, which although depend on additional mechanisms (Friedman, 1993) shows similarities with memory for sequences as well.

These data are also in agreement with neurophysiological studies showing that replay of newly acquired memory representations during sleep promotes their consolidation (Fischer *et al*, 2005;Walker *et al*, 2005;Takashima *et al*, 2006). Findings in animals and humans testing declarative and spatial memory relying on hippocampal function, as does temporal memory (Nadasdy, 2000;Fujii *et al*, 2004;Morrone *et al*, 2005;Howard *et al*, 2005;Dragoi & Buzsaki, 2006), have shown that single neuron or neuronal networks are reactivated during sleep in a similar way as during encoding, a process taking place primarily during SWS (Bland, 1986;Skaggs & McNaughton, 1996;Lee & Wilson, 2002;Moelle *et al*, 2006) although REM sleep has also been reported (Louie & Wilson, 2001). Thus, this finding suggests that the replay of memory during sleep apart from strengthening those traces could be the key mechanism that explains how temporal order is embedded and maintained in the trace of an episodic memory. This is also in agreement with the view of Waugh (1970) who viewed the asymmetry in retrieval direction of serial lists as an epiphenomenon resulting from rehearsal of the sequences. An intriguing finding on animal exploratory spatial behavior is that apparently, replay of the novel experiences, already takes place in the wake animal immediately after the behavior was exhibited, but that in contrast to the patterns observed during sleep this reply is in a backward direction (Foster & Wilson, 2006). This finding is particularly puzzling since one would expect that in this case the backward direction of retrieval should not be inferior to the forward direction especially not in the wake group. Although the reason this is not the case needs further investigation it could be that this backward replay immediately after arriving at a certain place simply reflects the need for an animal to also be able to find its way back. Hence, this reversed replay in spatial navigation might trigger a process relevant for that specific behavior that is not relevant for other forms of sequential or episodic memory.

Recently it has also been argued that signs of reprocessing during sleep merely reflect residual activity in neuron assemblies used previously for encoding, whereas the enhancing effect of sleep on memory results from a general down scaling of synaptic efficiency (Tononi & Cirelli, 2006). That view predicts that downscaling erases weak synaptic connections below a certain threshold whereas strong connections survive. This contradicts the present and other findings of a greater sleep-dependent gain for the weaker rather than stronger associations. Although this view is consistent with our finding that backward associations did not profit from sleep, the sleep and wake group did not differ from each other in the backward associations and even though the sleep group was worse than the wake group in the 1st position at backward retrieval this difference did not reach significance. Thus, in the light of our data an erasure of the weak associations as a result of consolidation is not fully supported.

Future research should look into the effect of sleep on the consolidation of word lists learned according to the B-A, C-A paradigm. By probing memory for the B and C words by presenting A, it could be investigated if sleep not only undoes the interference emerging after this type of learning, but more importantly if a general enhancement can be observed after sleep. This would point to the possibility that under certain circumstances the reorganization of memory traces (Wagner *et al*, 2004) could result in a reversal of those traces. This possibility can also be investigated using an A,B,C task like Study 3, were after learning subjects are informed that memory will be tested backward as well. Those experiments would pose towards a prospective nature of sleep dependent memory consolidation.

In conclusion the data can be summarized as follows: sleep seems to specifically support the consolidation of explicitly learned material through covert replay taking place during SWS. Consolidation thus, was shown to be an active process and its enhancing effect on memory cannot be explained merely by the reduced extra experimental interference during sleep (although sleep does protect from that). Sleep preferentially supports consolidation of the weaker memory traces but only to the extent that replay occurs in the same direction as during encoding (i.e. forward).

Zusammenfassung

Verschiedenste Befunde belegen, dass die Konsolidierung neu erworbenen Wissens vom nachfolgenden Schlaf profitiert. Die Reaktivierung neuer Gedächtnisspuren im Schlaf könnte dabei den Schlüsselmechanismus für die verstärkte Konsolidierung darstellen. Ziel der vorliegenden Arbeit war es verschiedene psychologische Bedingungen der schlafbezogenen Gedächtniskonsolidierung zu untersuchen.

In Studie 1 wurden die Effekte des Schlafes auf die Bildung expliziten (Recollection) und impliziten (Familiarity) Gedächtnisses in einer Wortlisten-Disskriminierungsaufgabe miteinander verglichen. Im Gegensatz zur Wachheit verbesserte der Schlaf nach dem Lernen die expliziten Gedächtnisinhalte (Recollection) während implizites Wissen (Familiarity) nicht vom Schlaf profitierte. Darüber hinaus wurde die explizite Recollection insbesondere vom Schlaf der ersten Nachthälfte verstärkt.

Aufgrund dieser Befunde konzentrierten wir uns in den beiden folgenden Studien auf das explizite Gedächtnis. Mittels Manipulation der Enkodierungsstärke untersuchten wir in der zweiten Studie die Frage, inwiefern der Schlaf eine passive oder eine aktive Rolle im Gedächtniskonsolidierungsprozess spielt. In Anlehnung an zwei ältere Studien (Ekstrand, 1967; Ekstrand, Sullivan, Parker, & West, 1971) untersuchten wir außerdem die Bedeutung retroaktiver Interferenz für die schlafbezogene Konsolidierung deklarativer Gedächtnisinhalte. Unter Verwendung des „A-B, A-C“ (Wortpaarlisten) – Paradigmas, konnte gezeigt werden, dass die negativen Effekte, die durch retroaktive Interferenz beim Enkodieren entstehen, durch nachfolgenden Schlaf verringert werden können. Verschiedene Wachkontrollbedingungen zeigten keine vergleichbaren Effekte. Unter Verwendung nicht - interferierender Wortpaarlisten („A-B, C-D“) konnte gezeigt werden, dass die Konsolidierung beider Listen dann vom Schlaf in gleicher Weise profitiert, wenn die Enkodierung weniger stark war (durch weniger und kürzere Präsentation der Stimuli beim Lernen).

Basierend auf diesen Ergebnissen wurde in Studie 3 untersucht, inwiefern die zeitliche Komponente einer Gedächtnisspur von Reaktivierungsprozessen im Schlaf profitieren kann. Zwei Versuchspersonengruppen (eine Schlaf- und eine Wachgruppe) lernten eine Liste von 32 nicht - assoziierten Worttripletts. Nach 2 Nächten (Schlaf vs. Wach +

Recovery-Nacht) wurden die Tripletts entweder vorwärts oder rückwärts abgefragt. Vorwärts getestete Tripletts wurden in beiden Gruppen besser erinnert, wobei dieser Effekt nur für die am schwächsten enkodierten Worte gefunden werden konnte. Dieser Befund unterstützt die Annahme, dass durch die Reaktivierung neu erworbener Gedächtnisspuren im Schlaf, Informationen über die zeitliche Abfolge der Inhalte erhalten bleiben.

Zusammenfassend können die Ergebnisse wie folgt beschrieben werden: Schlaf scheint die Konsolidierung von explizit gelerntem Material durch einen im Tiefschlaf stattfindenden Reaktivierungsprozess zu verstärken. Es konnte gezeigt werden, dass die schlafbezogene Konsolidierung ein aktiver Prozess zu sein scheint und nicht durch das bloße Fehlen von Interferenz während des Schlafes erklärt werden kann. Schlaf scheint außerdem vorzugsweise die Konsolidierung schwächerer Gedächtnisspuren zu verstärken, allerdings nur dann, wenn Lernen und Abfrage in derselben Richtung stattfinden (d.h. vorwärts).

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