MUNICH
SEPTEMBER 30, 2014

Neuroimaging and Behavioral Investigations of Memory Consolidation during Sleep on Time Scales from Hours to Months

~

Dissertation der Graduate School of Systemic Neurosciences
der Ludwig-Maximilians-Universität München

Dissertation by Melanie Graetsch

Dissertation of the Graduate School
of Systemic Neurosciences of Ludwig-Maximilians-University Munich

Graduate School of
Systemic Neurosciences

LMU Munich

First reviewer: Prof. Dr. Steffen Gais

Second reviewer: Prof. Dr. Stefan Glasauer

Third reviewer: Prof. Björn Rasch

Day of Disputation: 03-Mar-2015

To my sister

Abstract

Introduction: Successful storage of memory can be divided into three fundamental processes: encoding, consolidation and retrieval. During encoding, information is acquired e.g. in a learning session of an experiment. New mnemonic traces are formed in the brain. When the information needs to be remembered e.g. at the retrieval session of an experiment, memory needs to be recovered. Since encoding does not lead to instantaneous permanent storage of the learned material, a form of memory stabilization is necessary. A person's freshly acquired memory is initially fragile until the memory trace is reinforced through a process of consolidation. While learning and retrieval must occur during wakefulness, memory consolidation can occur during sleep.

One question that is still under debate in sleep literature is whether a period of sleep, in comparison to wakefulness, significantly and persistently benefits the consolidation of recently and explicitly acquired declarative information (such that memory retrieval after a period of sleep is significantly better than retrieval after a period of wakefulness). A further problem regarding the benefit of sleep for memory is the discrepancy between functional and behavioral findings: Sleep associated changes are possibly a *covert* process and changes on the anatomical level are not necessarily congruent with behavioral results. Another question concerns memory consolidation in the long run. In humans, the medial temporal lobe, especially the hippocampus, is an important brain structure involved in declarative memory retrieval. Through the process of consolidation, declarative memory has been found to become independent of the hippocampus over time. Yet, human imaging studies investigating memory retrieval for a longer period of time (several months) are scarce. Another gap of knowledge lies in the role of the hippocampus. Several different hypotheses about its role exist: The multiple trace theory, established by Nadel and Moscovitch (1997), states that personally experienced episodes stay hippocampus dependent, whereas semanticized memories become independent over time. O'Keefe et al. (1978) proposed that the hippocampus is permanently accessed for spatial memory retrieval. According to Eichenbaum (2000), the hippocampus binds new information coupled with an episode into a network of existing memory traces.

This thesis focuses on long-term memory. The major focus lies on declarative memory, whereas the minor focus lies on non-declarative memory. All five studies of this thesis investigate declarative memory and the last study (study 5) additionally investigates non-declarative memory.

Study 1:

Objective: To investigate the relation between episodic (declarative) memory and sleep *versus* sleep deprivation on the functional and behavioral level. The aim is to do the investigation on a time scale of 2 ½ months. **Methods**: The analysis was based on a between-group (factor: sleep / wake), within-

subject (factor: autobiographical task / spatial task) design. Each subject learned two episodic memory tasks (word associations): an autobiographical task and a spatial task. Brain activity (using a 3T MRT) and behavioral performances were measured at 3 times: 1) Immediately after learning; 2) after a night of sleep/wake and two recovery nights of sleep; 3) 2 ½ months after learning. **Results:** No sleep related changes in hippocampal activation could be concluded from the neuroimaging results. Supporting this, behavioral results (free recall) showed no difference between sleep and sleep deprivation groups. Recall results showed no difference between the sleep group and the sleep deprivation group.

Study 2: Presuming that sleep supports hippocampus dependent declarative memory, but given the results of study 1, it was important to investigate the role of the hippocampus.

Objective: This study focused on the role of the hippocampus in declarative memory retrieval, given the different hypotheses (mentioned above) about its role. **Methods**: Using a between-group design, hippocampal involvement during free recall at an early stage after encoding was compared between sequential, spatial and autobiographical learning strategies. (Study 2 was not a sleep-study). Free recall performance of concrete nouns was measured on the functional as well as behavioral level. **Results:** Not all episodic memory traces depended equally on the hippocampus when information was retrieved in free recall: Whereas recall of autobiographical memory relied on the hippocampus after consolidation, recall of spatially and sequentially associated information did not. Functional conjunction analyses showed that brain areas mutually involved in *all* tasks tested, were: the precuneus (medial parietal cortex), medial occipital gyrus and superior parietal lobe (SPL).

Studies 3 – 5: The specific mechanisms underlying the process of memory consolidation are still not clarified. It has been suggested that a positive effect of sleep on memory occurs when a sensitive set of requirements is met, although to date, pinpointing the exact requirements has not been possible from sleep literature.

Study 3:

Objective: The question to be answered was: Is the *type* of retrieval, that is, *cued recall* or *recognition,* crucial for an effect of sleep on declarative memory? **Methods**: The following parameters were applied: i) Cued recall and recognition as the type of retrieval test; ii) Circadian rhythm: Learning either in the morning or in the evening; iii) The retention period between learning and the post-conditional test was kept constant at 12 hours; iv) Interference learning was used; v) The learning material was restricted to non-sense syllables. **Results:** A beneficial effect of sleep on memory retrieval 12 hours after learning non-sense syllables occurred only when syllables were tested via *cued recall*. However, results were influenced by circadian rhythm effects with better test scores in the morning than in the evening.

Study 4:

Objective: Same as in study 3, but controlling for the circadian rhythm effects by using nap sleep instead of nocturnal sleep. **Methods**: Circadian rhythm effects were controlled by choosing a 60 minute nap sleep paradigm, in which encoding and retrieval both took place at the same time of day (in the afternoon), for both the sleep and wake conditions. The two types of retrieval in relation to nap sleep and wakefulness were examined: *cued recall* and *recognition*. The following parameters were applied: i) Cued recall and recognition for the type of retrieval test; ii) Circadian rhythm: Learning in the afternoon; iii) The retention period between learning and the post-conditional test was kept constant at three hours (including a 60 minute nap or time spent awake); iv) Interference learning was used; v) The learning material consisted of concrete German nouns. **Results:** subjects did not perform significantly better after a period of napping compared to a period of wakefulness, neither for words tested via cued recall nor words tested via recognition. A sleep benefit on the behavioral level did not show to be specific to the type of retrieval test.

Study 5:

Objective: To examine whether a sleep benefit occurs between a critical period of 12 to 144 hours post learning. In addition to declarative memory, the relation between sleep and procedural memory is tested, using a motor sequence (finger tapping) task. **Methods**: Subjects learned a procedural and a declarative task. The following parameters were applied: i) Free recall for the declarative and procedural retrieval tests; ii) In contrast to the other studies, total sleep deprivation *and* daytime wakefulness were used as wake condition iii) The retention period between learning and testing was 12, 72 or 144 hours (3 groups); iv) Interference learning was not used for the declarative task (a main and new motor sequence task were learned); v) The learning material was restricted to non-sense syllables. **Results:** No beneficial post-learning effect of sleep could be detected in the declarative and procedural tasks over the retention interval of up to six days. Results of study 5 demonstrated that sleep after learning did not lead to better performance of motor skills than wakefulness after learning.

Conclusion: From the results of the five studies of this thesis, it can be concluded that declarative and procedural memories are consolidated equally well over a period of wakefulness compared to a period of sleep. The type of retrieval, circadian rhythm, retention period, interference, and the type of material might all contribute to a set of variables influencing the benefit of sleep on memory. It can also be assumed that the human brain is capable of compensating a night of sleep deprivation without significant behavioral deficits during retrieval of verbal declarative and motor skill tasks, whether memory is tested shortly after encoding (a few hours), after days or after months.

Table of Contents

Table of Figures

Table of Figures

Tables

Abbreviations

Abbreviation	Term
BOLD	blood oxygen level-dependent
EPI	echo planar imaging
fMRI	functional magnetic resonance imaging
FWE	Family wise error
MNI	Montreal Neurological Institute
MRI	magnetic resonance imaging
MRT	magnetic resonance tomography
non-REM	non-rapid eye movement
NREM	non-rapid eye movement
PET	Positron emission tomography
REM	rapid eye movement

1 General Introduction

In the process of learning, memory is indispensable for humans and animals. Memory is studied by a variety of different disciplines and research groups. Memory is not a unified feature, but instead can be separated into “sensory”, “short-term” and “long-term” memory, as was clarified by Atkinson and Shiffrin (1968) on amnesia. Sensory systems, such as the visual system, are able to store sensory information for a short time. For example, Purdy and Olmstead (1984) found that the visual system stores letters and shapes for a quarter of a second. Thereafter, sensory information was “transferred” to visual short-term memory storage. Items of short-term memory are stored for up to roughly half a minute (Peterson and Peterson, 1959) and according to Miller (1956) the storage capacity is limited to approximately seven “chunks”, for instance seven words. In the process of consolidation of memory, freshly acquired memory is “moved” from short-term memory storage to long-term memory storage through a process of systemic and synaptic changes. On the synaptic level, learning is subserved by neuronal alterations, such as an increase in synaptic strength. Long-term potentiation (LTP) was first reported in depth by Bliss and Lomo (1973) who found that high-frequency electric stimulation of a chemical synapse located in the hippocampus led to a long-lasting increase in synaptic strength. Long-term memory can still be retrieved after several minutes, hours, or even years. For freshly acquired memory to be retrievable after an extensive amount of time, memory must be consolidated.

1.1 Declarative and Non-declarative Memory

Early studies on patients with medial temporal lobe lesions (Squire, 1986, 1987) gave rise to the two-class model of memory (Squire and Zola, 1996). This model separates long-term memory into declarative and non-declarative memory (Figure 1), depending on whether patients learned and retrieved items through a “conscious” or “unconscious” mnemonic process.

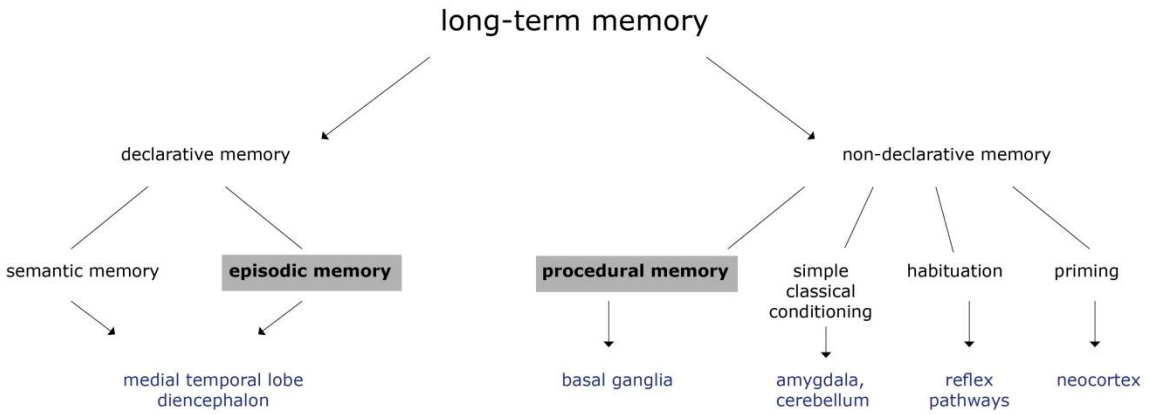


Figure 1: Classification of memory categories by Squire and Zola (1996)
Long-term memory can be divided into declarative and non-declarative memory. According to the model, declarative memory, also known as explicit memory, requires conscious recall and can be divided into semantic (facts) and episodic (events) memory. Non-declarative memory, also termed implicit memory, does not require conscious recollection. It is accessed "unconsciously". It comprises the categories procedural memory (skill learning and habit learning), simple classical conditioning, habituation and priming. The bottom row indicates the brain regions found to be required by the different types of memory. This thesis mainly investigates declarative memory. Among the two types of declarative memory, episodic memory (highlighted in grey) is studied. According to the memory model, episodic memory depends on the medial temporal lobe structures. Therefore, this thesis especially focuses on functions of the medial temporal lobe. A minor focus of this thesis is non-declarative memory. Among the different types of non-declarative memory, procedural memory (highlighted in grey) is studied in this thesis (study 5).

Functionally, the declarative memory system relies upon the medial temporal lobe, which comprises the hippocampal formation[1] and amygdala as well as cortical regions[2] (Eichenbaum et al., 1996; Henson et al., 2003). According to the model by Squire and Zola (1996), declarative or "explicit" memory is accessible to conscious memory. The model splits declarative memory into two subclasses: semantic memory (facts and general knowledge) and episodic memory (events). When episodic memory is accessed, contextual detail of the stored event, such as what, when and where something happened, is remembered. For more detailed information see also Squire (1986) and Tulving (1985).

Non-declarative or "implicit" memory comprises procedural memory, simple classical conditioning (Pavlov, 1927), habituation and priming (Squire and Zola, 1996). Procedural memory is accessed only through performance and comprises motor skills, such as playing the piano or riding a bicycle, and habit learning (e.g. probabilistic classification tasks (Squire and Zola, 1996)). Functionally, procedural memory has been shown to mainly rely upon the basal ganglia[3]. However, hippocampal involvement

[1] The hippocampal formation comprises the cornu ammonis, dentate gyrus, and subiculum.

[2] The cortical regions of the medial temporal lobe include the parahippocampal, perirhinal and entorhinal cortices.

[3] The basal ganglia comprise the striatum, globus pallidus, substantia nigra and the subthalamic nucleus.

in procedural tasks has been reported (Albouy et al., 2008); Poldrack and Packard (2003) give a detailed review on the memory systems involved in declarative and non-declarative tasks.

The memory model by Squire and Zola (1996), which is based on consciousness, is challenged by several scientists who have created their own versions of a memory model; to name a few: Henke (2010); Lewis and Durrant (2011); Moscovitch et al. (2005). For example, Henke (2010) proposes that declarative and non-declarative memory be based on the involved processing operations (Figure 2). The model depicts the neural structures according to the different learning stimuli and processing demands (see legend of Figure 2).

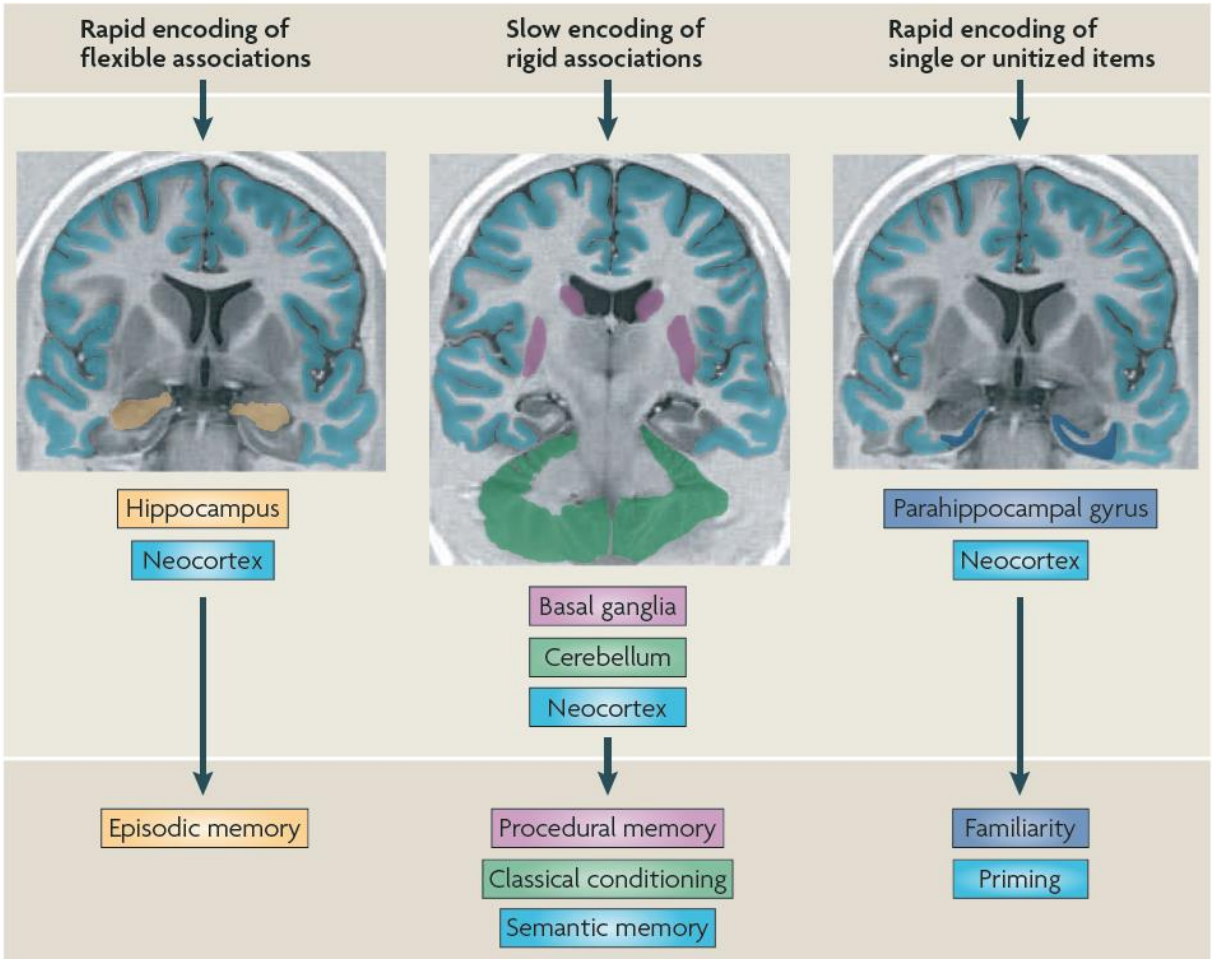


Figure 2: The long-term memory model by Henke (2010)
The model classifies memory into three categories, based on the processing modes which are required in specific learning situations: rapid encoding of flexible associations, slow encoding of rigid associations, and rapid encoding of single unitized items. Rapid encoding of flexible associations: Episodic memory is acquired in a single learning trial. Storage of this type of memory relies on the hippocampus and neocortex. Slow encoding of rigid associations: Procedural memory, classical conditioning and semantic memory require multiple learning repetitions in order for memory traces to be established. For this processing mode, brain structures depend on the basal ganglia, cerebellum and neocortex. Rapid encoding of single unitized items: Similar to the first processing mode, only one learning trial is enough for facilitated response to a stimulus based on previous exposure (priming) or for an item to be perceived as familiar (familiarity). Information storage depends on the parahippocampal gyrus and neocortex and information is stored in a unitized fashion. Thus, memory processes can be subdivided into (i) rapid versus slow memory acquisition, (ii) associative versus single-item learning, and (iii) flexible versus rigid representations. (Reprinted by permission from Macmillan Publishers Ltd [Nature Reviews Neuroscience]; copyright (Henke, 2010)).

1.2 Memory and Sleep

A vast amount of literature[4] focused on the relation between sleep and memory in humans and animals. However, the role of sleep in memory is continuously under debate. Before giving more details on the debate, an introduction to sleep is given here for a better understanding of the studies conducted in this dissertation.

1.2.1 Sleep Stages

In a polysomnography, a person is monitored during sleep. Measured are the electrical activity along the scalp (using electroencephalography, EEG), eye movements (using electrooculography, EOG) and electrical activity of facial muscles (using electromyography, EMG). Rechtschaffen and Kales (1968) were the first to establish sleep scoring guidelines in order to separate sleep into stages. The result, a hypnogram (Figure 3), shows the different sleep stages throughout a night. Sleep can roughly be divided into rapid eye movement (REM) sleep and non-rapid eye movement sleep (non-REM or NREM) sleep. As its name says, REM sleep is characterized by rapid movement of the eyes. This stage is accompanied by reduced muscle tone. Non-REM sleep is divided into stages 1, 2, 3 and 4. Stages 3 and 4 are collectively called slow wave sleep (SWS)[5].

[4] Diekelmann et al. (2011); Fishbein et al. (1966); Gais et al. (2007); Hennevin et al. (1995); Jenkins and Dallenbach (1924); Laureys et al. (2002); Lewis et al. (2011b); Maquet (2001); S. Mednick et al. (2003); S. C. Mednick et al. (2008); S. C. Mednick et al. (2002); Nishida and Walker (2007); P. Orban et al. (2006); Payne and Nadel (2004); Rieth et al. (2010); Smith et al. (1974); U. Wagner et al. (2004); Walker et al. (2003a); Walker et al. (2002); Walker et al. (2003b); Walker and Stickgold (2004); Erin J Wamsley et al. (2010); E. J. Wamsley et al. (2010); Wilhelm et al. (2011a); Witt et al. (2010); Yoo et al. (2007)

[5] Not all polysomnographs are scored according to the original rules by Rechtschaffen and Kales. Due to debate, e.g. about the division of slow wave sleep into one or two stages or the definition of the onset of REM sleep periods (Silber et al., 2007), the American Academy of Sleep Medicine has revised the scoring rules (Iber et al., 2007) and regularly provides updated scoring manuals.

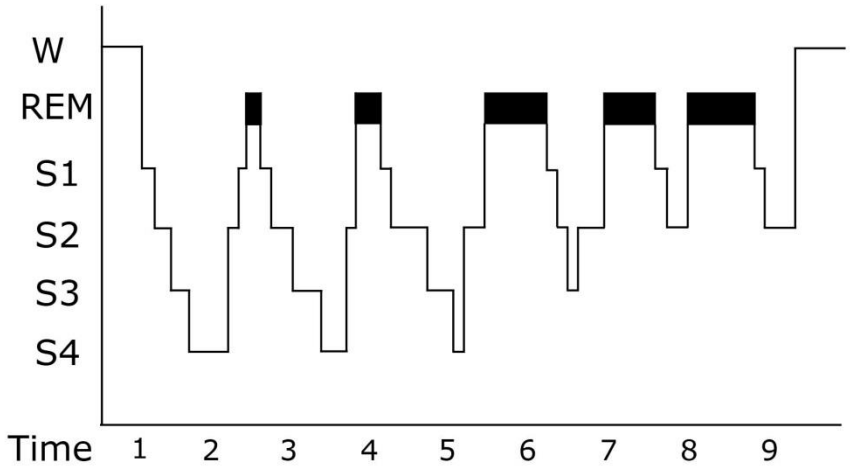


Figure 3: Sleep cycle: A hypnogram of 8 hours of sleep
Depicted are wakefulness (W) and the stages of sleep (REM = REM sleep; non-REM stages: S1 = sleep stage 1, S2 = sleep stage 2, S3 = sleep stage 3, S4 = sleep stage 4) and sleep duration (time). When a person falls asleep (time = 1), he/she quickly reaches slow wave sleep (phases 3 and 4). After approximately one hour (time = 2), the human brain enters REM sleep (thick lines). One sleep cycle has a duration of approximately one and a half hours. The brain alternates between REM and non-REM phases, whereas the duration of REM sleep increases and the duration of slow wave sleep decreases throughout the sleep cycles of the night. The subject to whom this diagram belongs was awoken after 8 hours of sleep (time = 9) from stage 2 sleep. (Figure modified with permission from U. Wagner (2004)).

In a regular night of sleep we go through four to six sleep cycles, each taking between 90 to 120 minutes. Figure 3 depicts a person's sleep within eight hours. Each sleep cycle is composed of non-REM sleep and REM sleep. When falling asleep, the brain of a healthy person changes from a wake state to sleep stage 1 (not passing through REM sleep!), then to stages 2, 3, 4 and back up until he/she ends in REM sleep (Figure 3, thick lines). The hypnogram above shows that the person completed five sleep cycles and was woken after having started a sixth cycle. The duration of non-REM sleep and REM phases do not stay constant for every sleep cycle during the night: In earlier cycles, REM sleep phases are shorter, in later cycles the phases are longer. On the contrary, non-REM sleep phases decrease in length with each cycle. In an entire night's sleep episode (of e.g. 8 hours), the amount of slow wave sleep is much higher than REM sleep and we spend only roughly 20 percent of a sleep episode in REM sleep (Horne, 2000).

1.2.2 Sleep Oscillations

In the transition from wake to sleep states, the neuronal activity of the brain changes dramatically. EEG oscillations in the two brain structures, cortex and thalamus, connected through reciprocal projections, become slower, larger in amplitude and especially more synchronous. The high-frequency waking patterns of beta waves (12 – 30 Hz) and alpha waves (8 – 12 Hz) are replaced by increasing theta activity (4 – 7 Hz) in the hippocampus (G. Orban et al., 2006). Theta waves are typical

for stages 1 and 2. Furthermore, in stage 2, spontaneous cellular firing produces K-complexes (Loomis et al., 1938) and sleep spindles (12 - 16 Hz) in the cortex.

Sleep spindles are generated by reticular nucleus (RE) neurons located in the thalamus. RE neurons enact on thalamocortical neurons and de-inactivate a low calcium current, resulting in burst firing. The firing induces rhythmic excitatory postsynaptic potentials in the cortex, where spindles are measured in an EEG (Kokkinos and Kostopoulos, 2011).

Depolarization of non-synchronous pyramidal cells in the hippocampus leads to sharp-waves (150-200 Hz), causing cellular firing to become synchronous and giving rise to field oscillations called ripples (150 – 250 Hz). These sharp wave ripples have been detected in the pyramidal layer of the animal hippocampus during slow wave sleep but also in phases of quiet wakefulness (for a review see Girardeau and Zugaro (2011)).

Slow oscillations occur in stage 3 and are most abundant in stage 4. Slow wave sleep is characterized by synchronization of neuronal firing (0.1 – 3.5 Hz) which can be measured in the cortex (Steriade et al., 1993). The slow oscillation contains two phases, an “up” state, which is a prolonged depolarization, and a “down” state. The “up” state is linked to a short cellular firing, whereas in the second phase, the cell does not fire, hence the name “down” state. Slow oscillations, spindles and ripples are associated with increased brain activity and are related to the consolidation of memory (see next sections).

1.2.3 Sleep and Memory Consolidation

Successful storage of memory can be divided into three fundamental processes: encoding, consolidation and retrieval (Figure 4, upper row). During encoding, information is acquired e.g. in a learning session of an experiment, and new mnemonic traces are formed from the incoming information. When the information needs to be remembered e.g. at the retrieval session of an experiment, memory needs to be recovered. Since encoding does not lead to instantaneous permanent storage of the learned material, a form of memory stabilization is necessary. Moreover, immediately after learning, memories are susceptible towards interference, i.e. competing information leading to memory loss through overwriting (Figure 4, “forgetting”). Barnes and Underwood (1959) proposed that memory loss can result from failure of proper storage or from response competition at the time point of memory retrieval. For newly acquired information to become stable and retrievable after longer time intervals, memory must be consolidated (Nadel and Moscovitch, 1997). Already at the turn of the 19th century Ribot (1882) and Müller and Pilzecker (1900) who conducted studies on retrograde amnesia, saw that a form of consolidation exists that protects memories from disrupting interferences. Consolidation is a period after acquisition which

serves to stabilize newly encoded information into a more robust form (McGaugh, 2000; Müller and Pilzecker, 1900).

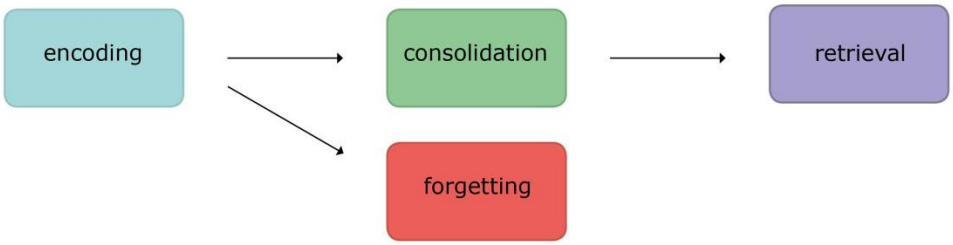


Figure 4: The memory process
In the encoding step, information is learned. The freshly learned information is vulnerable towards forgetting. In the successful memory storage process, the information is consolidated, counteracting the process of forgetting. Consolidation of memory occurs during sleep but also during periods of wakefulness. At the retrieval stage, information is remembered. For this thesis, fMRI and behavioral tests were conducted at the retrieval stage to give insights about previous effects of sleep, compared to wakefulness, on memory.

Rapidly acquired information is quickly forgotten if not consolidated. Ebbinghaus (1983) was one of the first to study the attention span of human memory and recorded the rate of forgetting of non-sense syllables and published the first forgetting function (see study 3 for more information). Forgetting is also an interesting topic studied in animals. For example, when rats had experienced only one trial of a spatial task (in this case a Morris water maze task) and an electroconvulsive shock was applied immediately or after 15 seconds post acquisition, rats completely forgot the newly learned information. In contrast, when the shock was applied after more than 30 seconds, the Morris water maze task could be performed without significant memory loss (Bohbot et al., 1996). This showed that memory consolidation (in this case of spatial memory) seems to have a critical time period, in which it is vulnerable towards disruption. Thereafter, memories have been consolidated into a stable form.

1.2.3.1 Memory Consolidation on the Systemic and Cellular Level

It is widely accepted that two levels of consolidation exist: consolidation on a systemic level (active system consolidation, 1.2.3.1.1) and consolidation on a cellular level (synaptic consolidation, also called homeostasis, 1.2.3.1.4).

1.2.3.1.1 Active System Consolidation

The concept of active system consolidation originates from the model of consolidation by Frankland and Bontempi (2005). In their model, two types of complementary storage systems for memory exist, a temporary storage for fast learning and a long-term storage for slow learning. For the case of declarative memory, the hippocampus has been proposed to serve as temporary storage and the

neocortex as long-term storage [for a review see Diekelmann and Born (2010)]. Whereas short term consolidation takes only seconds, long-term consolidation is much slower, but longer lasting. According to the standard theory of consolidation (Frankland and Bontempi, 2005), cortical areas are necessary when motor, perceptual or cognitive tasks are learned. Then, the hippocampus serves as an integrator, binding together the information stored and distributed in primary and associative cortical areas. Over time and through frequent reactivation of the learned information, cortico-cortical connections are strengthened and memory traces become hippocampus *independent* over time. It is this gradual modification to cortical dependence that allows for long-term security of memory (Eichenbaum et al., 1996; Squire and Alvarez, 1995).

1.2.3.1.2 Medial Temporal Lobe Discoveries through Patient H.M.

The theory of system consolidation stems from the discoveries made with patients suffering from damage to the medial temporal lobe, such as the famous patient Henry Gustav Molaison (H.M.), whose memory functions were studied intensely over five decades, e.g. by Scoville and Milner (2000) and Corkin (1984). In an effort to relieve the patient of intractable epileptic seizures, a bilaterial medial temporal lobe resection was performed, and the patient's hippocampus, parahippocampal gyrus and amygdala were surgically removed. From early neurocognitive observations, a link between the hippocampus and declarative memory could be concluded. H.M. suffered from anterograde amnesia and in cognitive tests provided evidence for severe impairment in the encoding of declarative information which he acquired after surgery. In contrast, encoding of procedural memory stayed intact. Furthermore, H.M. suffered from temporally graded retrograde amnesia. He showed deficits during retrieval of memories acquired shortly before the removal of large parts of the medial temporal lobe. However, remote memories, such as those acquired during childhood, were spared. H.M. also showed no deficits in experiments of working memory, such as recalling previously shown numbers. It was concluded that the hippocampus plays an important role in the formation of new declarative long-term memories. Furthermore, memory retrieval becomes independent of the hippocampus over time.

1.2.3.1.3 Reactivation

Active system consolidation comprises the reactivation of the memory traces temporally saved in the hippocampus. Pavlides and Winson (1989) were the first to discover reactivation in rat hippocampal neuronal ensembles during sleep following spatial exploration of an environment. Firing patterns of hippocampal place cells[6] during sleep were similar to those observed during the previous spatial learning task (when the animal was awake), but no reactivation of the place cells could be seen when

[6] Detailed information see Appendix

rats had *not* been exposed to the learning task (Lee and Wilson, 2002). Post-learning reactivations in the animal brain have been detected during slow wave sleep (Kudrimoti et al., 1999) but also during REM sleep (Louie and Wilson, 2001). In the sleeping brain of humans, reactivation of the same cortical areas that had been active during prior visuomotor skill learning task, were observed in a PET[7] investigation conducted by Maquet et al. (2000). Thus, during post-learning sleep the brain processes previous learning episodes through reactivation of the memory traces.

Traces selectively reactivated during sleep are integrated into an existing network of long term memories in the neocortex, leading to plastic changes in the brain (Staba et al., 2002). During sleep memory traces are replayed, i.e. cells are reactivated during sleep in the same temporal order as they were active during wakefulness (Buhry et al., 2011). For example, Lee and Wilson (2002) discovered firing patterns of hippocampal cell ensembles of rats that occurred in the same temporal sequence during post-learning slow wave sleep as during previous spatial exploration. It is assumed that this replay, leading to synaptic reorganization, serves as an important part of the consolidation process (McClelland et al., 1995).

It must be noted here that memory reactivation has been studied not only in sleep phases but also in wake phases. In their recent investigation Oudiette et al. (2013) found that reactivation during wakefulness strengthens memories, but reactivation during sleep not only strengthens but also associates related memories. Furthermore, in comparison to wake states, reactivation during sleep was found to reduce retroactive (Drosopoulos et al., 2007a; Ekstrand, 1967) and proactive (Abel and Bauml, 2013) interference. (When learning a list of word pairs, e.g. tulip – car, or A – B, and then learning a second list, e.g. tulip – zebra or A – C, then retroactive interference refers to the word pairs of the first list, or A – B, and proactive interference refers to the second list or A – C). For more information about interference, see study 3.

1.2.3.1.4 Synaptic Consolidation

The standard theory of consolidation also proposes that synaptic consolidation occurs. On one hand, it is hypothesized that during sleep, synaptic weights, which are necessary for future learning episodes during wakefulness, need to be "reset" because wakefulness requires an increase of synaptic strength (Hanlon et al., 2011). Thus, synaptic downscaling would prevent the saturation of synapses and prepare for further learning phases (Tononi and Cirelli, 2006). On the other hand, it is hypothesized that Hebbian synaptic upscaling occurs in order to stabilize memories acquired in the past: Already in 1949, (Hebb), famous for his learning theory (Hebbian learning), proposed that reverbatory activity between cells initiates a process making memory traces permanent. Synaptic

[7] Detailed information see Appendix

upscaling originates from the discovery of the upregulation of levels of calcium-dependent proteins, which is impaired during sleep deprivation (Vecsey et al., 2009). It has been suggested that synaptic consolidation occurs during REM sleep phases, whereas system consolidation occurs during slow wave sleep (Diekelmann and Born, 2010), whose functions are discussed in detail in the next section.

1.2.3.2 REM Sleep and Memory Consolidation

On one hand, REM sleep deprivation was related to memory impairment (Smith, 1995, 2001), on the other hand, it was criticized that the impairment was the result of stress but not clearly sleep (Robert P. Vertes and Eastman, 2000). The idea that only REM but not non-REM sleep plays a beneficial role in memory consolidation has also been expressed (Pearlman, 1981). Whereas many authors propose that REM sleep aids the consolidation of procedural memory, improving e.g. perceptual discrimination (Karni et al., 1994) or visuomotor skills (Maquet et al., 2000), just as many authors fail to find an effect (for an example of a REM deprivation study see Donchin et al. (2002), for a review see Horne (2000)). The inverse, a relation between a decrease in REM sleep and improved procedural performance has also been discovered [for a review see (R. P. Vertes, 2004)]: of REM sleep with antidepressant drugs (serotonin re-uptake inhibitors) resulted in the improvement of a finger sequence task (a motoric task). Others argue that REM sleep supports the consolidation of declarative material (Empson and Clarke, 1970; Tilley and Empson, 1978): Empson and Clarke (1970) showed that REM sleep deprivation leads to poorer recall of short stories, but slow wave sleep deprivation does not. Later, Tilley and Empson (1978) could see the same specific detrimental memory effect after post-learning REM deprivation for lists of words from different categories.

The idea that REM serves to consolidate declarative memory has been refuted (R. P. Vertes, 2004). Some patients with brainstem lesions, resulting in REM sleep elimination or in REM reduction, exhibit no cognitive disorders. For example, a young man whose brainstem was damaged due to a gunshot wound, lived well without ever entering REM phases during sleep (R. P. Vertes and Siegel, 2005). To date, the role of REM sleep in memory consolidation is still under debate (Siegel, 2003).

1.2.3.3 The functions of REM and non-REM sleep - The Dual Process Theory

The majority of the published findings on sleep and memory consolidation support the view that sleep in comparison to wakefulness aids memory retrieval [for a review see (Diekelmann et al., 2009)]. However, there is some controversy regarding the memory enhancing benefit of sleep (R. P. Vertes, 2004; R. P. Vertes and Siegel, 2005). It remains difficult to pinpoint a specific role to a single sleep stage (Gianluca Ficca and Salzarulo, 2004). Different interpretations of the role of the sleep stages exist, one of the most cited being the dual process hypothesis (Peigneux et al., 2001). It states that REM sleep supports the memory consolidation of procedural tasks, whereas non-REM sleep aids the memory consolidation of declarative tasks. Thus, the effect of REM and non-REM sleep on

memory is task-dependent. It has even been proposed that the declarative and procedural systems work in a disconnected fashion during sleep, the advantage being independent system operation (R. M. Brown and Robertson, 2007). Plihal and Born (1997) made use of the discovery that when looking at a hypnogram and splitting the night into two halves, slow wave sleep is more dominant in the first half than in the second one, whereas REM sleep is more prominent in the second half than in the first one (compare with Figure 3). Based on the observation that the first half of the night ("early" sleep) is rich in slow wave sleep and the second half ("late" sleep) is rich in REM sleep, studies investigated the two halves separately and in regard to declarative and procedural memory. For example, participants in the study of Plihal and Born (1997) learned a declarative (paired associates) and procedural task (mirror tracing) and were tested either after early sleep (after the first three hours), or after late sleep. In the late sleep condition, subjects slept during the first half of the night, learned, and were tested after completion of the second half of the night. Two wake control groups were also tested: An early wake group stayed awake for three hours after learning and was tested. A late wake group slept during the first half of the night, learned, stayed awake during the second half of the night and was tested. Since subjects performed significantly better at declarative memory recall in the early sleep condition and significantly better at the procedural task in the late sleep condition, authors concluded that the period of nocturnal sleep influences the effect of sleep on memory. However, the design of dividing sleep into two halves to study the roles of sleep stages has been criticized (Gianluca Ficca and Salzarulo, 2004). Non-REM sleep also occurs in the late half of the night and REM sleep also occurs in the first half. One way of pinpointing the effect of REM or non-REM sleep is to depress it with specific drugs (see next section). A simple way of testing the effect of non-REM sleep is to test the learned material already after short sleep durations (nap sleep) in which subjects are woken before the onset of the first REM sleep phase. A nap sleep study was performed and is reported in this thesis (see study 4).

Overall, findings on both animals and humans about the role of non-REM and REM sleep in memory are not consistent (see Gianluca Ficca and Salzarulo (2004) for a review). To date the majority of sleep studies support the idea that non-REM sleep facilitates declarative memory consolidation whereas REM sleep promotes procedural memory consolidation e.g. Gais and Born (2004); Kuriyama et al. (2004); Plihal and Born (1997); Rasch et al. (2007); Takashima et al. (2006). Yet, the dual process model has been questioned due to its attribution of *opposite* roles towards REM and non-REM sleep states.

1.2.3.4 Non-REM Sleep and Memory Consolidation

Interest in the function of non-REM sleep rose in the 1980's (Buzsaki, 1984). Similar to the relation between REM and memory consolidation, studies investigating non-REM sleep activity after learning phases could not deliver uniform results about its function.

Slow wave sleep has been proposed to be an optimal time for declarative memory consolidation. Besides studies dividing sleep into early and late phases see section 1.2.3.2, substantial support for learning-dependent changes in the brain during slow wave sleep was found in studies not dividing sleep into two halves: In a PET study by Peigneux et al. (2004) subjects had learned a virtual maze task (spatial navigation task), which depends on the hippocampus. Especially during post-learning slow wave sleep, increased hippocampal blood flow, previously seen during learning, re-appeared. Furthermore, the authors detected a positive correlation between the increased brain activity in the hippocampus during slow wave sleep and improved post-sleep performance at the navigation task. It is possible that during slow wave sleep neurons, involved in the coding of spatial information, are re-activated, contributing to improvement when the task is once again performed during wakefulness.

The amount of slow wave sleep has been related to post-sleep performance on declarative tasks. For example, Takashima et al. (2006) conducted a nap sleep study in which subjects memorized photographs of natural landscapes, took a nap, then memorized new photographs and were then tested on old (before sleep) and new (after sleep) photographs. Results showed that a correlation between the duration of slow wave sleep and test performance existed for photographs learned before, but not after sleep. Authors thus concluded that slow wave sleep promotes the consolidation of declarative memory.

Contrary to the dual-process theory, non-REM sleep does not only support declarative memory. For example, Yotsumoto et al. (2009) found enhancement of activation during non-REM sleep following a visual perceptual learning task (non-declarative memory). Specifically, fMRI results showed significantly higher activation in the location necessary for the visual task, which was the primary visual cortex known as V1. The amount of V1 reactivation correlated with an improved performance at post-sleep testing. Further evidence for the relation between non-REM sleep and non-declarative memory was found by Huber et al. (2004) whose study is discussed in further de in the next section.

1.2.3.4.1 Learning has a regionally specific effect on post-learning non-REM sleep

Learning can induce slow wave sleep in specific brain regions that were necessary for the learning task: For example, an EEG study on humans by Huber et al. (2004) demonstrated a local increase in the intensity of slow wave sleep which correlated with improved performance of a motor rotation task that had been performed before sleep and was tested after sleep. Subjects showed significantly more slow wave activity in the parietal cortex after the motor adaptation task than after a no-rotor

task. Authors concluded that the learning task triggered a local increase of slow wave activity. Further support for the induction of regional slow wave sleep is given by a study on chicken (Nelini et al., 2010). Chicken that had been exposed to a spatial learning task, selecting the one of four boxes containing food showed more post-learning local (here: unihemispheric) sleep than chicken that had not been exposed to the task. Authors associate the increase in local post-learning sleep with spatial memory consolidation.

1.2.3.4.1.1 Neuronal Communication between the Hippocampus and Cortex

On the cellular level, communication between the cortex and hippocampus has been observed during non-REM sleep. For example, Ji and Wilson (2007) studied firing sequences of cells in the hippocampus and the visual cortex of rats during active exploration (wakefulness) and post-learning slow wave sleep. The examined animals were trained to sleep for one to two hours, then ran in an alternation task on a figure-8 shaped maze, and slept again for one to two hours. Electrophysiological recordings during slow wave sleep showed a replay of the same firing patterns that had been recorded during previous maze exploration. Authors assume that a dialog between the hippocampus and cortex during post-learning sleep reflects the consolidation of previously acquired episodic memory. From EEG recordings it was further implied that a feed-forward interaction from the cortex to the hippocampus exists, since firing of cells in the cortex preceded firing in the hippocampus.

1.2.3.4.2 The role of Sharp-wave ripples and Spindles in Memory Consolidation

Besides slow oscillations, a post-learning sleep benefit has been ascribed to spindles and sharp-wave ripples. Sleep spindles are related to memory consolidation concerning procedural as well as declarative tasks (e.g. Rasch et al. (2009) and Piosczyk et al. (2013) respectively). Spindles characterize non-REM sleep, are typical for stage 2 sleep, but also occur during other stages, e.g. in the transition between non-REM and REM sleep. An example for the relation between stage 2 sleep and a procedural task is a study by Smith and MacNeill (1994). Here, the deprivation of stage 2 sleep led to impairment in the performance of a pursuit rotor task. Spindle density, especially that of fast spindles (14 – 16 Hz) in comparison to slow spindles (12 – 14 Hz) is affected during sleep after learning, which was shown e.g. for motor sequence tasks (Barakat et al., 2011) and a mirror tracing task (Tamaki et al., 2009). For more details on the role of spindles in procedural memory, see the review by Fogel and Smith (2011). Furthermore, the endogenous circadian rhythm and the amount of pre-sleep wakefulness influence spindle activity during sleep (Dijk and Czeisler, 1995; Fogel and Smith, 2006). Spindles have also been related to learning potential and intelligence. Spindle activity has been positively correlated with cognitive ability, such as the ability to solve complex problems (Schabus et al., 2006). On the contrary it has been found that spindles of mentally disabled children

have higher amplitudes and longer durations compared to healthy subjects (Gibbs and Gibbs, 1962), possibly due to dysfunction of molecular gating mechanisms between the thalamus and cortex.

Ripples in the neocortex temporally coincide with spindles in the hippocampus. Chrobak and Buzsaki (1996) recorded electrical activity in hippocampal cells of freely behaving rats. They found that ripples in the entorhinal cortex were phase-related, occurring 5-30 msec after the occurrence of ripples in the hippocampus (in CA1 neurons). This act of phase-related synchrony gave rise to the idea that memory consolidation is enabled through a "transfer" in the direction from the hippocampus to cortical regions. The findings further support the theory by Squire and Alvarez (1995) who proposed that memory representations in the cortex are gradually established through structures of the medial temporal lobe.

1.2.4 The medial temporal lobe

The medial temporal lobe has been found to play a role in encoding, consolidation and retrieval processes. Structures of the medial temporal lobe include the hippocampal complex, amygdala, parahippocampal cortex, entorhinal cortex and perirhinal cortex (Henson et al., 2003). Together with the neocortex, the medial temporal lobe forms a neuronal connection to process information. Findings on the involvement of the anterior and posterior part of the medial temporal lobe are controversial. Several fMRI studies provide evidence that the posterior part of the medial temporal lobe is involved in memory encoding, (for a review see Schacter and Wagner (1999)). Stern et al. (1996) found significantly higher fMRI signal intensity in the posterior hippocampus and parahippocampal gyrus when subjects encoded novel pictures than when they encoded familiar pictures (pictures that had been shown repeatedly). The conclusion from various PET studies ascribes the opposite role to the two parts of the medial temporal lobe: Lepage et al. (1998) concluded from over four dozen PET studies that the anterior medial temporal lobe is associated with encoding and the posterior part is associated with retrieval of episodes.

1.2.5 Neuroanatomy of the Declarative Memory System: The Limbic System

Several areas of the temporal lobe, including the hippocampus, are part of the limbic system. The limbic system (Figure 5) plays an important role in memory storage, but also in the processing of emotions.

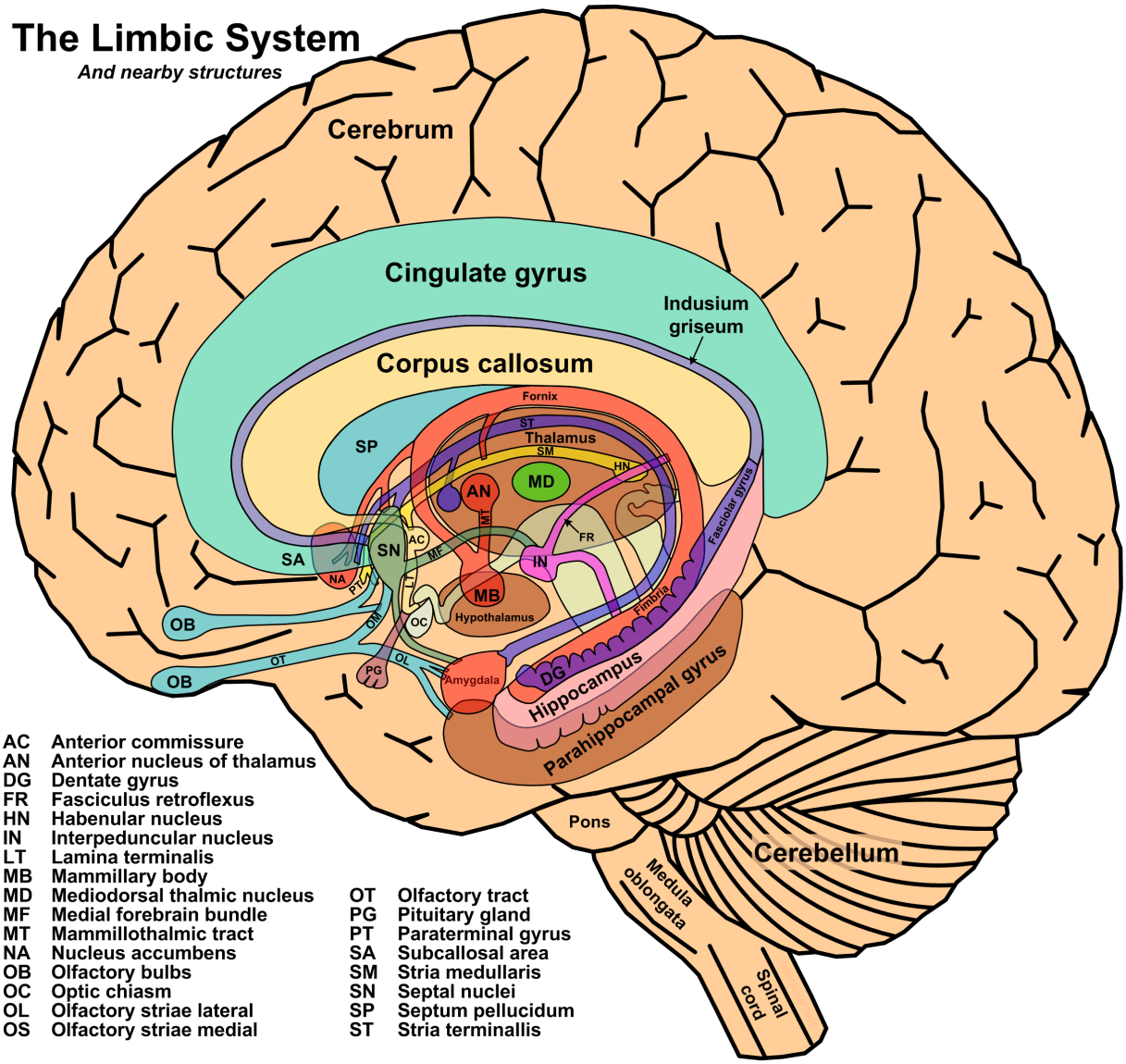


Figure 5: Anatomy of the limbic system
Embedded in the limbic system are structures of the medial temporal lobe: The hippocampus as well as parahippocampal, entorhinal and perirhinal regions. With permission from Taylor (2012).

At the core of the limbic system lies the Papez circuit. It comprises the entorhinal cortex, hippocampus, mammillary bodies, anterior nucleus of the thalamus, cingulate gyrus and parahippocampus. The main afferent connection to the hippocampus is called the perforant pathway, in which glutamatergic fibers project from the entorhinal cortex to the hippocampus. The main efferent pathway of the hippocampus is the fornix (Wilson and Keil, 1999).

1.2.5.1 Memory consolidation during off-line periods

After encoding, during "off-line" periods, the medial temporal lobe (see section "The medial temporal lobe") plays a role in processing information. Peigneux et al. (2006) showed that during wakefulness in the first two hours after acquisition of information, the brain processes newly encoded memories. Subjects performed an auditory oddball task while in the MRT, which served as the baseline task for fMRI analysis and then learned a declarative task (spatial navigation task in a

virtual environment) or a procedural task (a multiple choice reaction time task) outside the MRT. Thereafter, subjects were tested on the oddball task. Comparing the blood oxygen level-dependent (BOLD) signals of the two oddball sessions, experimenters looked for changes in regional brain activity and detected learning related changes in brain activity over the time spent awake. Furthermore, after declarative learning, brain activity was higher in both the hippocampus and parahippocampus than before learning. Cerebral activity in these two areas was also significantly higher after the declarative task than after the procedural task. Thus, from the study the importance of the medial temporal lobe in declarative memory can be inferred.

R. M. Brown and Robertson (2007) propose that during off-line periods, the procedural and declarative memory systems are flexible in their connections and can either act independently of each other or interact. The caudate nucleus has been found to support non-declarative habit learning (Packard and Knowlton, 2002). Results from Poldrack et al. (2001) and Voermans et al. (2004) gave rise to assumption that the consolidation of declarative and non-declarative memories may be supported by *separate* systems which *interact* during wakefulness. Voermans et al. (2004) found that both systems act in parallel and in a non-competitive fashion. This conclusion was made from fMRI analyses of a navigation a task (indoor routes in virtual environments), comparing healthy subjects to patients with mild or moderate Huntington's disease.

The two systems can also behave in an overlapping manner, working together: Schendan et al. (2003) detected medial temporal lobe involvement in both procedural and declarative encoding using a learning task that was either encoded implicitly or explicitly, respectively. In their fMRI study, Schendan et al. (2003) investigated the involvement of the medial temporal lobe in the serial reaction time task, originally developed by Nissen and Bullemer (1987). Subjects were either engaged in an implicit (procedural) or an explicit (declarative) learning task. In the implicit learning task, subjects were not informed about an underlying rule. In the explicit learning task, subjects were informed about an existing pattern and learned the sequence consciously. Authors observed medial temporal lobe involvement regardless of the implicit vs. explicit character of the memory.

R. M. Brown and Robertson (2007) also see the declarative and procedural systems as functionally uncoupled during sleep. Massimini et al. (2005) recorded brain responses to transcranial magnetic stimulation (TMS) and found evidence for a breakdown of cortical activity during non-REM sleep. When functional connectivity in the brain decreases during sleep, procedural and declarative memory systems can act *independently*.

Overall, sleep literature offers a wide range of answers towards the question of the relation between sleep and the consolidation of human long-term memory, ranging from better retrieval after sleep than after a period of wakefulness, to REM or non-REM specific effects, to no benefit for both types

of long-term memory (declarative as well as procedural memory). According to a wide range of literature, the medial temporal lobe, especially the hippocampus, is an important brain structure involved in declarative memory processing. However, no consensus about the role of the hippocampus has been found (for more details see the introductions in studies 1 and 2). Furthermore, it has been proposed that the time until declarative memory becomes independent of the hippocampus ranges between weeks to years (McClelland et al., 1995; Norman and O'Reilly, 2003; G. Orban et al., 2006; Rudy and O'Reilly, 2001). However, few studies examine the retrieval process of episodic memory after a long delay (i.e. several months). Yet another discrepancy in sleep literature arises from the different methods used to detect sleep related memory changes. For example, it has been proposed that the consolidation of memory is a covert process (P. Orban et al., 2006), detectable on the anatomical but not always on the behavioral level, since the brain is able to compensate possible deficits, e.g. from sleep deprivation.

1.3 Sleep and Memory Paradigms

In the literature of cognitive psychology memory tests are administered in order to measure the effect of a task. In this thesis, memory paradigms serve to measure the effect of sleep on memory consolidation in comparison to wakefulness. It has been argued that a benefit from post-learning sleep depends on the *type* of retrieval test (see Introduction of studies 3 and 4 for more detail). A benefit from sleep after learning has been found more consistently in recall, especially cued recall paradigms than in recognition paradigms (for a review see Diekelmann et al. (2009)). In *free recall*[8] paradigms subjects study a list of items (e.g. words) and in a test need to retrieve as many items as possible with no specific cue support. In contrast, *cued recall* is a paradigm in which a cue, such as one word of a pair of words, is presented, and the stimulus (the second word) needs to be recalled by the subject. Another paradigm is the *recognition* test. As the name indicates, the stimulus needs to be recognized. Thus, the stimulus is presented amongst a variety of possible answers and the subject who is tested needs to choose the correct stimulus, e.g. as in a multiple choice test. Many other types of retrieval tests exist. However, in this thesis, only the three – free recall, cued recall and recognition – are applied in the declarative memory tests (for more information on the method of the tests used, see the Materials and Methods sections of studies 3 to 5). Studies scrutinizing recognition and recall processes gave rise to a dual-process[9] model: Two independent memory processes exist, namely recollection and familiarity. Whereas the former involves the recovery of events in contextual detail, the latter simply provides a sense of oldness.

[8] Detailed information see Appendix

[9] Not to be confused with the dual-process model contrasting the functions of non-REM and REM sleep, see section 1.2.3.2.

1.4 Functional Magnetic Resonance Imaging (fMRI)

Since functional magnetic resonance imaging is one of the major methods with which the data for this thesis were obtained, an overview about the technique is given here. Functional magnetic resonance imaging is a non-invasive neuroimaging technique using strong electromagnetic fields to measure the magnetic resonance signal of hydrogen atoms in different tissues and fluids of the body in order to create a spatial image, e.g. of the brain. Haemodynamic changes (the changes in blood flow and blood oxygenation) are related to neural activity: For example, when solving a cognitive task, neurons in certain brain regions necessary to solve the task become active and require oxygen which is delivered by haemoglobin in capillary blood cells. Thus, local blood flow to these brain areas increases. Whereas an oxygenated haemoglobin molecule is diamagnetic, a deoxygenated haemoglobin is paramagnetic. In functional magnetic resonance tomography, this difference in magnetism is used to locate brain activation. The blood oxygen level dependent contrast, abbreviated as BOLD contrast, is measured and used to demonstrate variations in brain perfusion (in case of brain imaging) related to neuronal activity.

Successful magnetic resonance imaging requires three magnets: a primary magnet, gradient coils and radiofrequency coils. The magnets are assembled in a MRT in the shape of a loop, which surrounds the person lying in the tomograph. The primary magnet, a superconducting electromagnet, has a magnetic field (measured in the units Tesla) that is strong enough to penetrate the body (non-invasively). For the studies of this thesis a 3-Tesla MRT was used. To create the primary magnet, which is the largest part of the tomograph, electrical wire (niobium titanium alloy embedded in copper) is coiled into a loop. Since resistance must be reduced to have strong magnetism, the wires of the magnet are bathed in cold, liquid helium. A stable magnetic field is created perpendicular to the loop. Gradient coils, which are resistant electromagnets, are of lower strength than the primary magnet and enable accurate changes of the magnetic field. The radiofrequency coils are composed of a loop of conducting material and produce an oscillating magnetic field. Then, radiofrequency is turned on and off (the first pulse is usually a so-called 90 ° pulse), and the resonance of certain atoms in the human body is measured: The magnetic resonance imaging technique makes use of the body's high percentage of hydrogen atoms. All tissues, such as muscle and fat, and fluid (in this case cerebrospinal fluid) contain hydrogen atoms. The proton in the nuclei of a hydrogen atom has a positive electrical charge, possesses a magnetic spin and has its own magnetic field. When a person lies in the strong magnetic field of the tomograph, the body's protons precess (spins around in the shape of a cone) in one of two directions (parallel or antiparallel to the magnetic field of the tomograph). The stronger the magnetic field, the higher the proton's precession frequency, which can be calculated using the Larmor equation ($\omega_0 = \gamma * B_0$; where ω_0 is the precession frequency, γ is the gyromagnetic ratio and B_0 strength of the magnetic field of, in this case, the tomograph). Since

the magnetic field of the protons of the subject are longitudinal to the magnetic field (in parallel) of the tomograph, the magnetization of the person cannot be measured and thus a radio wave is sent: When the radiofrequency system of the tomograph is turned on, the radiofrequency coils emit a radiofrequency pulse which energetically excites the protons, decreasing longitudinal magnetization. The radiofrequency pulse has the same frequency as the protons, which is the Larmor frequency. Furthermore, protons change the orientation of the spin so that they precess in the same direction, leading to transversal magnetization. Precession in the same phase causes resonance. The atoms continue resonating when the radiofrequency coils are turned off. The nuclear magnetic resonance signal which is emitted when the protons return from the excited to their natural state (increasing longitudinal magnetization), and when their spins de-synchronize and lose phase coherence (decreasing transversal magnetization). The time it takes for longitudinal magnetization to recover (longitudinal relaxation time, T1) and the time needed for transversal magnetization to decrease and disappear (transversal relaxation time, T2) are measured.

The different tissues and fluids exhibit different relaxation times[10]. Tissues with a high content of water molecules, which move rapidly, have a long T1 and a long T2. In fat molecules, which move slowly, protons can easily give off thermal energy and thus T1 of fat is short and also T2 of fat is shorter than T2 of liquids/water, such as cerebrospinal fluid. A third relaxation time can be measured: T2* is the time constant for free induction decay (FID), which occurs when a single radiofrequency pulse (e.g. a 90 degree pulse) is turned off. For this thesis, functional images were obtained using a T2*-weighted sequence. Such a sequence sensitive to T2* decay shows more magnetic resonance signal in brain areas with oxygenated blood than with deoxygenated blood. In magnetic resonance imaging usually three relaxation times (T1, T2 and T2*) are measured. Relaxation times of the different tissues are used to create brain images displaying the different tissues in shades of grey.

In neuroimaging, a challenge is to acquire images with the best tissue contrast and best resolution but at the highest speed possible. Besides relaxation times, pulse sequences contribute to the varying degrees of tissue contrasts in brain images. Not only one radiofrequency pulse but a sequence of pulses is sent. The type of pulse (e.g. 90° or 180°) and the time interval between successive pulses can be chosen depending on the tissue characteristic one wants to emphasize. There are several parameters one has to choose for a sequence: TR, TE, flip angle, turbo factor, field of view matrix. These factors influence the electric signal that is measured by the MRT, which is the magnetic resonance signal or MR signal. TR (given in milliseconds) stands for the time after which the

[10] For MRI it is necessary to keep in mind that T1 depends on the composition, structure and surroundings of the tissue. For example, in a stronger magnetic field, T1 is longer than in a weaker magnetic field because protons precess faster in stronger magnetic fields.

pulse is repeated. TE is the time after which a spin echo occurs: For example, when the radiofrequency is turned off and protons start to dephase, we can send a 180° pulse after time TE, the direction of the spins changes (by 180°) and the before dephasing protons refocus. The signal, which is the spin echo, is collected.

Several hundred different sequences exist, but sequences can be divided into two main categories: spin echo sequences and gradient echo sequences. For example, in this thesis, in order to obtain anatomical images in studies 1 and 2, the gradient echo sequence FSPGR (fast spoiled gradient recalled) was used, and for functional images an echoplanar imaging (EPI) sequence was applied. The two main sequences differ in the type of echo that is recorded. Spin echo sequences involve a 180° pulse (the net magnetization is tilted 180°). Gradient echo sequences lack this 180° pulse. Their aim is to acquire the images with high speed. The EPI sequence offers the highest speed in acquisition needing only 100 milliseconds per slice. The EPI sequence is a type of gradient echo sequence with balanced gradients. For functional image acquisition in the studies of this thesis (see acquisition protocols 2.2.3.4 and 3.2.3.4), the EPI sequence involved a single radiofrequency pulse for excitations of atoms without a preceding magnetization preparation and is thus termed a T2* weighted sequence (Stephan et al., 2010).

1.5 Aim of Thesis

The main goal of this thesis is to investigate whether the actual state of sleep is necessary for long term memory consolidation, or whether wakefulness allows for an equal level of retention. The goal of the first study (study 1) is to compare human retrieval performance of episodic memory after sleep to sleep deprivation, and to investigate whether the brain state after learning is related to performance differences after a long time delay (of two and a half months). Functional magnetic resonance imaging (fMRI) as well as behavioral measures are applied in order to explore the relation between sleep and memory on the behavioral and anatomical levels, enabling conclusions about the possibly covert process of sleep and memory (as mentioned above). Furthermore, overt[11] recall had been considered unsuitable for fMRI designs due to movement artifacts during speech and uncontrolled latencies in free recall events (Oztekin et al., 2010). The fMRI studies of this thesis are among the first to successfully use an event related overt recall procedure in fMRI examinations (see sections Materials and Methods of studies 1 and 2 for further details).

Presuming that sleep supports hippocampus dependent declarative memory, but given the results of study 1, it was important to investigate in more detail the role of the hippocampus. Thus, in study 2, the role of the hippocampus in declarative memory retrieval, independent of post-acquisitional brain

[11] In all of the tests administered in the magnetic resonance tomograph, subjects recalled answers *verbally*; spoken recall.

states (sleep or wakefulness), was scrutinized. Since results of study 2 showed that not all episodic memory traces depend on the hippocampus when information is retrieved, behavioral studies were performed to examine different parameters that might influence the effect of sleep on human memory (studies 3, 4 and 5). The goal of these behavioral studies was to test whether a positive effect of sleep depends on: the type of learning material, the type of retrieval test, the duration of sleep, interference or the time interval between learning and retrieval (see individual introductions of each study for more detail). Albeit a minor part of this thesis, procedural memory retrieval was investigated in one of the five studies (study 5) in order to clarify whether the lack of significant differences between performance after sleep versus wakefulness is specific to declarative tasks.

2 Study 1 –The Relation between Episodic Memory and Sleep on a Functional and Behavioral Level, after Short and Long Delays

2.1 Study 1 – Introduction

A person's freshly acquired memory is initially fragile until the memory trace is reinforced through a process of consolidation. While the processes of learning and recall occur during wakefulness, consolidation can occur during sleep (Diekelmann and Born, 2010). It is still under debate whether a period of sleep, in comparison to wakefulness, significantly and persistently benefits the consolidation of recently and explicitly acquired declarative information, such as word associations [for a review see Walker (2008b)].

Two main theories of consolidation exist: Synaptic consolidation and system consolidation, reflecting localization at the neuronal and systems level, respectively (Dudai, 2004). Whereas the former assumes global synaptic downscaling to serve the homeostatic need of energy recovery, the latter proposes active reactivation of selected regions. The process of synaptic consolidation can occur within minutes or hours, whereas consolidation on a systems-level may take up to years (Molle and Born, 2011). In the view of active system consolidation, declarative memory, which is initially dependent on the hippocampus, is reactivated during sleep, especially slow wave sleep, and is gradually integrated into a cortical network of existing long-term memories (McClelland et al., 1995). At night time, decreased levels of acetylcholine during slow wave sleep facilitate feedback synapses between the hippocampus and neocortex, supporting system consolidation (Hasselmo, 1999).

The effects of sleep on learning and memory have been investigated with functional neuroimaging techniques. Increased activity in the hippocampal formation during slow wave sleep following intensive learning of a declarative task was observed (for a review see Dang-Vu et al. (2010)). It has been explained by two theories: Either an experience-dependent *reactivation* occurs in the hippocampus or the brain region is still active due to high demands in the previous hours (use-dependent processing). The latter theory proposed by Krueger et al. (1995) however, is opposed by more recent EEG, PET and fMRI studies (Rudoy et al., 2009; Thomas et al., 2000; Vyazovskiy et al., 2000). Furthermore, in an fMRI study involving an object location task and an external olfactory stimulus, Rasch et al. (2007) demonstrated a functional role of reactivation: that external cueing of memory traces enhanced later performance. A causal relationship between this "replay" of memory during post-training sleep and consolidation was concluded. A correlation between the amount of reactivation during sleep and behavioral improvement of a declarative spatial learning task was observed (in a PET study) by Peigneux et al. (2004): The stronger the regional cerebral blood flow in

the hippocampus and parahippocampal gyrus had been during NREM sleep, the better subjects performed on a virtual navigation task during a post-sleep recall.

Changes on the anatomical level are not always congruent with behavioral results! In another virtual navigation task, navigation was not better when sleep followed training (P. Orban et al., 2006) than after a night of sleep deprivation, although significant differences in brain activity could be observed: Activity in the caudate nucleus and several neocortical areas were significantly higher in the sleep group. This discrepancy between functional and behavioral findings allowed authors to conclude that sleep associated changes seem to be covert. In the mentioned study by Rasch et al. (2007) significantly improved recall of the declarative task was found for subjects who had slept after learning *and* received the same external stimulus (odor) during sleep that had been presented during the encoding phase. Important to note is that subjects who did not receive the stimulus during sleep, showed no improvement compared to those who spent the consolidation period awake. Thus, the question arises whether the true benefit of sleep is hidden on the behavioral level, if the task is not coupled to an external stimulus. In this case, functional imaging would shed light upon the question.

Human imaging studies investigating the long-term (what is meant here with "long-term" is a time of several months after initial encoding) effects of sleep deprivation on the recall of explicitly learned declarative information are scarce. Research results from two labs, those of Takashima et al. (2006) and Gais et al. (2007) found that as activity in the hippocampus decreased, activity in the medial prefrontal cortex increased over the course of time. In the study by Takashima et al. (2006), BOLD responses to recently learned pictures and those learned before a nap sleep of 90 minutes were analyzed. Over a period of three months, activation for items which subjects had been highly confident about having learned before sleep, and recognized correctly, increased in the medial prefrontal cortex. At the same time, a decrease in hippocampal activation could be found. Furthermore, a linear relationship between the duration of slow wave sleep and subjects' picture recognition performance of items learned before sleep, was found until one month after learning. In the study by Gais et al. (2007), system consolidation of declarative memory was investigated over a period of six months. In congruence with results from Takashima et al., word recall was associated with the medial prefrontal cortex and no longer with the hippocampus. In contrast to Takashima et al. (2006) who observed decreased hippocampal involvement as the number of correct picture recognitions, rose already a few hours after learning pairs of concrete nouns by forming a mental image (of the nouns), Gais et al. (2007) found an increase in hippocampal activation during correct word recall two days after learning. The difference between the study by Gais et al. (2007) and the study by Takashima et al. (2006) can mainly be ascribed to the sleep/wake conditions. Whereas the former study involved the two conditions sleep and sleep deprivation, the latter tested rest/nap sleep (2-3 hours) but did not include a wake control.

The aim of study 1 is to assess functional and behavioral effects of sleep on declarative memory performance and to characterize its time course over a period of two and half months. Similar to the study by Gais et al. (2007), study 1 allows the comparison of brain activity during recall of declarative material that had been acquired before a period of sleep or sleep deprivation. Furthermore, sleep or sleep deprivation occurred during the first night following learning in both studies. New to study 1, in contrast to the study by Gais et al. (2007) was the *task* associated with declarative learning: In study 1 a spatial and an autobiographical[12] task were chosen. According to Burgess et al. (2002) the encoding of personally experienced memory involves the same memory system as spatial memory, that is the hippocampus. Both the spatial and autobiographical tasks of study 1 contain aspects of episodic memory, thus both tasks are considered to fall into the category of episodic declarative memory. In the present study, functional MRI is used to distinguish brain activity related to free recall of episodic memory associations after three time intervals: Shortly after learning, after three nights and after two and a half months. The role of sleep in memory consolidation is investigated by comparing a group of subjects who slept for a full night with a group who was sleep deprived (total sleep deprivation) in the first night following the learning session. According to the above mentioned theory that sleep enables an integration of memory traces from hippocampal to cortical storage sites through sleep (McClelland et al., 1995), it is expected to see a difference in the location of brain activity at the time of recall of declarative material after sleep compared to sleep deprivation. Furthermore, from the study by Gais et al. (2007) it can be expected that long lasting differences on the functional level will be seen between the two groups examined in study 1.

[12] Autobiographical memory is the memory of personally experienced events.

2.2 Study 1 – Materials and Methods

The present study was conducted in accordance with the principles described in the Declaration of Helsinki and approved by the ethics committee of the department of psychology of the Ludwig-Maximilians-University Munich (LMU).

	Day 1	Night 1	Day 2	Night 2	Day 3	Night 3	Day 4			2 ½ months later
Sleep Group	Learning and Recall 1	Sleep	Wake	Sleep	Wake	Sleep	Recall 2			Recall 3
Sleep Deprivation Group	Learning and Recall 1	Wake	Wake	Sleep	Wake	Sleep	Recall 2			Recall 3

Figure 6: Study 1 – Study design
On the first day of the study, subjects learned two long-term episodic memory tasks: an autobiographical and a spatial task (the order of tasks was counterbalanced). They were then tested immediately with a free recall test, in the MRT. Thereafter, subjects of the sleep group went home to sleep for a full night, whereas subjects of the sleep deprivation group stayed in the laboratory and were deprived of sleep for a full night (Night 1). All subjects slept for a full night in night 2 and 3. On the fourth day, all subjects returned for MRT recall. All subjects returned two and a half months later to once again recall the two memory tasks in the MRT.

2.2.1 Subjects

Twenty-five right-handed subjects gave informed written consent to participate in the study for monetary compensation. One subject was excluded from analysis because she did not participate in two thirds of the study. Thus, for the remaining 24 subjects (15 female, 9 male, mean age ± S.D.: 24 ± 3 years) data analysis was completed for recall 1 and recall 2. Four subjects could not participate in the third recall of the experiment. Thus, for recall 3, data of the remaining 20 subjects (12 female, 8 male, age ± S.D.: 24 ± 3 years) were analyzed. In the first and second recall, both sleep and sleep deprivation groups contained 12 subjects (9 female and 3 male in the sleep deprivation group, 6 female and 6 male subjects in the sleep group), whereas in the third recall, 9 of the participating subjects (4 female, 5 male) were part of the sleep group and 11 of the sleep deprivation group (8 female, 3 male). Subjects were free of psychoactive medication and had not engaged in activities resulting in a disruption of the regular sleep cycle, such as shift- or night-time work, transmeridian travel, etc. (Bass, 2012) during the 6 weeks prior to the study. All subjects were native German speakers, non-smokers and reported to be free of psychiatric and sleep disorders. In order to prove right-handedness, all subjects completed a handedness questionnaire (Oldfield, 1971). On the days of the study, subjects abstained from caffeinated drinks and caffeinated food. Subjects kept sleep logs (see Appendix), documenting the bed-time and arousal, starting 5 days prior to the study until the end of day 4.

2.2.1.1 Learning Session

Subjects learned words by associating them with either a place (spatial task) or a personal memory (autobiographical task). The order of the starting task was counterbalanced across subjects, gender and date in each of the two groups. After the first task, subjects took a 10 min break before starting with the second task.

2.2.2 Learning Materials

Sixty-six mono- or bisyllabic concrete and highly imaginable German words, such as “Stuhl” (engl.: chair) served as learning material. All words were selected from the CELEX lexical database created by the Instituut voor Nederlandse Lexicografie (INL) and the Max-Planck-Institute, Nijmegen (Baayen et al., 1995). Each subject learned the same words (see Appendix). Just before the learning session, the words were randomly distributed to 2 lists, each containing 33 words, using Matlab (version R2010, The MathWorks, Inc.). One list served as the learning material for the autobiographical task, the other for the spatial task. Words were presented in white letters (font Arial, size 45) on a black background on a TFT-LCD screen with a diagonal of 15 ½ inches. The presentation rate was 6 seconds for the first presentation and 4 seconds for repetitions. In the spatial task, the interstimulus-interval was 0.1 second. There was no fixed interstimulus-interval in the autobiographical task. Subjects sat in a room, well lit by daylight, at a comfortable viewing distance approximately half a meter from the screen.

2.2.3 Procedure

2.2.3.1.1 Spatial task

In the spatial task, subjects learned a new route which they had never taken before. The route was a ten-minute walk inside and outside the building of the department of biology of the LMU in Martinsried. Along this route, the instructor pointed out 33 locations, e.g. the cafeteria of the university building, a certain lantern, etc., which the subjects needed to keep in mind. Thereafter in a quiet room, subjects were shown 33 words on a monitor (see section 2.2.1.1), which were all concrete objects, such as “chair”, “bottle”, etc. (in German). The subjects’ task was to visually place each object at a location from the route, such that the first object learned was visually placed at the first location of the route, the second object at the second location, etc. (Figure 7).

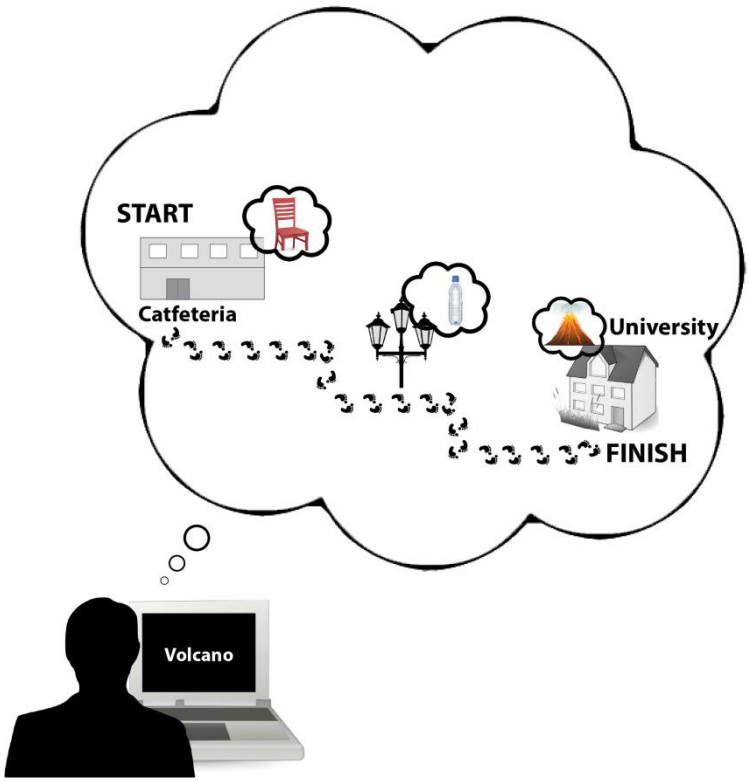


Figure 7: Study 1 – Method - Spatial task
Subjects associated words with a spatial location. First, subjects learned a new route outside and inside the department of biology of the LMU in Martinsried, Germany. Subjects started at the cafeteria and had to memorize 33 locations along their route, such as a lamp or the main building. For each location a German noun, which was a concrete object, such as “volcano”, was presented on a monitor. The task was to mentally place the objects next to the locations learned on the route. Thus, the first object learned was mentally placed at the first location of the route. The graphic above depicts three of the 33 words learned (in color) and three of the 33 locations of the route (in shades of grey). Subjects associated 33 novel spatial locations with 33 words of objects.

Once subjects could remember 100% of the objects when given the location, subjects underwent a free recall test. During the recall tests, subjects visually followed the route and verbally recalled the word associated with each location without the help of a cue from the instructor. Subjects repeated the free recall test until they could correctly remember at least 90% of the words.

2.2.3.1.2 Autobiographical task

In the autobiographical task, subjects first remembered 33 events they had experienced throughout the day, starting in the morning and ending before the start of the learning session. Events had to be experienced personally and be unique, thus not every-day events. (Beforehand, subjects had been told to spend the day of the experiment actively.) Then, each of the 33 events was associated with a word. Words were presented on a monitor (see section 2.2.1.1). All words had to be visualized as objects (Figure 8).

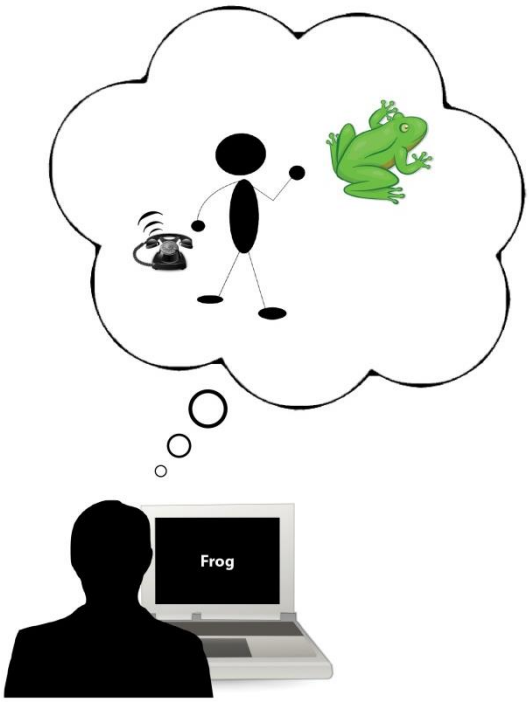


Figure 8: Study 1 – Method - Autobiographical task
Subjects associated words of objects with autobiographic episodes. Subjects recalled 33 events that had occurred throughout their day, e.g. "in the morning, my telephone rang". Each episode was then associated with a word of an object, such as "frog", which subjects had to visualize. For example, the telephone episode was associated with "frog". All words of objects were concrete German nouns and were shown on a monitor in a quiet room. Subjects associated 33 autobiographic events with 33 words of objects.

Once subjects associated 100% of the words correctly when the instructor called out the events, subjects underwent a free recall test. During the free recall test, subjects remembered an event and verbally recalled the associated word without help from the instructor. For the purpose of randomization, subjects were not allowed to recall the events in the same order of occurrence throughout the day. Subjects passed the free recall test when they had correctly remembered at least 90% of the words.

2.2.3.2 Magnetic Resonance Tomography (MRT) Recall

Subjects participated in MRT recalls on three different days, recall 1, recall 2 and recall 3. Recall 1 took place approximately half an hour after completion of the learning session. Recall 2 was

performed 3 days later (on day 4 of the experiment). Recall 3 was performed approximately two and a half months later. Subjects underwent each recall in the MR tomograph at the Department of Neuroradiology at the University Clinic Grosshadern, Munich. All subjects received written as well as verbal instructions before the recall procedure in the MRT began.

When in the MRT, subjects took part in six runs of free recall (three for each task). The order of the tasks was the same as during the learning phase, which had been randomized among subjects before the start of the study. Before the start of a run, subjects focused on a plus sign that served as a fixation point on the screen until the first fMRI pulse triggered the start of the recall. The instruction words "remember" and "speak" were presented on the screen. For the spatial task, when "remember" appeared, subjects had to visualize the location of the route and the associated word they had learned. After a minimum of three seconds of remembering when subjects were ready to overtly recall the word, subjects pressed a button using a two-button MR compatible response device (Lumina LP-400 Cedrus Corp., San Pedro, CA, USA). The instructional word "speak" appeared on the monitor and subjects vocalized the word over an optical microphone (Sennheiser MO 2000, Hannover, Germany). (The optical microphone was connected to a USB audio interface, E-MU, Dublin, Ireland. Sound was recorded with Matlab, version R2009b, The MathWorks, Inc., Natick, MA, USA). Once the word was spoken, subjects pressed a button and the instructional word "remember" appeared for the subject to think about the next location and associated word. Subjects decided themselves when it was time to end the round of recall because they had remembered and said all words they knew.. After every 10 words, subjects underwent an odd-even judgment task for 20 seconds. Here, numbers between 1 and 8 were presented on the screen and had to be categorized as even or odd by pressing a button on the key pad within two seconds. (The correct answer for an odd number was the left key of the key pad, the correct answer for an even number was the right key). The odd-even judgment task was chosen as a baseline task because it was found to suppress hippocampal activity (Stark and Squire, 2001).

For the autobiographical task, the same recall procedure was applied in the MRT, except that subjects remembered events instead of locations.

2.2.3.3 Sleep and Sleep Deprivation Procedures

In order to prevent influences on the recall performance, subjects were not informed about whether they would spend the night awake or asleep until the recall procedure was finished. Those subjects who were part of the sleep group were released and slept at home as usual. Those subjects who were part of the sleep deprivation group were accompanied to the sleep laboratory at the Department of Psychology, LMU, Munich, where they spent the night awake in the experimenter's company. Typical activities included watching PG-rated videos and playing board games. Reading or

studying was not allowed in order to avoid additional learning, possibly interfering with the previous learning material. Subjects of the sleep deprivation group wore an actimeter (ActiSleep Sleep Monitor, ActiGraph LLC., Pensacola, USA) on the left arm. The actimeter recorded motor activity and served to ensure that subjects had no brief periods of sleep during the experimental periods of sleep deprivation. After the night of sleep deprivation (Figure 6: Night 1), subjects spent the following day (Day 2) awake and were allowed two recovery nights (Night 2 and Night 3) before returning for a second MRT recall (Day 4) at the University Clinic Grosshadern, Munich.

2.2.3.4 Magnetic Resonance Imaging (MRI) Acquisition Protocol

Subjects performed the MRI recalls in a 3-Tesla GE Signa HDx whole-body MRT (General Electric Healthcare, Milwaukee, Wisconsin, USA), the head positioned in an eight channel head coil. Subjects viewed visual stimuli (image resolution: 1024 x 768 pixels, FOV: 25° horizontal; 19° vertical) through a front surface mirror, positioned at a 45 degree angle. Visual stimuli were projected from an LCD beamer (Christie LX40, Christie Digital Systems, Mönchengladbach, Germany) to a semi-transparent back-projected screen via three mirrors. Functional images were obtained using an echoplanar imaging (EPI) T2*-weighted sequence. Images were made up of 34 slices, not entirely covering the cerebellum, with a slice thickness of 3.5 mm (repetition time: 2.616s, time echo: 40 ms, flip angle: 90 degrees, matrix: 96 x 96, field of view: 220 mm x 220 mm). Structural (T1-weighted) images were taken once for every subject on the first day, applying a 3 D gradient echo sequence (FSPGR fast spoiled gradient recalled) with a voxel size of 0.86 mm x 0.86mm and a slice thickness of 1.4 mm (matrix: 256 x 256, field of view: 220 mm x 220 mm) with 0.7 mm oversampling in the z-direction.

2.2.4 MRI Data Analysis

Data were analyzed using the Statistical Parametric Mapping toolbox (SPM8 UCL, UK) for Matlab (version R2010, The MathWorks, Inc.). Data included events from three different days of recall (recall 1, recall 2, and recall 3). Each recall was made up of six runs, three autobiographical and three spatial runs. Since in this event-related design, subjects chose when to end a run, the number of events recorded per run varied depending on individual performance. Analysis was performed on correctly recalled words; events of incorrect words did not enter the analysis. Due to possible effects of spin saturation, the first five images of each session (dummy scans) were not analyzed. Before preprocessing, the anterior commissure (AC) was chosen as the origin for structural (anatomical) images and for functional (echo planar imaging, EPI) images collected on days 1, 2 and 3. Images from all three days of recall were preprocessed together in five steps (realignment, coregistration, segmentation, normalization and smoothing). Spatial disorientations occur in the images due to the fact that a body disrupts the magnetic field, rendering it inhomogeneous. For the realignment step, a two-pass procedure was chosen. First, functional images of all runs were realigned to the first image

of the first run. Then, images were realigned to the mean. In the coregistration step, the structural image acquired at the end of the first session, was coregistered to the mean EPI image. Using the subject's own structural image allowed a better identification of activations in the functional image. Since warps derived from a higher resolution (structural image), this coregistration allowed a more precise spatial image for the normalization that followed. In the segmentation step, structural images were segmented into white and gray matter. In the normalization step, the resulting segmentation parameters were used to segment functional images and convert them into MNI (Montreal Neurological Institute) standard coordinate template echo planar image (Evans et al., 1993). For improvements of the signal-to-noise ratio and to reduce false positive results, smoothing of functional images was performed with an 8 mm full-width at half maximum or FWHM isotropic Gaussian kernel. Low-frequency noise and slow signal drifts with a period longer than 128 seconds were removed using the standard high-pass filter (Ashburner et al., 2007).

Analysis at the first and second level was performed using SPM 8 (UCL, UK). On the first level, the single subject statistical analysis, the general linear model was applied to generate parametric maps. The design matrix, defining the experimental design, contained three regressors: correct word recall, speech and key press. The regressors were modeled as events and convolved with the HRF impulse response. For movement (in the x-, y- and z-direction as well as yaw, pitch and roll) six parameters and one constant (resulting from the mean activity at all time points at MRT recall) were included in the design matrix. For inferences about the population from which the subjects for this study were drawn, a random-effects analysis (RFX) was made. For the second level model of this study, each subject's contrast images corresponding to the parameter estimates for correct word recall were taken as summary measure. An event was excluded from analysis, if its duration was longer than five seconds. A complete run was excluded, if it contained less than seven events. The second level model included all three recall sessions (recall 1, recall 2, and recall 3), learning tasks (spatial and autobiographical tasks). T-tests were performed on contrast images from the single subject level in order to analyze differences between sleep and wake groups as well as between the learning tasks and recall sessions. Applying the conjunction null (Nichols et al., 2005), conjunction analyses were made to detect mutual activity between the recall sessions and tasks. The SPM 8 Anatomy Toolbox, part of Matlab (versions R2010 and R2012a, The MathWorks, Inc.) was used to find areas of activity and the tables in the results section list coordinates in MNI space unless otherwise stated.

2.2.5 Behavioral Data Analysis

For the learning session and each of the three MRT recalls, the amount of correctly recalled words was calculated. The sound files which had been recorded via the optical microphone during MRT recalls, contained each subject's verbal responses. These files were extracted using Matlab (version

R2010, The MathWorks, Inc.). Auditory noise (from the tomograph) was removed using the program Audacity (version 1.2.6, SourceForge.net). Behavioral data were statistically analyzed using Microsoft Excel 2007. For a comparison between groups, the independent two-sample t-test was performed, and for within-group comparisons, the paired t-test was performed. ANOVA were calculated using IBM SPSS Statistics (version 17.0). In the first and second recall the sleep deprivation group contained 12 subjects and in the third recall the sleep deprivation group contained 11 subjects (see 2.2.1). On the day of the third recall, one subject could not participate. For behavioral analysis, sample size was 11 for all three recalls performed by the sleep deprivation group. Data from one subject was excluded from behavioral analysis for the first and second recall due to technical difficulties with the optical microphone. In the first and second recall the sleep group contained 12 subjects and in the third recall the sleep group contained 9 subjects (see 2.2.1). For behavioral analysis, sample size of the sleep group was 12 for recalls 1 and 2, and sample size was 9 for recall 3. Thus, no data was lost due to technical difficulties in the behavioral analysis. The data of three subjects were not gathered for recall 3 (two and a half months after the learning session) because these subjects could not participate in the study due to personal reasons, such as having moved to another city.

2.3 Study 1 – Results

2.3.1 Behavioral Results

During learning, there was no significant difference between the sleep and wake groups and also no significant difference between the autobiographical and spatial tasks. Subjects needed an average of 4.00 ± 0.14 repetitions to correctly remember 100% of the events they had experienced throughout the day, and 3.83 ± 0.14 repetitions of the route to be able to recall 100% of its locations correctly (and in the correct order). After word association, subjects needed 3.17 ± 0.12 repetitions to correctly recall the words of the autobiographical task and 3.09 ± 0.15 repetitions in the spatial task, in order to meet the learning criterion for procession with MRI recall.

In the MRI recall, subjects freely recalled the spatial and autobiographical information they had learned on day 1 of the study during each of the three runs. Three runs were performed because at least three were assumed necessary for proper fMRI analysis. On each day, recall was composed of three runs of spatial and three runs of autobiographical recall. Overall, subjects performed 18 recalls (in the MRT) in the entire study. Results show that the performance of the sleep group did not differ significantly from performance of the sleep deprivation group: Figure 9 shows that results of the sleep deprivation group (light shades of a color) are similar to the results of the sleep group (dark shades of a color). This holds true for all three days of recall (recall 1, recall 2 and recall 3).

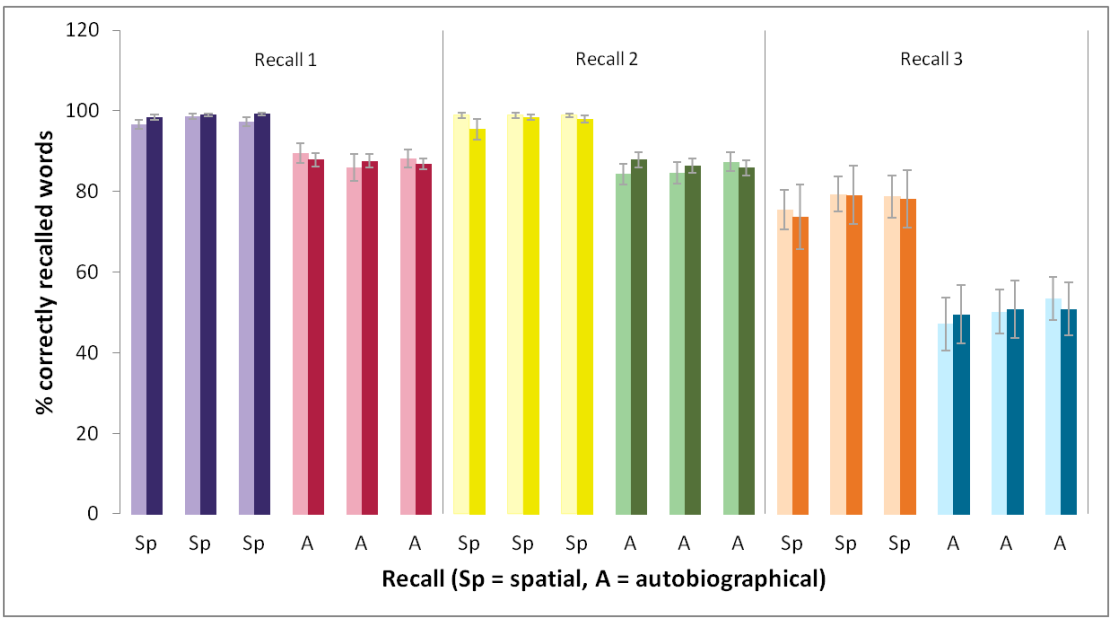


Figure 9: Study 1 – Behavioral results – Percentage of correctly recalled words – sleep deprivation vs. sleep
Shown are the percentages of correctly recalled words of a total of 33 words learned. Subjects performed recalls on three different days, recall 1 occurred on day 1 shortly after learning, recall 2 took place on day 4 and after the conditions of sleep deprivation or sleep had been performed, and recall 3 was performed approximately 2 ½ months after learning. On each day, a recall session consisted of six recalls, three spatial (Sp) and three autobiographical (A) recalls. Bars in light shades (of a color) depict average percentage of results of the sleep deprivation group. Bars in dark shades depict results of the sleep group. Error bars indicate the standard error of the mean. N = 11 for all recalls of the sleep deprivation group. In the sleep group n = 12 for recalls 1 and 2, and n = 9 for recall 3.

Moreover, memory can also be displayed in terms of words forgotten (Figure 10). After recall 1 subjects either slept or stayed awake. Regarding recall 2 in Figure 10, it becomes clear that there is no significant difference between the sleep and wake groups. Thus, no significantly higher amount of words was forgotten, whether subjects slept or stayed awake in night 1. Furthermore, the graphic shows that only few words were forgotten in recall 1 and recall 2. The autobiographical task was more difficult than the spatial task since more words were forgotten in that task. Subjects did not forget significantly more words over the time of four days (recall 2) than approximately half an hour after learning the words (recall 1). Naturally, over a period of two and half months subjects had forgotten a significant amount of words both in the sleep deprivation group (spatial recall: $p < 0.01$; autobiographical recall: $p < 0.01$, both $T = 4.30$, paired two-sample t-test) and in the sleep group (spatial recall: $p < 0.01$; autobiographical recall: $p < 0.001$, both $T = 4.30$, paired two-sample t-test) compared to recall 1. Similarly, when comparing recall 3 to recall 2 subjects had also forgotten a significant amount of words both in the sleep deprivation group (spatial recall: $p < 0.01$; autobiographical recall: $p < 0.001$, both $T = 4.30$, paired two-sample t-test) and in the sleep group (both spatial and autobiographical recall: $p = 0.001$; both $T = 4.30$, paired two-sample t-test).

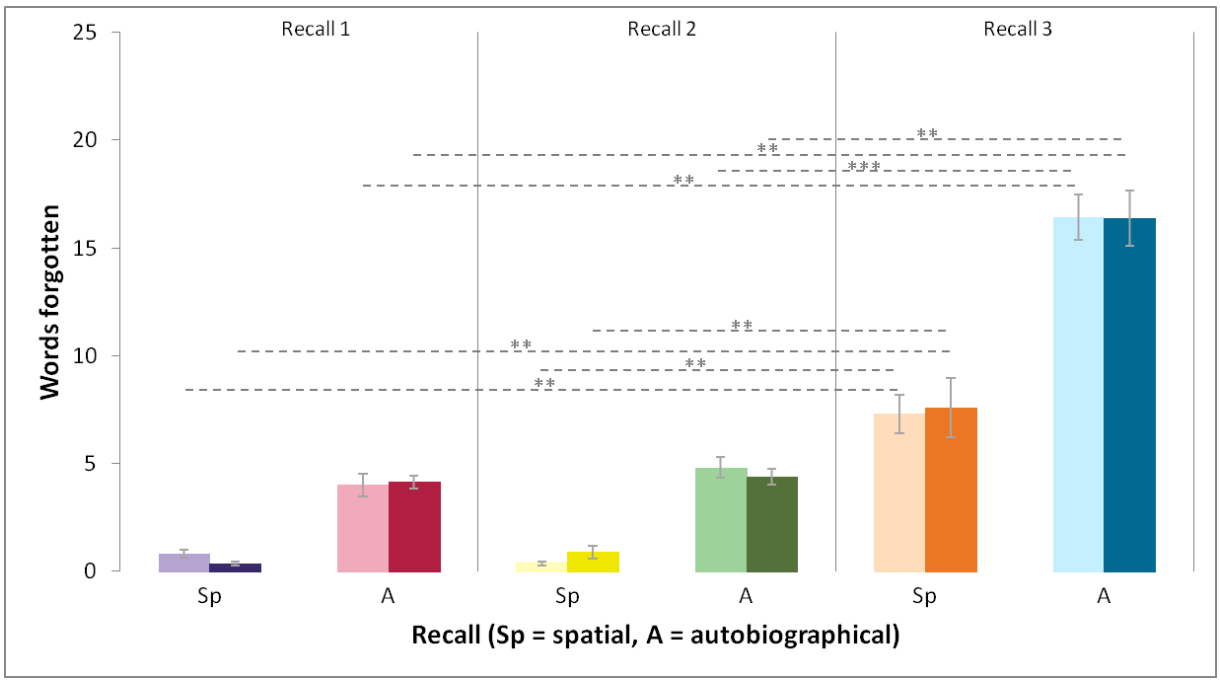


Figure 10: Study 1 – Behavioral results – Forgetting
The graphs present the number of words not recalled or not recalled correctly of a total of 33 words. Shown is the mean (and standard error of the mean) of all three runs of spatial (Sp) and autobiographical (A) recalls performed on each day. Subjects performed recalls on three different days, recall 1 occurred on day 1 shortly after learning, recall 2 took place on day 4 and after the sleep deprivation / sleep condition had been performed, and recall 3 was performed approximately 2 ½ months after learning. Bars in light shades (of a color) depict results of the sleep deprivation group. Bars in dark shades depict results of the sleep group. N = 11 for all recalls of the sleep deprivation group. In the sleep group n = 12 for recalls 1 and 2, and n = 9 for recall 3.

2.3.2 Results of Neuroimaging

2.3.2.1 Sleep compared to sleep deprivation

In order to see what difference sleep had on brain activations, the two groups, sleep and sleep deprivation, were compared using several contrasts whose results are reported in the following paragraphs. None of the contrasts resulted in family wise error (FWE)[13] corrected activity. On day 4 (recall 2) when subjects recalled the autobiographical information for the first time after the sleep / sleep deprivation condition, significant activations in the middle temporal gyrus (both hemispheres p = 0.008, uncorrected) and left fusiform gyrus (p = 0.001, uncorrected) were found when contrasting the sleep with the sleep deprivation group (Figure 11 and Table 1). In the same contrast, the postcentral (p = 0.003, uncorrected) and lingual (p = 0.006, uncorrected) gyri were also found to be significantly active (Figure 11 and Table 1).

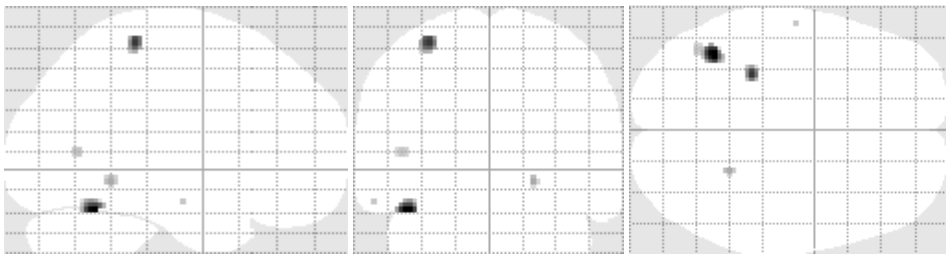


Figure 11: Study 1 – Functional results – Glass brain showing the contrast sleep – sleep deprivation in recall 2, autobiographical task
Views of graphics from left to right: Sagittal, coronal, axial.

Table 1: Study 1 – Functional results – Autobiographical task: Activations for the contrast sleep – sleep deprivation in recall 2

BA	Region	Hemisphere (R = right; L = left)	Coordinates (x,y,z) in mm	z-score (voxel level)	Cluster size (number of voxels)	Threshold p (uncorrected)
37	fusiform gyrus	L	-42, -54, -20	2.98	47	<0.01
1	postcentral gyrus	L	-30, -34, 64	2.81	38	<0.01
18	lingual gyrus	R	22, -46, -6	2.49	8	<0.01
39	middle temporal gyrus	L	-44, -62, 10	2.41	11	<0.01
21	middle temporal gyrus	L	-58, -10, -16	2.40	1	<0.01

[13] Detailed information see Appendix

When subtracting activations of the sleep group from those of the sleep deprivation group for the autobiographical recall in recall 2, results were limited to the precentral (p = 0.001, uncorrected) and calcarine gyri (p = 0.008, uncorrected).

Significant activations were found in the fusiform gyrus (BA 37: p = 0.0002; BA 19: p = 0.0003, both uncorrected) when making comparisons between sleep and sleep deprivation groups during recall 2 in the spatial task (Figure 12 and Table 2). No activations were found with FWE corrections. For the spatial task, contrasts subtracting activations of the sleep from those of the sleep deprivation group (thus, sleep deprivation – sleep) yielded no activity below a p-value of 0.001, however, activity was found below p = 0.01 in the right hemisphere of the hippocampus (p = 0.007, uncorrected) and the left hemisphere of the cingulate gyrus (p = 0.001, uncorrected).

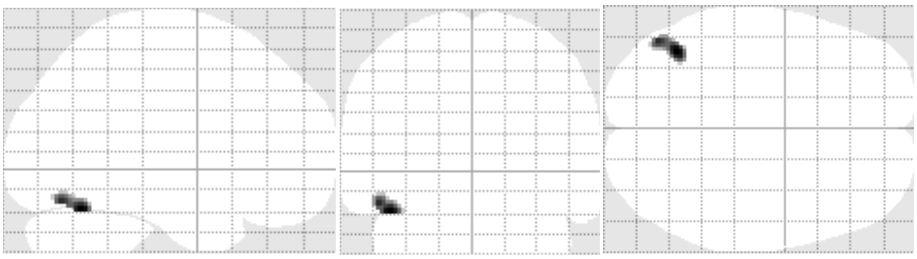


Figure 12: Study 1 – Functional results – Glass brain showing the contrast sleep – sleep deprivation in recall 2, spatial task
Views of graphics from left to right: Sagittal, coronal, axial.

Table 2: Study 1 – Functional results – Spatial task: Activations for the contrast sleep – sleep deprivation in recall 2

BA	Region	Hemisphere (R = right; L = left)	Coordinates (x,y,z) in mm	z-score (voxel level)	Cluster size (number of voxels)	Threshold p (uncorrected)
37	fusiform gyrus	L	-42, -60, -20	3.52	86	< 0.001
19	fusiform gyrus	L	-48, -70, -16	3.43		< 0.001

During the last autobiographical recall which took place 2½ months post learning, activity in the middle temporal gyrus was found when subtracting activity of the sleep group from the sleep deprivation group. Significance in all Brodmann areas (21, 22, 37 and 39) were below p = 0.001, uncorrected, and none of these areas are considered to be part of the hippocampus (BAs 27, 28, 34-36). Recall of spatial information after two and a half months resulted in the activation of the middle frontal gyrus (BA 6 and BA 10, p < 0.01, uncorrected) and calcarine gyrus (BA 17, p < 0.01, uncorrected) when subtracting activity in the sleep group from the activity in the sleep deprivation group. When subtracting activity of the sleep deprivation group from activity in the sleep group, the middle occipital gyrus (BA 18) and cingulate gyrus (BA 33) were active (both p < 0.01, uncorrected) for the autobiographical recall; recalling spatial information, the cingulate gyrus (BA 32) and supramarginal gyrus (BA 40) were active (both p < 0.01).

2.3.2.2 Differences between recalls 1 and 2

Contrasts between recalls prior to and after sleep showed no significant difference in the autobiographical task. In the sleep deprivation group, activation in the precuneus (BAs 7 and 31) and, amongst others, also in the fusiform gyrus (BA 37) were more pronounced in the recall prior to sleep deprivation than afterwards (Figure 13). Table 3 lists all activations (all activations $p < 0.001$, uncorrected).

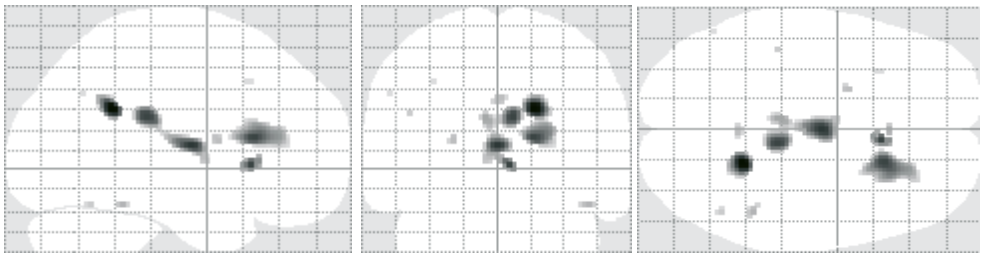


Figure 13: Study 1 – Functional results – Glass brain showing the contrast recall 1 – recall 2 for the sleep deprivation group, autobiographical task
Views of graphics from left to right: Sagittal, coronal, axial.

Table 3: Study 1 – Functional results – Activations for the contrast recall 1 – recall 2 for the sleep deprivation group, autobiographical task

BA	Region	Hemisphere (R = right; L = left)	Coordinates (x,y,z) in mm	z-score (voxel level)	Cluster size (number of voxels)	Threshold p (uncorrected)
31	Precuneus	R	18, -48, 30	4.10	103	<0.001
	Thalamus	middle	0, -8, 12	3.84	145	<0.001
	Caudate	R	6, 22, 2	3.78	17	<0.001
23	Posterior cingulate	R	8, -30, 26	3.78	133	<0.001
23	Cingulate gyrus	L	-6, -18, 26	3.31		<0.001
	Caudate	R	18, 24, 16	3.77	286	<0.001
	Anterior cingulate	middle	0, 26, 6	3.29	3	<0.001
37	Inferior temporal gyrus	R	46, -42, -18	3.27	6	<0.001
	Putamen	L	-22, 6, 14	3.22	5	<0.001
7	Precuneus	R	2, -50, 36	3.22	9	<0.001
40	Inferior parietal lobule	L	-44, -30, 28	3.17	1	<0.001
37	Fusiform gyrus	R	46, -58, -18	3.16	3	<0.001
8	Middle frontal gyrus	L	-33, 20, 44	3.10	2	<0.001
39	Inferior parietal lobule	L	-54, -62, 38	3.09	1	<0.001

When recalling the learned route, the parahippocampal gyrus and a small area in the frontal gyrus were significantly ($p < 0.001$, uncorrected) more active after sleep deprivation (recall 2) than immediately after learning (recall 1) as Figure 14 shows. Table 4 lists the two activations (both activations $p < 0.001$, uncorrected).

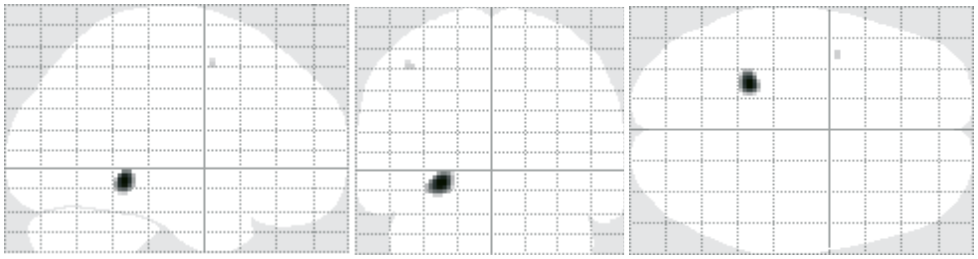


Figure 14: Study 1 – Functional results – Glass brain showing the contrast recall 2 – recall 1 for the sleep deprivation group, spatial task
Views of graphics from left to right: Sagittal, coronal, axial.

Table 4: Study 1 – Functional results – Activations for the contrast recall 2 – recall 1 for the sleep deprivation group, spatial task

BA	Region	Hemisphere (R = right; L = left)	Coordinates (x,y,z) in mm	z-score (voxel level)	Cluster size (number of voxels)	Threshold p (uncorrected)
n.a./36	Hippocampus / Parahippocampal gyrus	L	-26, -40, -6	3.73	95	<0.001
6	Middle frontal gyrus	L	-42, 4, 52	3.14	3	<0.001

Spatial recall after sleep (recall 2) had higher demands on the frontal lobe ($p < 0.001$, uncorrected) than immediately after learning (recall 1) as Figure 15 shows.

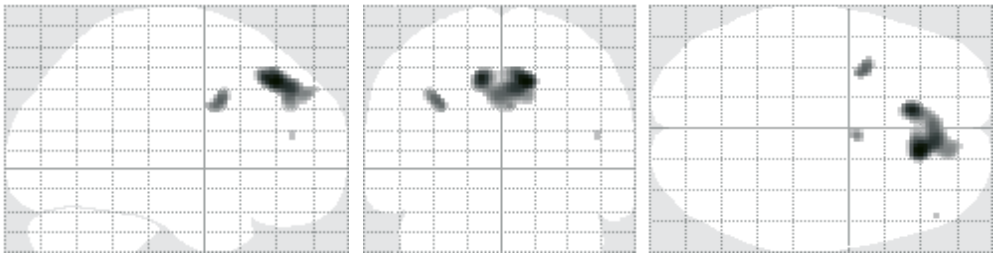


Figure 15: Study 1 – Functional results – Glass brain showing the contrast recall 2 – recall 1 for the sleep group, spatial task
Views of graphics from left to right: Sagittal, coronal, axial.

Table 5 lists the activations (activations $p < 0.001$, uncorrected) shown in Figure 15.

Table 5: Study 1 – Functional results – Activations for the contrast recall 2 – recall 1 for the sleep group, spatial task

BA	Region	Hemisphere (R = right; L = left)	Coordinates (x,y,z) in mm	z-score (voxel level)	Cluster size (number of voxels)	Threshold p (uncorrected)
8	Superior frontal gyrus	R	12, 36, 44	3.57	590	<0.001
8	Medial frontal gyrus	L	-10, 32, 46	3.50		<0.001
9	Precentral gyrus	L	-32, 8, 34	3.35		<0.001
24	Cingulate gyrus	R	4, 4, 32	3.26	11	<0.001
46	Middle frontal gyrus	R	48, 44, 16	3.15	2	<0.001

Note that uncorrected hippocampal activity was found in recall 1 ($p = 0.006$ for the sum of both sleep and wake subjects), but only in the spatial task, not the autobiographic task. However, contrasts between the two tasks (autobiographic – spatial tasks and spatial – autobiographic) during recall 1 did not yield hippocampal activity.

2.3.2.2.1 Differences between recalls 2 and 3

There were no significant FWE corrected differences between the activations during recall after the time span of two and a half months (recall 3) and after three days (recall 2). An uncorrected ($p < 0.001$) significant activation was found for the middle temporal gyrus (BA 21) and the parahippocampal gyrus (BA 34) when contrasting spatial recall 2 with recall 3 of the sleep group (recall 2 minus recall 3).

2.3.2.3 Differences between recalls 1 and 3

Overall, BOLD responses were higher in the middle temporal gyrus (BA 21 and 39) immediately after encoding than after two and a half months. BOLD responses in the middle temporal gyrus were

higher during recall 1 than during recall 3 for autobiographical associations (sleep and wake: $p < 0.01$ uncorrected) and for spatial associations independent of the brain state after acquisition (sleep: $p < 0.01$ and wake: $p < 0.001$, both uncorrected). For spatial memories, no contrast supported higher hippocampal activity when comparing recent to remote memories. However, when learning in the autobiographical task was followed by wakefulness in night 1, the hippocampus showed higher BOLD responses in recall 3 than in recall 1 ($p < 0.01$ uncorrected).

2.3.2.4 Conjunction Analyses

A conjunction analysis revealed that the precuneus (BA 7) is the common area activated in both sleep and sleep deprivation groups during both the autobiographical and spatial tasks and on all three days of recall ($p < 0.05$, FWE corrected, Figure 16). Activity was found in both hemispheres.

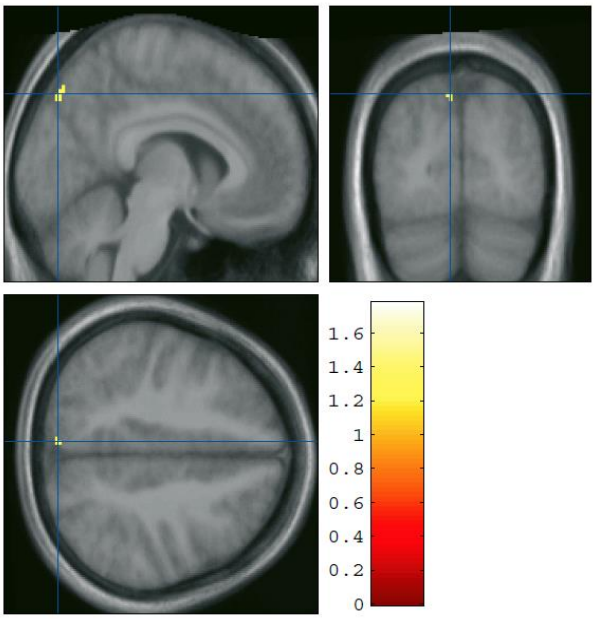


Figure 16: Study 1 – Functional results – Conjunction of all three days of recall in the sleep and sleep deprivation groups for both spatial and autobiographical task
Common activation occurred in the precuneus (BA 7) at $p < 0.05$, FWE corrected.

The conjunction of all spatial recalls (Figure 17) shows that in addition to BA 7, a further Brodmann area, also part of the precuneus, BA 31, is active.

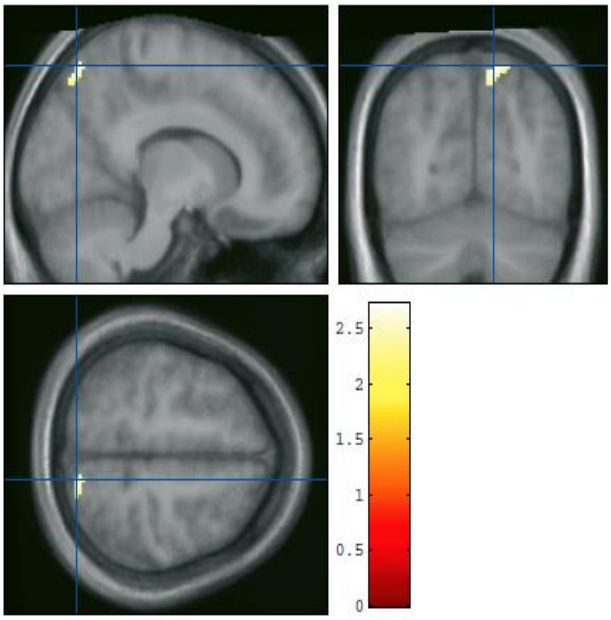


Figure 17: Study 1 – Functional results – Conjunction of all three days of recall in the sleep and sleep deprivation groups for the spatial task
Common activation occurred in the precuneus (BA 7 and 31) at p < 0.05, FWE corrected.

In addition to the precuneus, common and FWE corrected ($p < 0.05$) activation in all recalls of autobiographical information activated the superior parietal lobule, middle occipital gyrus, lingual gyrus, cerebellum, precentral gyrus and angular gyrus (Figure 18).

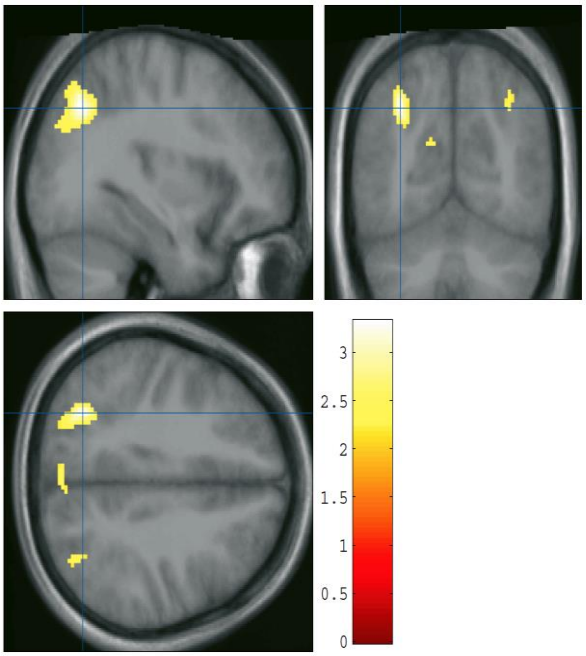


Figure 18: Study 1 – Functional results – Conjunction of all three days of recall in the sleep and sleep deprivation groups for the autobiographical task
Common activation occurred in the precuneus (BA 7 and 31) reaching into the superior parietal lobule (BA 7), middle occipital gyrus (BA 19), lingual gyrus (BA 18), cerebellum, precentral gyrus (BA 6) and angular gyrus (BA 39) at p < 0.05, FWE corrected.

A conjunction of long term memory recall (recall 2 and 3) resulted in FWE corrected activity ($p < 0.05$) in the middle occipital lobe (BA 19) and precuneus (BA 7), extending into the superior parietal lobule (also BA 7) and is depicted in Figure 19.

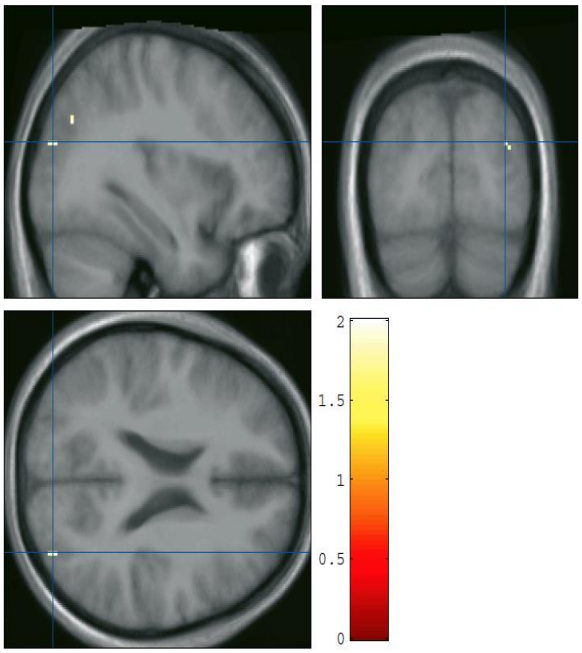


Figure 19: Study 1 – Functional results – Conjunction of recall 2 and 3 in the sleep and sleep deprivation groups for both tasks
Common activation occurred in the precuneus (BA 7) and middle occipital lobe (BA 19), at $p < 0.05$, FWE corrected.

2.4 Study 1 – Discussion

Study 1 aimed to capture positive effects of sleep after learning, on human declarative memory performance, focusing on the hippocampus. A special feature in study 1 compared to most literature investigating declarative memory was the long testing period of two and half months. Free recall of two episodic memory tasks (an autobiographical and a spatial task) was analyzed and compared between two conditions, sleep and sleep deprivation. In study 1 no sleep related changes in hippocampal activation could be concluded from the neuroimaging results. Supporting this, behavioral results showed no difference between sleep and sleep deprivation groups.

Recent reviews in favor of a positive effect of sleep on hippocampus dependent memory imply that sleep provides the special milieu needed to integrate freshly encoded memories, facilitating consolidation and thus allowing for permanent storage in neocortical areas (Dang-Vu et al., 2010; Diekelmann and Born, 2010; Diekelmann et al., 2011; Sadowski et al., 2011). However, in accordance with the behavioral results (discussed below) of the present study, this study's functional results show no difference between free recall of declarative associations after sleep compared to sleep deprivation, neither after three nights nor after two and a half months. However, uncorrected results hint at a covert change in brain activity from temporal to neocortical sites, similar to findings by P. Orban et al. (2006). Results of the present study show that when recalling newly learned route information after three full nights of sleep, cortical areas, specifically the frontal lobe, are significantly more active compared to immediate recall. Marr, one of the first to propose a model of system consolidation, suggested that the hippocampus acts as a temporary repository, quickly storing events encountered throughout the day, and later these memory traces are "transferred" to cortical areas (Marr, 1970, 1971).

No significant enhancement of neocortical areas could be found when learning was followed by a period of sleep deprivation. Within-group analyses showed higher activity in the fusiform gyrus and parahippocampus after 3 days of sleep deprivation than during immediate recall, for both autobiographical and spatial information. Such activity was not found in within-group analyses of the sleep group. This suggests that an intra-temporal lobe "transfer" had been initiated over a period of four days, yet relocation to neocortical areas had not been completed without post-training sleep during the first night. Between-group analyses showed that hippocampal activity was more prominent after sleep deprivation than after three full nights of sleep, thus it can be assumed that recall was not independent of the hippocampus after two recovery nights. Since activity was also found in the fusiform gyrus when recalling the learned route without having experienced sleep deprivation, it can be suggested that three nights of sleep are not enough for a full reorganization of declarative memory traces from temporal to cortical brain areas.

Due to the problem of fatigue after a night of sleep deprivation (Durmer and Dinges, 2005), in the present study, fMRI recall had been performed after two recovery nights. The problem with this design is that the immediate influence that sleep exerts on memory could not be obtained. Any reactivation, reorganization or reprocessing , possibly occurring in the first night after encoding, was not recorded. Evidence of neuronal reactivation during post-training sleep was found already in 1989 (Pavlides and Winson). In their experiment Pavlides and Winson found that when place cells in the hippocampus of rats were exposed to their place field during wakefulness, it resulted in increased firing activity, which influenced neural behavior during sleep. Increased activity during wakefulness led to increased activity when a period of sleep followed, but not when a period of wakefulness followed. Likewise, in a spatial memory study on humans, reactivation of the hippocampus and parahippocampus was found during post-training sleep (Peigneux et al., 2004).

Concerning remote memories, from the results of this study it cannot be ruled out that free recall of episodic information is completely independent of the temporal lobe after two and a half months, however significantly higher activity was found in temporal areas after immediate than after remote recall. According to the theory of system consolidation, reorganization of memory traces occurs as memories mature, such that after several months cortical areas, especially the medial prefrontal area, adopts the role which the hippocampus had played (Frankland and Bontempi, 2005). On one hand, supporting evidence for hippocampal independence throughout the formation of stable memories has been found e.g. by Gais (2007) and Takashima (2006). On the other hand, over the time course of one month, Bosshardt et al. (2005) found an increase in their subjects' hippocampal activity that was related to the retrieval of word pair associates. These findings, however, only apply to subjects which Bosshardt and colleagues categorized as "good learners". Thus, these results only give conclusions about a certain part of the general public.

In the present study, a conjunction analysis was performed in order to find the brain areas mutually involved in all recalls tested. Not differentiating between sleep and sleep deprivation, FWE corrected brain activity common to both types of episodic memory was the precuneus. Common to all fMRI retrievals was the visualization of associated objects and the process of free recall of declarative information. Similarly, the precuneus has been found active during memory retrieval and imagery (Cavanna and Trimble, 2006). Thus, it can be assumed that a possible reorganization of cerebral traces due to memory consolidation over the course of time does not mitigate the involvement of the precuneus.

Behavioral results of the learning session demonstrated no difference in difficulty between the autobiographical and spatial tasks. Recall results showed no difference between sleep and sleep deprivation groups. Interestingly, after four days, subjects remembered as many of the associations

as on the day they had been freshly learned. Similarly, in Takashima's study (Takashima et al., 2006) involving picture recognition, no significant worsening in memory performance was found between the first and second day of testing (for recent or remote items). According to Ebbinghaus (1983) the average percentage of remembered items is estimated to be below 40 percent, already after twenty-four hours. Thus the lack of significant forgetting in the present study can be explained by the overlearning that occurred. The strict learning procedure chosen in this study (see materials and methods) was necessary in order to assure enough events for proper fMRI analysis. Furthermore, the three runs of word recall for each task in the MRT most likely enhanced the overlearning.

Overall, in the present study, temporal lobe independence during free recall of episodic information after sleep or sleep deprivation cannot be ruled out, neither after a period of three days nor after two and a half months. However, it seems plausible that declarative memory consolidation underlies a covert process, enabling the brain to compensate possible consolidation deficits from sleep deprivation, leading to no insufficiencies in memory performance during recall. Relating to the findings of this study, sleep deprivation effects have been found to be less severe on cognitive performance than on fatigue and mood states (Pilcher and Huffcutt, 1996). The immediate effect of sleep deprivation on cognitive performance is reported in study 5 of this thesis, in which a memory test was performed immediately after sleep deprivation. Since only free recall could be tested in the present study, it is possible that behavioral results were influence by the type of recall. Thus, whether behavioral improvements after sleep depend on the type of retrieval test or whether the brain compensates sleep deprivation in a covert process, leading to no effects on the behavioral level is scrutinized in further behavioral studies.

3 Study 2 – The Contribution of the Hippocampus to the Recall of Autobiographical, but not Spatial Memory

3.1 Study 2 – Introduction

Human subjects can learn declarative information using different mnemonic strategies. However, it is unclear whether all forms of declarative memory show a similar dependence on the hippocampus, that is the *Cornu Ammonis* fields, dentate gyrus and subiculum (Goodrich-Hunsaker et al., 2009). In study 1 no sleep related changes in hippocampal activation could be concluded from the neuroimaging results. It is now important to confront the different views on the role of the hippocampus. It has been argued that the hippocampus is crucial for spatial information processing and storage (O'Keefe and Nadel, 1978), remembering object relations (Cohen, 1993) and autobiographical[14] memory (Nadel and Moscovitch, 1997). Acquisition and initial storage of declarative memory are also thought to draw on this structure (Squire, 1986). Thus, different theories about the function of the hippocampus exist, yet no unified conclusion has been found.

It remains unclear which declarative mnemonic processes involve the hippocampus and which function independently at the retrieval stage (Figure 4). The following paragraph summarizes the most important theories for the role of the hippocampus: From animal studies, the hippocampus is well known to serve as a cognitive map (O'Keefe and Nadel, 1978), providing the brain with a spatial reference (O'Keefe and Dostrovsky, 1971). Lesion studies show that the hippocampus is necessary for allocentric processing, in which animals make use of relative positions of distal cues in order to navigate e.g. in the Morris water maze (Aggleton and Pearce, 2001; Martin et al., 2005; Schenk and Morris, 1985). A homologue to place cells (O'Keefe and Dostrovsky, 1971), neurons found in the hippocampus which show preferential firing when an animal has reached a specific location in the environment, has also been detected in the human hippocampus: Intracranial EEG recordings from patients with temporal lobe epilepsy indicate the existence of place- and view-responsive neurons in the hippocampus and the parahippocampal region (Ekstrom et al., 2003). Further virtual navigation experiments suggest that spatial navigation becomes independent of the hippocampus after repeated practice and dependent on the striatum (P. Orban et al., 2006). This shift from so-called place to stimulus-response navigation has been found in studies on rodents (Q. Chang and Gold, 2003; Packard and McGaugh, 1996).

Autobiographical memory is severely affected by hippocampal lesions, as studies on the topic of retrograde amnesia reveal: In some patients the duration of retrograde amnesia affects memories throughout the entire lifetime (Nadel and Moscovitch, 1997). More recent evidence has evoked the

[14] Autobiographical memory is the memory of personally experienced events (Nadel and Moscovitch, 1997) .

theory that the hippocampus is important for the retrieval of episodic memories and is also active as long as the vivid and detailed parts of the memory trace are required (Moscovitch et al., 2006). As the vividness of recently acquired information fades over time, so does the involvement of the hippocampus during recall of this information. Hippocampal activity has been found to decrease with increasing remoteness of the information retrieved (Gilboa et al., 2004).

Besides the theories of the hippocampus acting in the retrieval process of spatial and episodic memory, several scientific groups attribute the function of the hippocampus to associations, linking neuronal activation from several different modalities (M. W. Brown and Aggleton, 2001; Eichenbaum et al., 1996; Kesner et al., 2000). Goodrich-Hunsaker and colleagues (2009) analyzed association tasks in amnesic patients with hippocampal damage and concluded that the hippocampus supports odor-place associations. Furthermore, it has been reported that hippocampal lesions in humans and animals impair object-place associations (Crane and Milner, 2005; Gilbert et al., 2008; Jo and Lee, 2010; Rolls et al., 2005). In their article, Crane and Milner (2005) see spatial learning as a sub-type of episodic learning. In everyday life it is important to remember where objects have been placed, thus associating objects to spatial locations. Authors investigated patients after having had unilateral temporal-lobe surgery, i.e. selective amygdalo-hippocampectomy and unilateral anterior temporal lobectomy. Selective amygdalo-hippocampectomy meant the removal of the amygdaloid nuclei, hippocampal formation and the parahippocampal gyrus in the left or right hemisphere. Unilateral anterior temporal lobectomy meant removal of most of the amygdala, varying amounts of the hippocampal formation, the parahippocampal gyrus, and the lateral neocortex. In a delayed recall task (the locations of figures on a board had to be memorized and reconstructed), authors found that both groups of patients performed worse across multiple trials than healthy volunteers. However, this performance deficit was found only in patients with lesions in the right hemisphere. Patients with lesions in the left hemisphere were spared. Furthermore, in patients having received unilateral anterior temporal lobectomy, this impairment was only found when the extent of hippocampal excision was large (at least 1.5 cm of hippocampus had been removed). From the study it was concluded that the hippocampus is necessary for building object-location representations in order to be able to recall spatial arrangements after a delay (here: four minutes). This function was specifically attributed to the right hemisphere.

Memory consolidation is the conversion of recently acquired information from a fragile state into permanent storage (Diekelmann et al., 2009). According to the standard model of systems-level consolidation (Squire, 1986), the hippocampus plays an active role in the encoding and retrieval of new declarative memories, such as routes, events and word lists, which are then integrated into neocortical networks. This classical theory considers consolidation to be a slow process, in which cortical networks are gradually strengthened over time (Alvarez and Squire, 1994; Marr, 1970;

McGaugh, 2000; Takashima et al., 2006). It has been proposed that the time until declarative memory becomes independent from the hippocampus ranges between weeks and years (McClelland et al., 1995; Norman and O'Reilly, 2003; P. Orban et al., 2006; Rudy and O'Reilly, 2001). More recently published findings challenge the view of the standard model, inspiring the theory of rapid consolidation (Sharon et al., 2011). The study by Sharon et al. is based on a process called fast mapping, introduced by R.W. Brown (1957) in which novel linguistic information is quickly acquired by embedding it into an existing semantic[15] context. According to the multiple trace theory (Moscovitch et al., 2006; Winocur et al., 2010), spatial as well as autobiographical memories stay dependent on temporal lobe structures instead of consolidating in the sense of the standard model of systems-level consolidation.

The hippocampus is involved in declarative memory retrieval (Hoscheidt et al., 2010). Yet, there are numerous theories about the function of this brain structure. The aim of study 2 is to examine hippocampal involvement in three different forms of episodic declarative memory, testing memory retrieval of an autobiographical, a spatial and a sequential memory task. In study 2 the same free recall paradigm and functional magnetic resonance imaging technique as in study 1 are applied. The first two tasks of study 2 are the same as in study 1, the third task (sequential task) is new in study 2 and serves as a control. In contrast to study 1, the effect of sleep versus wakefulness on memory retrieval is not included in study 2, in order to keep the factors influencing results to a minimum. As a reminder, episodic memory is categorized as declarative memory (Figure 1). When remembering an episode, a person retrieves the specific context in terms of space and time, thus is mentally present at the event. Remembering an episode can even be considered as a mental snapshot (Tulving, 1983). This study serves to clarify whether the hippocampus is involved in all or only specific types of episodic declarative memory.

[15] Detailed information see Appendix

3.2 Study 2 – Materials and Methods

The ethics committee of the department of psychology of the LMU approved this study, which was conducted in accordance with the principles of the Declaration of Helsinki. Prior to the study, experimenters informed subjects about the procedures and possible risks, but not about the hypothesis of the study.

3.2.1 Subjects

Forty healthy subjects gave informed written consent of participation for monetary compensation. All subjects were native German speakers, non-smokers and had normal or corrected-to-normal vision. Right-handedness had been determined using the Oldfield (Oldfield, 1971) handedness questionnaire. Six weeks prior to the study, subjects did not engage in activities disrupting the regular sleep cycle, such as transmeridian travel, shift- or night-time work. On the day of participation subjects abstained from the intake of alcohol and caffeine. Data of 3 subjects were excluded before analysis due to subjects' intake of psychoactive drugs (two subjects) and anatomical abnormalities (one subject). The remaining 37 subjects (21 female, 15 male, mean age ± S.D.: 24 ± 3 years) were free of medication influencing the central nervous system.

3.2.2 Learning Materials

Forty words were selected from the CELEX lexical database created by the Instituut voor Nederlandse Lexicografie (INL) and the Max-Planck-Institute, Nijmegen (Baayen et al., 1995). All words were mono- or bisyllabic, highly imaginable and concrete German nouns, such as "Schwan" (English: swan). In order for maximal comparison of results, all subjects learned the same 40 words (see Appendix). Words were presented on a TFT-LCD screen with a diagonal of 15 ½ inches and presented in white letters of the font Arial and size 45, on a black background using Matlab (version R2010, The MathWorks, Inc., USA) and the Cogent 2000 toolbox (UCL, UK). Subjects sat at a comfortable reading distance of approximately half a meter from the screen. Throughout the learning session, subjects were seated in a quiet room at the University Clinic Grosshadern.

3.2.3 Procedure

The experimenter gave verbal instructions, and subjects read written instructions before starting the learning phase. Subjects learned forty concrete nouns, either by associating each word with a place (spatial task), a personally experienced event (autobiographical task), or the next word on the list (sequential task). Each subject was assigned to one of three groups according to the association strategy, staying unaware of the existence of the other association strategies. Of the 37 subjects, 14 learned the spatial task, 12 the autobiographical task and 11 the sequential task.

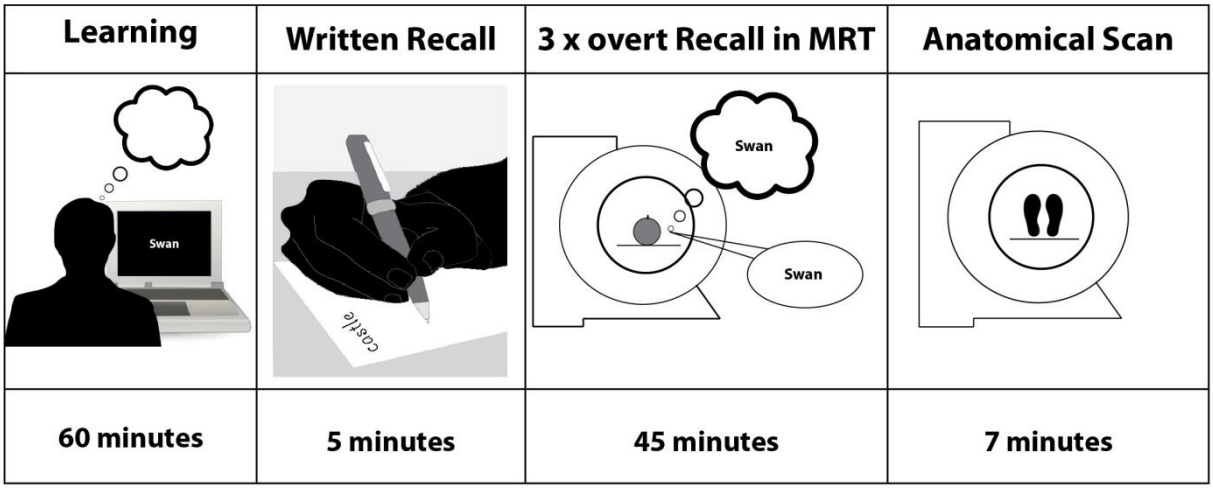


Figure 20: Study 2 – Study design
Learning: Subjects learned an episodic memory task. See section 3.2.3.1 for more details. Written recall: Subjects were tested immediately after learning in a written free recall procedure. See section 3.2.3.2 for more details. (A break of about half an hour followed.) 3x overt Recall in MRT: In three consecutive runs, subjects were tested on the task through an overt recall procedure; see section 3.2.3.3. Furthermore, functional magnetic resonance images of the brain were acquired and subjects' recall performances (behavioral data) were measured using an optical microphone (see section 3.2.3.4). Anatomical scan: anatomical images of the brain were taken to improve fMRI analysis (see 3.2.3.5).

The rate of word presentation depended on the task and presentation round. For the first round of word presentation, the time each word was presented was as follows: Seven seconds in the spatial task, 20 seconds in the autobiographical task, and 5 seconds in the sequential task. For all other rounds of presentation (repetitions), the time of presentation was 5 seconds. The interstimulus-interval was 0.5 seconds. The order of word presentation stayed constant in the spatial and sequential task throughout all repetitions, but was randomized in the autobiographical task. Subjects who participated in the spatial or sequential tasks learned the same order of words.

3.2.3.1 Learning Tasks

3.2.3.1.1 Sequential Task

The task here was to associate each word with the next word of the list, so that the first word served as the cue for the second word, which served as a cue for the third word, etc. The order of the words served as cues and subjects formed a mental "chain of words" (Figure 21). For the duration of word presentation (see section Learning Material) subjects repeated the words (rote repetition) by whispering them. Subjects were asked to repeat words as quickly as possible for avoidance of other associations and thus more strategy-specific memorization.

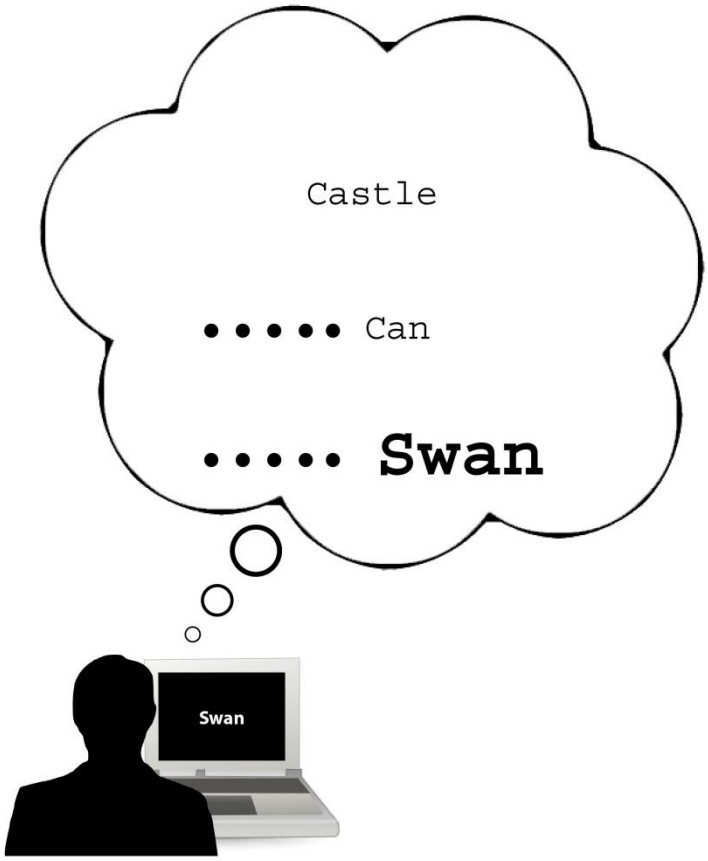


Figure 21: Study 2 – Method – Sequential task
Subjects formed a sequence of words by associating each word (presented on a monitor in front of them) with the next word that appeared on the monitor. As long as the word was presented, subjects repeated the word verbally as quickly as possible.

3.2.3.1.2 Spatial Task

Together with the experimenter the subjects chose a well known route (e.g. the way from home to university) which they had taken (walked, jogged or biked) numerous times in their life. Subjects were asked to mentally take the route and point out forty salient locations along the route (e.g. the door of the subject's house, mailbox, etc.). Once subjects could clearly re-locate the forty locations in their mind they verbally repeated the route with its forty locations twice to the experimenter in order to assure remembrance of the route. Thereafter, subjects were shown a list of forty words, one-by-one on a monitor. Each word had to be visualized as an object and be mentally placed at the location of the route, so that each location could be associated with a word. The first word of the list was placed at the first location of the route, the second word with the second location, etc. (Figure 22).

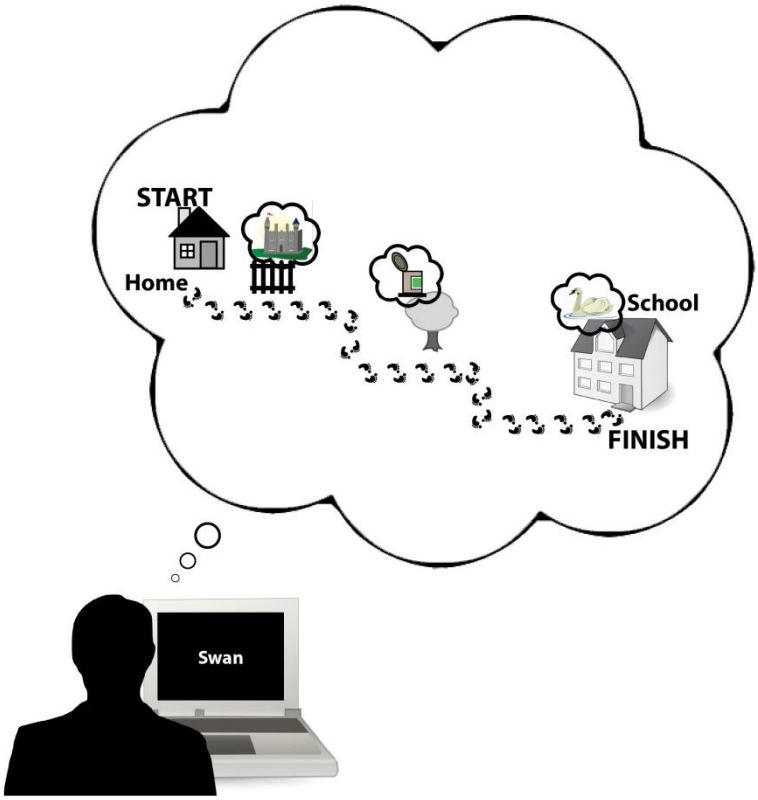


Figure 22: Study 2 – Method – Spatial task
Subjects first chose a well-known and often used route and picked forty locations on the route. In this example, the subject chose the route from his home to his school. Subjects then associated each word (presented on the monitor in front of them) with a location of a well known route. Each presented word was visualized as an object and mentally placed at a location of the route. The first presented word needed to be associated with the first location of the route, the second word with the second location of the route, etc. and the last presented word was visually associated with the last location of the route. In this example, the last presented word is swan. The subject mentally places a swan at the last location of his route, which is his school building. In this way, forty words were associated with forty spatial locations.

3.2.3.1.3 Autobiographical Task

Subjects had to associate each word shown on a monitor with a short personally experienced event. Events could be of any age (from childhood to presence) as long as subjects could imagine the experienced event vividly and mentally re-live it. For each word subjects needed to choose a different event. Subjects were informed that they would later have to describe the events. As in the spatial task, subjects were encouraged to visualize the words as objects. Subjects were encouraged to choose unique rather than every-day events (Figure 23).

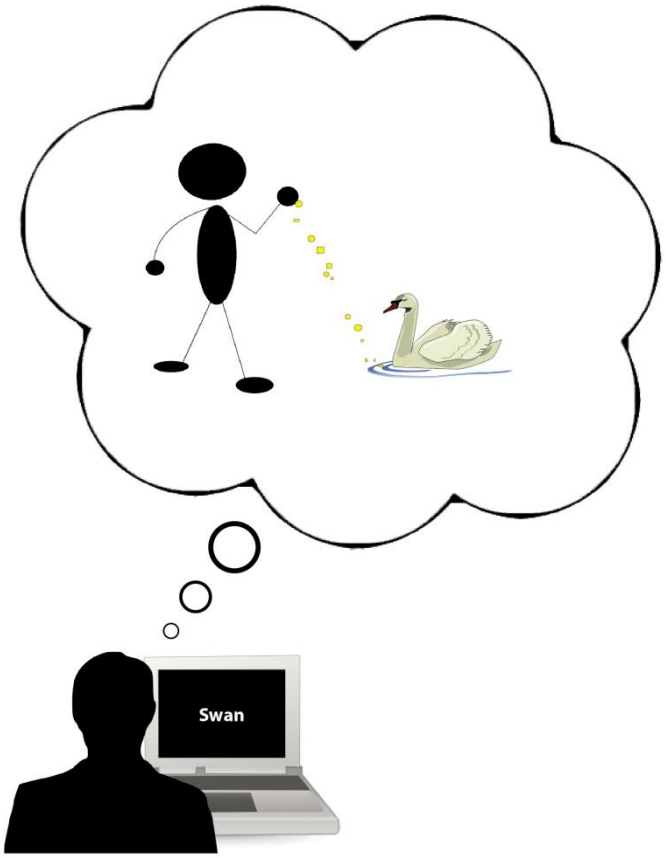


Figure 23: Study 2 – Method – Autobiographical task
Subjects associated each word (presented on the monitor in front of them) with a unique real-life situation that occurred in the past and involved the subject. In this example, the subject is presented the word "swan". He thinks of and visualizes a situation involving him and a swan, in this case, the subject fed the swan. (Note: This graphic is drawn much simpler than the detailed situations remembered by subjects.)

3.2.3.2 Immediate Recall

The experimenter instructed the subjects to apply the learned association strategy throughout the experiment, even if the strategy is not the easiest form they would normally use to memorize a word list. In each task, after having associated the entire list of 40 words with a location / event / the next word on the list, subjects were tested on the words using a written free recall procedure (immediate recall). There was no time limit. The experimenter evaluated the test immediately. If subjects did not recall at least 90% (learning criterion) of the words, word presentation was repeated (see section Learning Materials for time of word presentation). Once subjects reached the learning criterion, they proceeded to the recall in the MRT.

3.2.3.3 MRT Recall

Approximately 30 minutes after having reached the learning criterion (described in the section Immediate Recall), subjects overtly recalled the 40 words or as many as they could remember while in the MRT. Beforehand, the experimenter gave written as well as verbal instructions to the subjects about the MRT recall procedure. A scanning session consisted of three events: Remember, speak and a baseline task. In the MRT, the instructional word “remember” appeared on a mirror back projection screen (see section MRT Acquisition Protocol). Here, subjects needed to remember the association. For example, for the first word learned in the spatial task, subjects thought about the first location, visualized it together with the learned word (also visualized as an object) which they had learned to associate with the location. The duration of at least three seconds was given for the process of remembering. However, subjects could take more time if necessary. Subjects signaled successful remembering by pressing a button (see section MRT Acquisition Protocol), whereupon they saw the instructional word “speak”. Here, subjects spoke the word they had learned. Overt recall allowed for the verification of correct remembering and better analysis of behavioral data. The events “remember” and “speak” were repeated for 10 words. Thereafter, the baseline task, an odd-even judgment task had to be completed. Here subjects saw numbers between one and eight and had two seconds to decide (by button press) whether the presented number was odd or even. This task was used because it has been proven to suppress hippocampal activity (Stark and Squire, 2001). The odd-even judgment task lasted for 20 seconds, then subjects continued with remembering and speaking of 10 words until another odd-even judgment task occurred, etc. Once subjects had retrieved all words they could remember, the session was complete. The run was repeated twice, thus each subject engaged in a total of three runs. For the autobiographical task subjects were required to use a different order of words for each run. After scanning, subjects of the autobiographical group described the events with which they had associated each word and reported how long ago the experience was encountered.

3.2.3.4 MRT Acquisition Protocol

Subjects performed the recall in a 3-Tesla whole-body magnetic resonance tomography (GE Signa HDx, General Electric Healthcare, Milwaukee, Wisconsin, USA) at the neuroradiology department of Prof. Brueckmann, University Clinic Grosshadern. Lying in a supine position in the center of the magnet with the head positioned in a standard eight channel head volume coil, subjects viewed stimuli, such as instructional text, through a mirror positioned at a 45 degree angle (image resolution: 1024 x 768 pixels, FOV: 25° horizontal; 19° vertical). Stimuli were presented to subjects using a mirror back projection technique: An LCD beamer (Christie LX40, Christie Digital Systems, Mönchengladbach, Germany) projected to a semi-transparent back-projected screen (positioned behind the head coil) via three front surface mirrors from a wave-guide through the Faraday cage. Magnetic resonance – compatible optical microphone with preamplifier (Sennheiser MO 2000, Hannover, Germany) connected to a USB audio interface, E-MU, Dublin, Ireland, was used to record the spoken words. Auditory data was recorded with Matlab (The MathWorks Inc., USA). For button presses in the MRT subjects used an MR compatible response device (Lumina LP-400 Cedrus Corp., San Pedro, CA, USA).

Functional images were acquired using a gradient echoplanar imaging (EPI) T2*-weighted sequence. Further parameters: Repetition time (TR): 2.616 s; time echo (TE): 40 ms; flip angle: 90°; matrix: 96 x 96, field of view (FOV): 220 mm x 220 mm. A volume contained 34 contiguous transverse slices. The cerebellum was not entirely covered. Slice thickness was 3.5 mm. Anatomical images were taken after completing the third run in the MRT. For this, a 3D fast spoiled gradient echo recalled (FSPGR) sequence with a voxel size of 0.86 mm x 0.86mm and a slice thickness of 1.4 mm (matrix: 256 x 256, field of view: 220 mm x 220 mm) with 0.7 mm oversampling in the z-direction was chosen. Acquisition of functional images took approximately 45 minutes (depending on the subject's recall abilities) and anatomical images were taken in seven minutes.

3.2.3.5 fMRI Data Analysis

The first five functional images of each run (dummy scans) were not analyzed due to possible effects of spin saturation. Furthermore, the images recorded for the last 2 words said by the subject at the end of each run were discarded due to increasing variance in response time towards the end of a run. For all other functional and structural (or anatomical) images the anterior commissure was chosen as the origin. Images were preprocessed in five steps (realignment, coregistration, segmentation, normalization, smoothing) using the Statistical Parametric Mapping toolbox (SPM5 UCL, UK) for Matlab. Since a subject disrupts the magnetic field, rendering it inhomogeneous, spatial disorientations occur in the images. In order to account for movement artifacts, all functional images were aligned to the mean functional image. A coregistration of structural images to the mean EPI

image was performed. Using the subject's own anatomy, this coregistration procedure allowed for a better identification of activations in the functional image. Warps derived from a higher resolution (anatomical image), allowing a more precise spatial image for the later normalization. Anatomical images were then segmented into white and gray matter. Segmentation parameters were used for normalizing of functional images to the MNI standard coordinate template echo planar image (Evans et al., 1993). To improve the signal-to-noise ratio and reduce false positive results, functional images were smoothed with an 8 mm full-width at half maximum (FWHM) isotropic Gaussian kernel. Low-frequency noise and slow signal drifts with a period longer than 128 seconds were removed using the standard high-pass filter (Ashburner et al., 2007).

First-and second level analyses were performed using SPM 8 (UCL, UK). The general linear model (GLM) was applied to generate parametric maps for single subject (first level) statistical analysis. The design matrix, defining the experimental design, contained three regressors: correct word recall, speech and key press. Regressors were modeled as events and convolved with the HRF impulse response. Six further parameters for movement and one constant (resulting from the mean activity at all time points at MRT recall) were included in the design matrix. In order to make inferences for the population from which the subjects for this study were drawn, a random-effects analysis (RFX) was made. For the second level model of this study, each subject's contrast images that corresponded to the parameter estimates for correct word recall were taken as summary measure. The one-factorial second level model comprised three levels, one for each learning task. T-tests were performed on contrast images from the single subject level in order to analyze differences between groups. A conjunction analysis, applying the conjunction null (Nichols et al., 2005), was performed to detect mutual activity between the three groups. Making use of the time course of the average response to a single event (correct recall) in a given voxel over the entire experiment, percent signal change values for the hippocampus compared to the entire brain were received. On the single subject level, parameter estimates for every voxel of the hippocampus at all times of the event correct recall had been made, averaged and compared to the global mean. In addition, the extraction of signal from a specified region of interest (ROI), the hippocampus, was performed. The SPM 8 Anatomy Toolbox, part of Matlab (version R2010, The MathWorks, Inc.) was used to find areas of activity and the tables in the results section list coordinates in MNI space unless otherwise stated. For the ROI analysis, the region defined as the hippocampus in the anatomical automatic labeling (AAL) toolbox (Tzourio-Mazoyer et al., 2002) was chosen with the WFU (Wake Forest University) PickAtlas, version 2.4 (Maldjian et al., 2003). Unless otherwise reported, results are based on voxels with a p-value below 0.05 with family wise error (FWE) correction. Bayesian inferences about effects specific to the hippocampus were made with the help of a posterior probability map. The posterior probability (p) equation is described by Friston and Penny (2003, p. 1241 equation 3). The equation

includes the variable γ, which is the threshold in standardized units and was set to 2.2 for this study. Posterior probabilities in the hippocampus reported here have a confidence of 99% if not otherwise stated.

SPM contains some limitations. SPM uses classical inferences. The p-value reflects the probability of receiving the observed data in the absence of an effect. If the p-value is below a certain threshold, the null hypothesis is rejected. In the classical approach, the goal is to keep the probability of incorrect rejection of the null hypothesis lowest. However, inferences over large brain volumes cannot be made with the classical approach, producing the problem of multiple comparisons (Friston and Penny, 2003). Since thousands of scans enter fMRI analysis, the degrees of freedom are high, easily making small activations statistically significant. In contrast to the classical approach, the Bayesian analysis is more sensitive in regard to large volumes (for more details see Friston et al. (2002)). In study 2, Bayesian estimations, which rely on posterior analyses, were completed for second level models.

3.2.3.6 Behavioral Analysis

Sound files containing verbal responses recorded during MRT recall were extracted using Matlab (version R2010, The MathWorks, Inc.), noise was removed with Audacity (version 1.2.6, SourceForge.net), and statistically analyzed using Microsoft Excel 2007 and IBM SPSS Statistics (version 17.0). Experimenters evaluated verbal responses of each subject and categorized them into incorrect and correct. Unless otherwise stated, statistical analyses were performed with the number of correct words recalled in the last run in the MRT, which was the third run. For each of the three tasks, the number of learning repetitions (logged during the learning sessions with Matlab, the MathWorks, Inc., version 17.0), percentage of correctly recalled words and the average time needed to recall words were calculated. If subjects chose every-day events as word-associations in the autobiographical task, words were excluded from analysis. Between group statistics were conducted applying pair-wise Tukey HSD post-hoc tests and ANOVAs.

3.2.3.7 An analysis of age of autobiographical memory

On the single subject level a correlation analysis served to investigate the existence of age related autobiographical memory activity. The age (in days) of each word recalled during the third run had been reported by subjects immediately following scanning. An additional first level model was created, containing the age for the corresponding events, which were parametrically modulated. A between group analysis was run to find correlations between voxels and the age of events.

3.3 Study 2 – Results

The null hypothesis for the neuroimaging analysis of this study states that the hippocampus is activated during the recall in each of the three learning tasks.

3.3.1 Results of Neuroimaging

The results of this study disprove the null hypothesis, showing that hippocampal activity was found only during recall of the autobiographical task, but not during recall of the spatial or sequential tasks.

3.3.1.1 Conjunction Analysis

A conjunction analysis was performed to see common activation during the recalls of all three learning tasks (Figure 24): The region found commonly and bilaterally active in all three tasks (spatial, autobiographical *and* sequential) was the posterior precuneus (Brodmann area, BA 6, $p<0.001$ left hemisphere; $p<0.05$ right hemisphere), extending into the superior parietal lobule (SPL). The middle occipital gyrus (BA 19) was found significantly active only in the left hemisphere ($p< 0.05$).

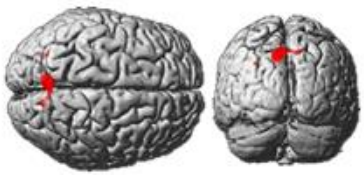


Figure 24: Study 2 – Functional results – Common activation during the recalls of all three learning tasks: Precuneus
The precuneus is shown in red.

3.3.1.2 Analysis of Individual Tasks

Scrutinizing the results of each group individually, the activation pattern in the sequential task was very similar to the results of the conjunction analysis. No other brain region besides the precuneus and the middle occipital gyrus was activated above the threshold of statistical significance.

Table 6: Study 2 – Functional results – Sequential task: Brain activation during MRT recall
Coordinates are reported according to the MNI space. All activations had a statistical p-value below 0.05 and were FWE-corrected. BA = Brodmann area.

BA of activated brain region	Activated brain region	Hemisphere (R = right; L = left)	Coordinates (x,y,z) in mm	Z-score (voxel level)	Cluster size (number of voxels)
7	Precuneus	L	-10, -74, 42	4.79	226
7	Precuneus	R	0, -70, 48	4.75	
7	Precuneus	R	12, -72, 48	4.34	84
19	Middle occipital gyrus	L	-30, -68, 38	4.56	41
19	Middle occipital gyrus	R	20, -74, 48	4.59	84

The brain areas activated during recall in the spatial task included the areas activated for recall of the sequential task (precuneus and middle occipital gyrus). In addition, the retrosplenial cortex (BA 30) was significantly activated in both hemispheres. Regions of activity for recall of the spatial task are as follows (Table 7).

Table 7: Study 2 – Functional results – Spatial task: Activation during MRT recall
Coordinates are reported according to the MNI space. All activations had a statistical p-value below 0.05 and were FWE-corrected. BA = Brodmann area.

BA of activated brain region	Activated brain region	Hemisphere (R = right; L = left)	Coordinates (x,y,z) in mm	Z-score (voxel level)	Cluster size (number of voxels)
7	Precuneus	L	-6, -66, 46	5.17	365
7	Precuneus	L	-10, -70, 52	4.87	
7	Precuneus	R	10, -66, 52	4.93	
19	Middle occipital gyrus	L	-34, -78, 32	5.37	208
19	Middle occipital gyrus	R	40, -78, 28	5.17	95
30	Retrosplenial cortex	L	-16, -58, 16	5.32	139
30	Retrosplenial cortex	R	14, -54, 16	4.80	60

Brain areas activated in the recall of the autobiographical task included all areas activated in the recall of the spatial task (precuneus, middle occipital gyrus and retrosplenial cortex). In contrast to the other two tasks, the hippocampus was activated in the recall of the autobiographical task. Regions activated in the recall of the autobiographical task are depicted in Figure 25.

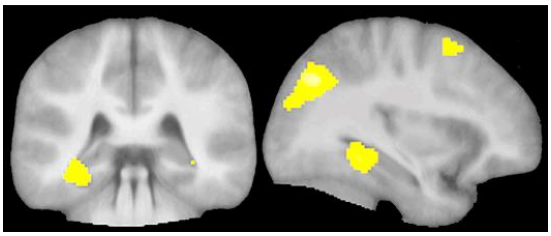


Figure 25: Study 2 – Functional results – Activation in the autobiographical task
Areas of activation see Table 8.

Table 8: Study 2 – Functional results – Autobiographical task: Activation during MRT recall
Coordinates are reported according to the MNI space. All activations had a statistical p-value below 0.05 and were FWE - corrected. BA = Brodmann area.

BA of activated brain region	Activated brain region	Hemisphere (R = right; L = left)	Coordinates (x,y,z) in mm	Z-score (voxel level)	Cluster size (number of voxels)
7	Precuneus	L	-6, -68, 40	6.33	2620
7	Precuneus	R	10, -72, 44	5.79	
19	Middle occipital gyrus	L	-32, -70, 38	5.75	395
19	Middle occipital gyrus	L	-34, -82, 24	5.22	
19	Middle occipital gyrus	R	34, -66, 34	5.09	395
19	Middle occipital gyrus	R	40, -76, 26	5.04	
30	Retrosplenial cortex	R	10, -54, 6	5.71	
	Caudate nucleus	L	-20, -24, 26	5.97	124
37	Fusiform gyrus	L	-34, -44, -8	5.49	269
18	Lingual gyrus	L	-2, -90, -10	4.59	
18	Lingual gyrus	R	8, -90, -10	4.90	329
	Cerebellum	L	-8, -84, -16	5.01	100
	Cerebellum	R	18, -80, -16	4.98	329
	Parahippocampal gyrus; probability for hippocampus: 80%	R	34, -42, -2	4.86	28
23	Posterior cingulated gyrus	L	0, -30, 24	4.35	10
6	Middle frontal gyrus	L	-30, 18, 58	5.03	152
9	Dorsolateral prefrontal cortex	L	-50, 26, 30	4.62	14

An analysis of the region of interest, the hippocampus, shows that during recall of the autobiographical task, the hippocampus was activated (Figure 26).

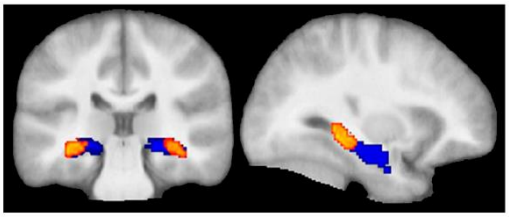
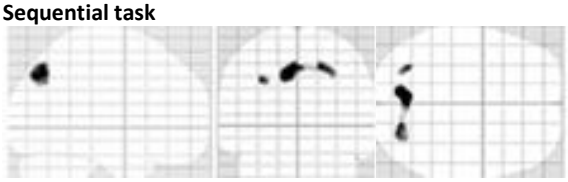


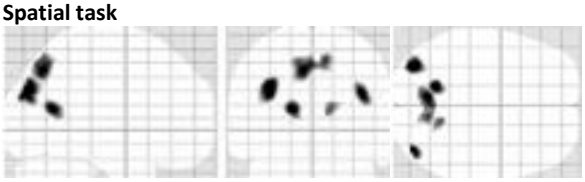
Figure 26: Study 2 – Functional results - Region of interest analysis fort the autobiographical task.
Yellow/red: Results of the region of interest analysis for the autobiographical task; blue: region of interest (hippocampus).

Overall, the areas of activation increase from sequential to spatial and to the autobiographical task (Figure 27).

Sequential task



Spatial task



Autobiographical task

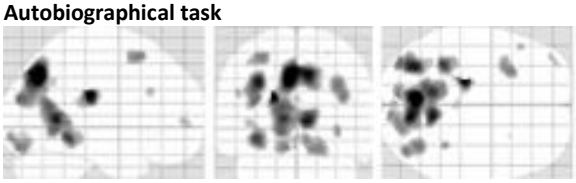


Figure 27: Study 2 – Functional results – Glass brains depicting areas of significant activity in each of the three tasks Areas of activity increase from the sequential to the spatial task, and also from the spatial to the autobiographical task. Views of graphics in each square: Sagittal (left), coronal (middle), axial (right).

Moreover, Figure 27 shows that the areas of activation of the sequential task are a subset of the areas of activation of the autobiographical task. In turn, the areas of activation of the sequential task are a subset of the areas of activation of the other two tasks.

Analysis on the group level resulted in significant differences between tasks. In the autobiographical task the left hemisphere of the hippocampus and the lingual gyrus (BA 19) were significantly activated in comparison to the spatial task ($p = 0.015$, $T = 5.47$; $p = 0.029$, $T = 5.21$ respectively). Comparing the autobiographical and sequential tasks, the fusiform gyrus (BA 37) was found to be significantly more active in the autobiographical task ($p = 0.026$; $T = 5.25$), see Figure 28.

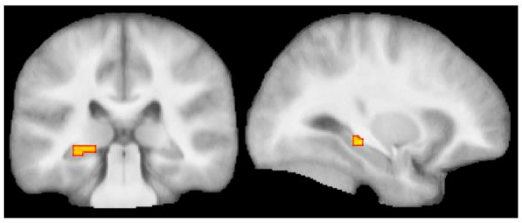


Figure 28: Study 2 – Functional results – Contrast autobiographical task minus sequential task

Selecting the hippocampus as the region of interest, the average percent signal change in this area (Figure 29) was 0.04 % ± 0.16 (mean ± S.E.) in the sequential task, -0.16 % ± 0.22 (mean ± S.E.) in the spatial task, and 0.68 % ± 0.16 (mean ± S.E.) in the autobiographical task.

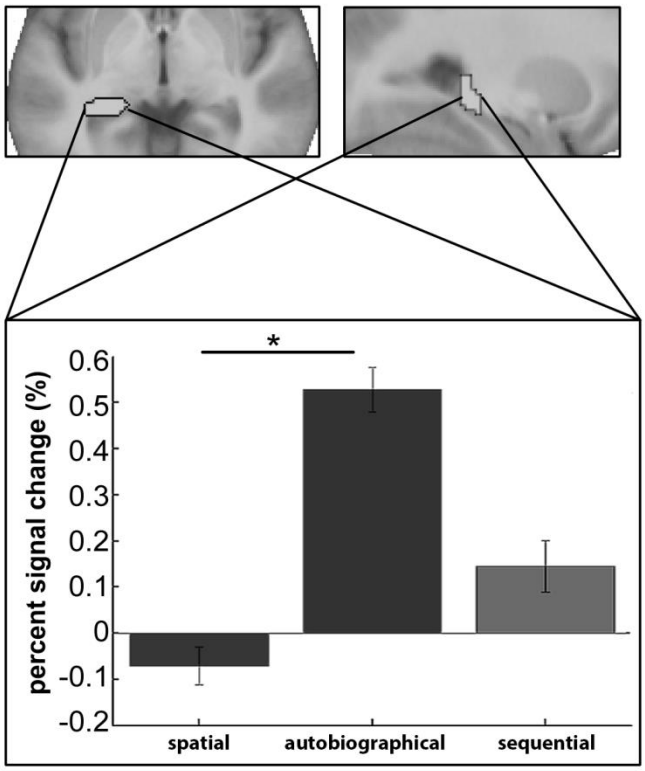


Figure 29: Study 2 – Functional results – Percent signal change
Top: Showing percent signal change in the area of the hippocampus that is activated in the autobiographical task.
Bottom: Corresponding values of percent signal change. All activations were FWE-corrected.

T-tests (assuming equal variances) showed that percent signal change in the autobiographical task is significantly higher compared to the sequential task ($p = 0.01$, $T(21) = 2.08$) and compared to the spatial task ($p = 0.007$, $T(24) = 2.06$). In the autobiographical task, percent signal change in the hippocampus was significantly higher than in the other two tasks (ANOVA, $F(2,34) = 5.36$, $p < 0.01$).

Posterior probability maps (PPM) enabled inferences about regionally specific effects for the hippocampus, in comparison to the global mean. Bayesian estimations ensured the lack of a significant statistical effect in the two tasks, spatial and sequential learning. Posterior probabilities were 0.01 in the sequential and 0.1 in the spatial task. In the autobiographical task however, the probability that an activation occurred in the hippocampus was at 84%. At the uncorrected level (not correcting for family wise errors, FWE), no hippocampal activations were found in the spatial or sequential task.

When investigating levels of difficulty between tasks, fMRI results as reported above did not change when adding a covariate for behavioral recall performance (see section Behavioral Level for behavioral results); the hippocampus was found active in the autobiographical but not in the other two tasks.

3.3.2 Age of Episodes

Analysis between the age of episodes chosen by participants of the autobiographical group and brain activity for the corresponding event did not reveal a significant correlation. When correlating the age of autobiographical events with the haemodynamic response in the hippocampus no relationships with a threshold below a p-value of 0.05 could be found.

3.3.3 Behavioral Level

Subjects in the spatial task had verbally repeated the well known route twice before associating 40 locations on the route with 40 concrete German words that were part of the learning task (pre-association rounds). The number of repetitions needed to reach the learning criterion of 90% (Figure 30) differed significantly between groups (ANOVA, $F(2,34) = 11.01$, $p < 0.001$). The Tukey-HSD (Tukey's honest significance test) post-hoc test revealed that spatial learning (without adding the two pre-association rounds) was significantly faster than sequential learning ($p = 0.0001$) and autobiographical learning was also significantly faster than sequential learning ($p = 0.044$). Approximately 30 minutes after having reached the learning criterion subjects overtly recalled the 40 words in the MRT. Thus, learning was finished approximately 30 minutes prior to MRT recall.

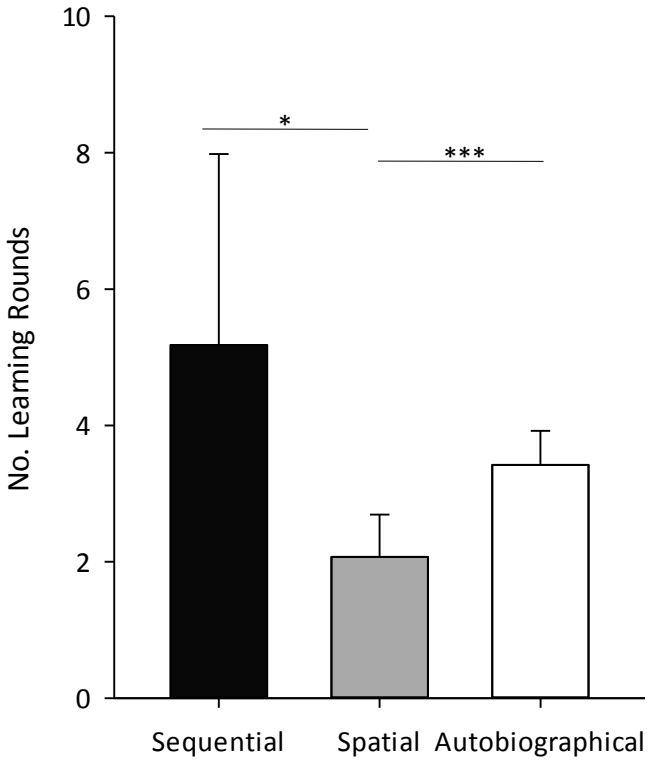


Figure 30: Study 2 – Behavioral results – Number of learning rounds
Number of learning rounds needed in each task to reach the learning criterion of at least 90% or 36 (of 40) correctly recalled words. Words (German mono- or bisyllabic concrete nouns) were recalled in a written free recall procedure. To complete the learning criterion, participants assigned to the sequential task needed 5.09 ± 2.81 (mean ± S.D.) learning rounds; participants in the spatial task needed 2.07 ± 0.62 (mean ± S.D.) and participants in the autobiographical task needed 3.42 ± 0.51 (mean ± S.D.) learning rounds. Number of participants per task: Sequential: 11; Spatial: 14; Autobiographical: 12.

During MRT recall, the percentage of correctly recalled words during MRT recall differed significantly between tasks (Figure 31), when comparing performance to the last of immediate recalls (ANOVA, $F(2,34) = 4.30$, $p = 0.02$). Recall performance in the spatial group was significantly better compared to the autobiographical group ($p = 0.02$, Tukey-HSD). There were no significant differences between the other groups.

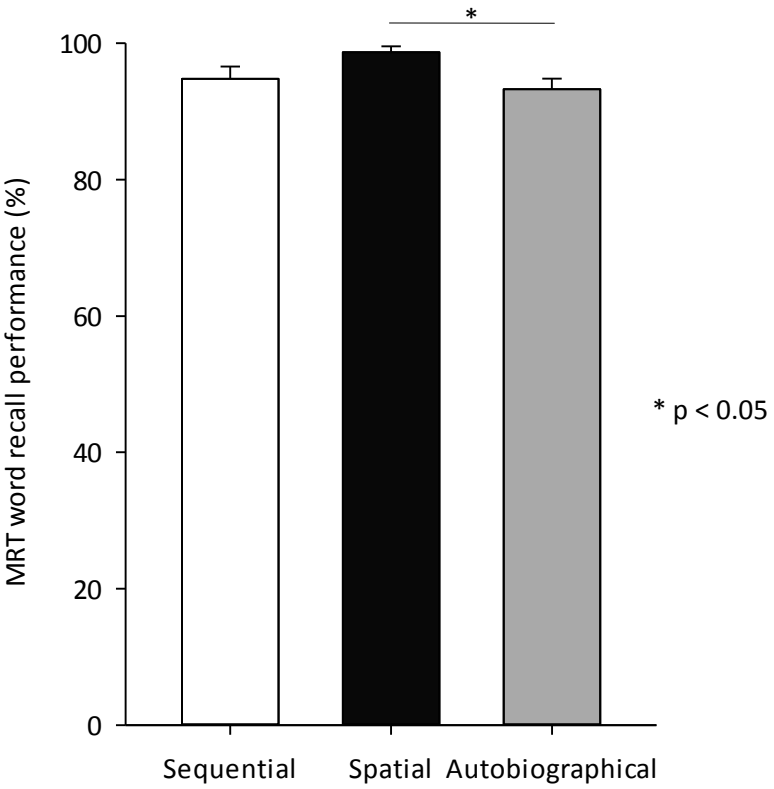


Figure 31: Study 2 – Behavioral results – Recall performance in MRT
Given is the percentage of correctly recalled words during the last run in the MRT compared to performance at the last immediate recall. Correct recall performance was: 94.78 ± 1.81 % in the sequential task, 98.70 ± 0.87 % in the spatial task and 93.28 ± 1.54 % in the autobiographical task. All percentages are mean ± S.E. Sample sizes: Sequential: 11; Spatial: 14; Autobiographical: 12.

The time needed by participants to remember a word association (average of three runs) did not differ between tasks (ANOVA, $F(2, 34) = 2.14$, $p = 0.13$).

3.4 Study 2 – Discussion

Using a between-group design, hippocampal involvement during free recall at an early stage after encoding was compared between sequential, spatial and autobiographical learning strategies. Free recall performance of concrete nouns was measured on the functional as well as behavioral level. As a result not all episodic memory traces depended equally on the hippocampus when information was retrieved in free recall: Whereas autobiographical memory recall relied on the hippocampus after consolidation, spatially and sequentially associated information did not. Functional conjunction analyses showed that brain areas mutually involved in all tasks tested, were: the precuneus (medial parietal cortex), medial occipital gyrus and superior parietal lobe (SPL).

Since the role of the hippocampus is still controversial, this study, using an fMRI design, tested three types of episodic memory that are all said to depend on the hippocampus. Specifically, hippocampal involvement during free recall at an early stage after encoding was compared between sequential, spatial and autobiographical learning strategies. This design presents the advantage of investigating mechanisms of episodic declarative memory processing with minimal cue support through free recall in the form of overt recall (subjects verbalized the answers while in the MRT). In the past, event related overt recall designs had been considered unsuitable for fMRI designs because of movement artifacts during loud speech and uncontrolled latencies in free recall events (Oztekin et al., 2010). This problem was solved in study 2 (and also in study 1) through the use of an optical microphone (see 3.2.3.4). With an optical microphone attached close to the mouth, subjects could speak out the answers they were thinking at a relatively small volume of speech, keeping the movement (e.g. of jaw muscles) to a minimum. The present study is among the first experiments to successfully use free overt recall in combination with an event related paradigm for testing memory processing.

The present study was constructed in such a way that all three learning strategies were identical aside from the type of word association. Common to all three tasks was that each subject implicitly encoded an episodic memory about the learning session. Furthermore, recall in all three tasks involved only simple, concrete nouns. Thus, the conjunction analysis delivered results about the brain areas that were mutually involved in all tasks, without being specific to one of the three types of episodic memories tested. As the results of the conjunction analysis show, free recall of concrete nouns, acquired shortly before, requires the precuneus (medial parietal cortex), medial occipital gyrus and superior parietal lobe (SPL). In brief, the functions of these areas are as follows: The precuneus plays an essential role in the retrieval processes of episodic memory (Cavanna and Trimble, 2006). The occipital lobe processes visual stimuli. When reading, information from the occipital lobe projects to Wernicke's area, which is located in the superior temporal gyrus and is responsible for language comprehension (Fox, 2005). The SPL is involved in visual information processing and motor control (Caminiti et al., 1996).

Importantly, the hippocampus was not found to be active during all but one tasks tested. The hippocampus seems to play a more specific role in declarative memory recall. In study 2, autobiographical memory recall relied on the hippocampus after consolidation whereas spatially and sequentially associated information did not. Other studies showing the lack of hippocampal involvement in episodic memory tasks support this notion (Cavanna and Trimble, 2006). Regions found significantly active in the conjunction analysis support the explicit retrieval of concrete words together with implicit retrieval of the episode in which the material was acquired. Considering the learning situation, many findings support the view that the precuneus plays an essential role in the retrieval processes of episodic memory (Cavanna and Trimble, 2006; Fletcher et al., 1995; Krause et al., 1999; A. D. Wagner et al., 2005). The functions of the precuneus in retrieval processes have been separated from the imagery content of the learned material (Krause et al., 1999): In their episodic retrieval study (recall of pictures and auditory words) Buckner and colleagues saw that the activation of the precuneus did not change with systematically varied imagery contributions. Another area active in all three tasks of the present study was BA 19, the middle occipital gyrus. It can be assumed that the concreteness of words and other visual aspects, such as the processing of graphic representation of text that was involved in the process of the MRT recall in the present study, were supported by the medial occipital gyrus (BA 19). Together with the visual processing area BA18, Brodmann area 19 is part of the peristriate cortex, which processes visual information arriving from the primary striate cortex (Edmond and Foroozan, 2006). When comparing representational to abstract paintings, BA 19 and the precuneus express significant activation (Vartanian and Goel, 2004). In the conjunction analysis of the present study, activity common to all strategies was also found in the superior parietal lobule, a region to which the precuneus projects information (Krause et al., 1999; Pandya and Barnes, 1987). The superior parietal lobule is known to be engaged in allocating attention during perceptional tasks and is linked to top-down processes in retrieval search tasks (Cabeza et al., 2008). Furthermore, the superior parietal lobule is activated in tasks involving words that have been experienced frequently prior to the experiment (Ciaramelli et al., 2008). The 40 nouns learned by all subjects in the present study were words of regular usage.

Regarding the outcome of single strategy analyses, results support the multiple trace theory, established by Nadel and Moscovitch (1997), which states that personally experienced episodes stay hippocampus dependent for a lifetime. Furthermore, Nadel and Moscovitch propose a semantization of information through the process of consolidation: Factual information is detached from the episode in which the information was initially acquired, and stored independently of the hippocampus. This view of episodic consolidation differs from the standard theory of system consolidation (Squire and Zola-Morgan, 1991) which assumes that the hippocampus and related medial temporal lobe structures quickly form a memory trace of the newly linked information. Over a

lengthy process of system consolidation the hippocampus becomes completely independent of the retrieval of the memory traces and will permanently require the neocortex. According to the standard model, retrieval of words associated with autobiographical material would not have activated the hippocampal formation. Thus, results of the present study contradict the theory about hippocampal independence in the retrieval of autobiographical memory traces. To date there have been numerous papers contradicting complete post-consolidation hippocampal independence in declarative memory retrieval.

The hypothesis that the hippocampus is permanently accessed for spatial memory retrieval, which O'Keefe et al. (1978) based on experiments with animals, cannot be supported by results of the present study. In concordance with results of the present study, Rosenbaum et al. (2005; 2000) who conducted spatial tasks on patients with neurological illnesses, support the view that well-learned routes are comparable to semantic memories and do not require the hippocampus for retrieval processes. Despite having lost their vividness and details, such environments are sufficient for proper navigation. In study 2 Bayesian analysis confirmed that recall of words associated with spatial material did not rely upon the hippocampus already half an hour after learning was finished. The same was true for sequentially memorized declarative material. Both the standard and multiple trace theories agree that non-autobiographical declarative memories no longer require the hippocampus after consolidation. Thus, it can be proposed that after a short period of intense learning, word associations had been consolidated thoroughly enough for consolidation to occur. It can thus be inferred that the consolidation which establishes within a few learning rounds, occurs rapidly. Remarkably, the areas activated in the sequential task were a subset of those activated in the spatial task, which were in turn a subset of the areas activated in the autobiographical task. It cannot be concluded that sequential learning was the easiest, spatial learning the intermediate, and autobiographical the most difficult learning strategy since the number of repetitions needed during learning was lowest in the spatial task. The larger amount of areas activated in MRT recall of autobiographical information can also not be related to different levels of difficulty of tasks. Behavioral recall performance between the autobiographical and sequential task were not significantly different. Thus, behavioral recall performance seems not to mirror brain activity.

Overall, findings demonstrate the hippocampus is not involved equally in the recall of all declarative memory. Memory traces for the three types of declarative memory tested in study 2 seem to have consolidated rapidly at an early stage after learning. Supporting the multiple trace theory, autobiographical memory recall relies on the hippocampus after consolidation whereas spatially and sequentially associated information does not. For all types of episodic declarative memory tested, the precuneus, SPL and middle occipital gyrus were found to play a role in free recall.

4 Study 3 – Is the Type of Retrieval crucial for an Effect of Sleep on Declarative Memory?

4.1 Study 3 – Introduction

The majority of the published findings on sleep and memory consolidation support the view that sleep in comparison to wakefulness aids memory retrieval [for a review see (Diekelmann et al., 2009)]. However, there is some controversy regarding the memory enhancing benefit of sleep (R. P. Vertes, 2004; R. P. Vertes and Siegel, 2005). Newly formed memory traces are initially fragile and are said to be stabilized over time through the process of consolidation (Diekelmann and Born, 2010). It remains difficult to pinpoint the factors needed for a reliable benefit from sleep on declarative memory. In study 1, due to the problem of fatigue after a night of sleep deprivation (Durmer and Dinges, 2005), fMRI recall had been performed after two recovery nights, not after the first night. The problem with this design was that the immediate influence that sleep exerted on memory could not be obtained. Thus, possible differences in declarative memory performance between sleep and sleep deprivation groups were not captured.

In the search of the specific set of requirements for a sleep benefit, it is necessary to look at several factors that might have influenced and overshadowed a beneficial effect of sleep, or hindered the beneficial effect of sleep from becoming visible, in study 1. Furthermore, for study 1 several possible confounds exist which could have impeded a beneficial effect of sleep on memory and which shall be improved in study 3: i) The type of retrieval test; ii) Circadian rhythm; iii) The retention period between learning and the first post-conditional test; iv) Interference; v) The learning material. The following sentences summarize the key elements of study 1: i. The type of retrieval used was a free recall; ii. Considering the circadian rhythm, study 1 did not allow for all subjects to learn at the exact same time of day. Sleep did not follow learning directly and also not with the same duration after scanning for each subject; iii) The retention period between learning and the first post-conditional test was three days; iv) An interference list was not learned; v) The type of material consisted of single concrete nouns.

In sum, the following key elements will be used for study 3: i) Cued recall and recognition for the type of retrieval test; ii) Circadian rhythm: Learning either in the morning or in the evening; iii) The retention period between learning and the post-conditional test is kept constant at 12 hours; iv) Interference is used; v) The learning material is restricted to non-sense syllables.

It has been suggested that a positive effect of sleep on memory consolidation occurs when a specific set of requirements is met, although to date, pinpointing the exact requirements has not been possible from past sleep literature (Diekelmann et al., 2009). The type of retrieval, circadian rhythm,

retention period, interference, and the type of material might all contribute to this set of variables influencing the benefit of sleep on memory. The present study cannot test all variables. Thus, the main focus of this study is on the *type* of retrieval test.

Concerning the different types of retrieval tests, free recall (used in study 1) is a common recall measure in experiments testing memory in humans, yet it might not achieve a visible enhancement of a post-sleep memory consolidation. In fact, studies using *cued recall* during retrieval testing seem to be more promising than those using free recall (Barrett and Ekstrand, 1972; Drosopoulos et al., 2007b; Ellenbogen et al., 2006; Fowler et al., 1973; Gais et al., 2007; Gais et al., 2006; Plihal and Born, 1997). In contrast to free recall, in which the learned information needs to be generated without a stimulus, in cued recall, a cue, such as one word of a two-word pair, aids memory recall. Ellenbogen et al. (2006) found that cued recall highly benefited from sleep in comparison to wakefulness. The authors concluded that sleep plays an active role in the consolidation of declarative memory.

Besides free and cued recall, a third test procedure typically used in declarative memory studies is recognition. Recognition is a type of retrieval of information, in which the subject knows that a word was previously learned without requiring recollection additional information about the context in which the information was encountered (Yonelinas, 1994). Dual-process models state that retrieval can be performed by using one of two independent processes, recollection or familiarity (Jacoby, 1991; Tulving, 1985). Whereas recollection pertains to remembering with contextual detail, re-living the episode in which information was acquired, familiarity occurs in the absence of detailed information such as time and space of the previous learning situation. Recognition was chosen as one of the two retrieval types to be tested in study 3, since there are only few reportings in literature using recognition as retrieval method in relation to declarative memory [for a review see Diekelmann et al. (2009)].

Furthermore, Ellenbogen et al. (2006) made use of *interference*. In fact, the authors detected a significant difference between sleep and wake groups only with the use of interference. In declarative memory paradigms using word lists, interference can be a *second* word list learned by subjects after the actual list of words to be memorized. Interference serves to render the memory trace unstable. Ellenbogen et al. (2006) who applied the classic AB-AC interference paradigm (Barnes and Underwood, 1959) and tested word pairs with a cued recall procedure demonstrated that sleep protects memories from “retroactive” interference or negative influence from competing information that is acquired *after* the information that needs to be remembered (Wixted, 2004). Without consolidation, the information is at risk of being overwritten by temporally following information.

Considering the type of material, most sleep studies on declarative memory involving interference test nouns or adjectives. Non-sense syllables have not received attention for several decades although several prominent studies on non-sense syllables found that this material benefits from a night of sleep (Jenkins and Dallenbach, 1924; Nesca and Koulack, 1994; Richardson and Gough, 1963). Already in 1924 Jenkins and Dallenbach discovered that if a period of sleep follows the encoding of such non-imaginable syllables, less forgetting occurred compared to the condition when a period of wakefulness ensued after the encoding phase (Jenkins and Dallenbach, 1924). In study 1 concrete nouns were tested. It is possible that a beneficial effect of sleep is sensible to the type of material. Ebbinghaus (1983) was the first to empirically test the effect of time on learning material and investigated what he categorized as simple material. Such simple material were trigrams, non-sense syllables composed of a consonant-vowel-consonant structure, e.g. “HOS”, which is not a word and therefore does not convey a meaning that could influence the effect of sleep on memory. The possibility to associate the newly learned material with existing memory is kept to a minimum with non-sense syllables. This led to the idea that non-sense syllables be tested in study 3.

If the hippocampus is necessary for associations, and sleep renders hippocampal memories stable, so that improvement becomes visible on the behavioral level, then a sleep benefit involving the hippocampus should be more pronounced in associated learning material, such as pairs, in comparison to single words (Aggleton and Pearce, 2001). Study 3 uses pairs of non-sense syllables. Thus, when using cued recall in study 3 for testing, it can be assumed that this type of retrieval engages the hippocampus. Possibly, a beneficial effect of sleep on memory is recognizable on the behavioral level, when cued recall is used as the type of retrieval due to hippocampal involvement.

Concerning the retention period between learning and testing, literature presents a wide range of *when* a beneficial effect of sleep occurs. Whereas Lahl et al. (2008) suggests that six minutes between learning and testing are sufficient for a beneficial effect of sleep to occur, Richardson and Gough (1963) report an effect after no earlier than six days. The retention period between the two recalls before and after the sleep condition of study 1 reported in this thesis amounts roughly to 72 hours. Due to the fact that most studies reporting a sleep benefit have a shorter retention time (Diekelmann et al., 2011; Tali Gorfine et al., 2007; Nesca and Koulack, 1994), it is necessary to look at the effect of sleep on declarative memory at an earlier time. Grosvenor and Lack (1984) found a positive beneficial effect of sleep after a retention period of four hours but not after six days.

The aim of study 3 is to investigate whether a declarative memory benefit of post learning night-time sleep compared to diurnal wakefulness is revealed after a 12 hour retention period. An important question of this study is whether a sleep benefit depends on the *type* of retrieval test. This behavioral

study focuses on testing pairs of non-sense syllables 12 hours after learning and after memory has been challenged with an interference task using the two retrieval tests: *cued recall* or *recognition*.

4.2 Study 3 – Materials and Methods

The present study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of the department of psychology of the LMU.

This study included three groups of participants who learned and were tested on pairs of non-sense syllables. All groups underwent two conditions, nocturnal sleep and diurnal wakefulness. The difference between the three lay in the type of retrieval test (cued recall vs. recognition).

4.2.1 Subjects

The number of participants in each of the three groups was 12, thus 36 in total (group I: 7 females, 5 males, mean age 25 ± 5 years (mean ± S.D.); group II: 9 females, 3 males, mean age 24 ± 2 years (mean ± S.D.); group III: 8 females, 4 males, mean age 23 ± 3 years (mean ± S.D.)). All subjects taking part in study 3 were free of medication and abstained from caffeine and alcohol on the days of participation. A prerequisite for participation was the absence of any sleep disorders. From all subjects a sleep log (see Appendix) was obtained, indicating total sleep time for the night prior to the days of examination.

4.2.2 Learning Materials

In all groups, the learning material was composed of meaningless 3-letter-syllables. After generating all possible combinations of 3-letter consonant-vowel-consonant combinations, syllables that formed words in German, English or French, or which formed abbreviations in German, were excluded. A pool of 711 syllables was left. For each condition, 60 different syllables were chosen randomly to create 3 lists (lists A, B and C) of 20 syllables. Syllables were presented in white letters with font type “Courier New” in size 80. Instructions were presented in the font “Arial” and in size 40. Syllables and instructional text was presented in white color on a black background on a 19 inch monitor using Matlab (version R2010, The MathWorks, Inc.) and the Cogent 2000 toolbox (UCL, UK). Subjects viewed the screen from a comfortable viewing distance of approximately half a meter.

4.2.3 Procedure

Participants of each group underwent two conditions, a wake condition and a sleep condition. During the wake condition, participants learned in the morning, stayed awake during the day, and returned for an interference list and test 12 hours later in the evening. In the sleep condition, participants learned in the evening, slept at home and returned for an interference list and test 12 hours later in the morning (Figure 32).

	8:00 pm-8:40 pm	8:40 pm-8:45 pm	11:00 pm-7:00 am	8:00 am-8:40 am	8:40 am-8:45 am	8:45am-8:50am
Sleep Condition	Learning	Test	Sleep	I-Learning	I-Test	Delayed Test
One Week Later						
	8:00 am-8:40 am	**8:40 am-8:45 am**	**8:45am-8:00pm**	**8:00 pm-8:40 pm**	**8:40 pm-8:45 pm**	**8:45pm-8:50pm**
Wake Condition	Learning	Test	Wake	I-Learning	I-Test	Delayed Test

Figure 32: Study 3 – Study design
Exemplary study design for all three groups of study 3. Each participant underwent two conditions, separated by at least one week. Upper row: In the sleep condition, participants learned a list of non-sense syllable pairs in the evening (Learning), were tested immediately (Test) and slept 7-8 hours during the night. Twelve hours after learning, subjects encoded an interference list of non-sense syllables (I-Learning) and were tested immediately (I-Test). Thereafter subjects were tested on the list of syllables (Delayed Test) that had been learned during "Learning". Bottom row: In the wake condition, participants learned in the morning, were tested immediately and spent the day awake. Twelve hours after learning, subjects encoded an interference list of non-sense syllables (I-Learning) in the evening and were tested immediately (I-Test). Finally, subjects were tested on the list of syllables (Delayed Test) that had been learned during "Learning".

The starting conditions (wake or sleep) were counterbalanced across subjects of a group. The order of the conditions was distributed equally and randomly[16] among subjects of a group. For the learning phase of the sleep condition, subjects arrived in the evening. Subjects could choose the starting time of the learning session among three time slots (8 p.m., 9 p.m. or 10 p.m.), whichever best fit their schedule. In the wake condition, subjects arrived in the morning and could choose three time slots: 8 a.m., 9 a.m. or 10 a.m. The instructor introduced the subjects to the sound-attenuated laboratory and asked subjects for their consent to take part in the study. Subjects were seated at an individual desk, completed a short questionnaire (see Appendix) including age, gender, bedtimes, etc. and were given verbal as well as written instructions for the study on a PC monitor. Thereafter, each subject learned and was tested on the pairs of non-sense syllables[17]. After completion in the evening (sleep condition), subjects went home to sleep. In the wake condition subjects spent the day awake, going

[16] No mathematical function was used to create randomness, instead, randomness was created to the best ability of the experimenters.

[17] In order to avoid human bias, only the computer (see Learning Materials) was used to present and test syllables.

about their daily chores. For the interference learning, interference test and delayed test, subjects arrived at the same laboratory exactly 12 hours later. A wash-out period of one week lay between the two conditions in order to avoid that the learning material from the first condition impinged with that of the second condition.

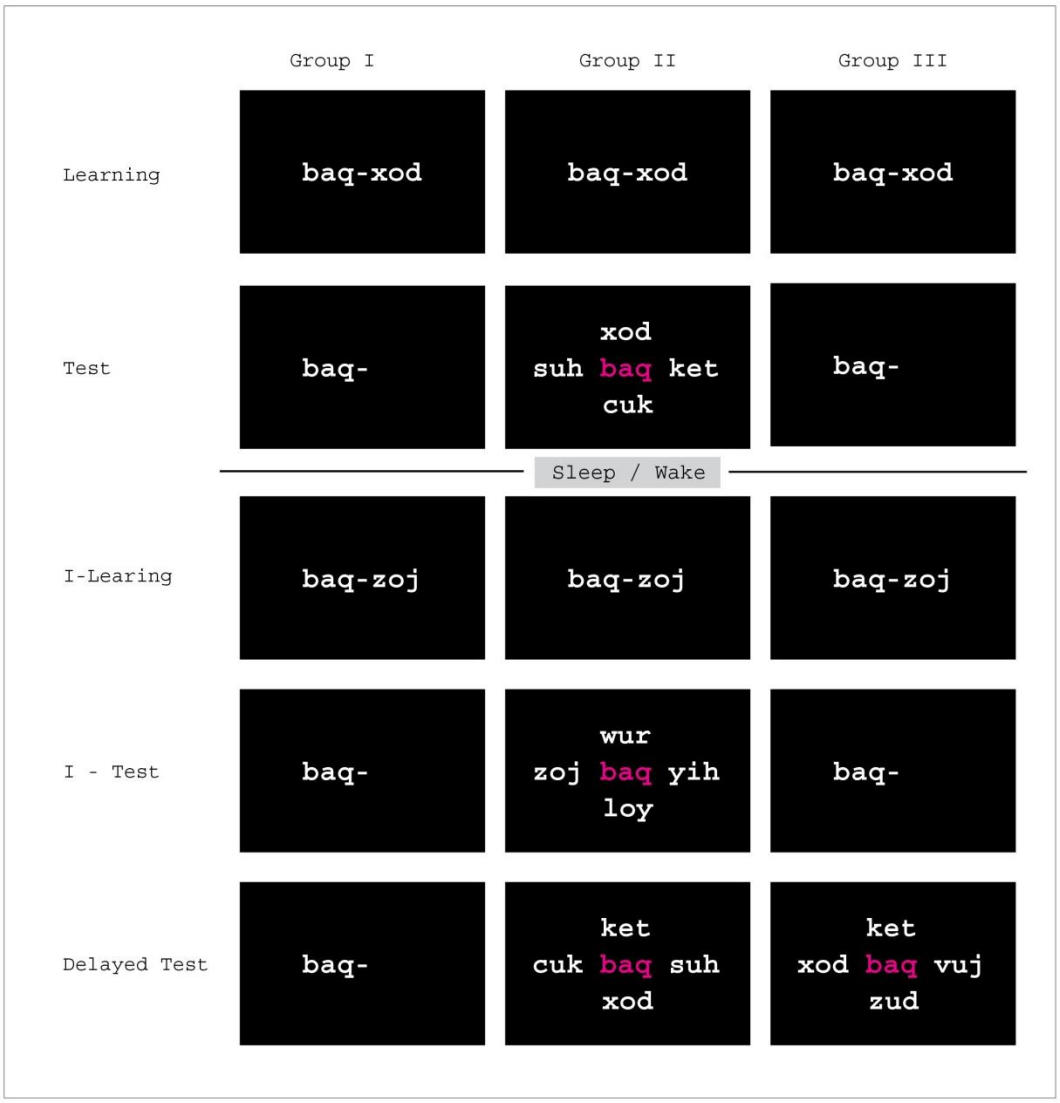


Figure 33: Study 3 – Method – Learning and testing
Panels display how non-sense syllables were presented to participants of each group during the different sessions. Sessions included learning (Learning), immediate test (Test), interference-learning (I-Learning), interference test (I-Test), and delayed test (Delayed Test). Subjects underwent sessions in the order of their appearance in the figure. Columns represent groups and rows represent sessions. The type of retrieval test differed between groups: Participants of group I were tested with cued recall in all three tests. Participants of group II received a recognition test for all three tests. Participants of group III were given both types: cued recall tests during the sessions Test and I-Test and recognition for the session Delayed Test. After the immediate test, a retention period took place, in which participants either went home to sleep (sleep condition) or stayed awake during the day (wake condition). Participants returned 12 hours later (calculated from the start of the learning session) to proceed with interference learning. The feedback test (see text) is not depicted here.

Participants started with a learning session (Figure 33, Learning). The goal of this phase was to learn 20 pairs of non-sense syllables. Syllables were from lists A and B (see section 4.2.2) and were presented as A-B pair. The syllable on the left side was from list A, the one on the right from list B. For example, Figure 33 in the session "Learning" for "Group I", syllable A is "baq" and syllable B is "xod". Each pair was presented in white lower case letters on a black background, for two seconds. The two syllables were presented next to each other, separated by a hyphen (e.g. baq – xod).

Participants were asked to vocalize the syllables repetitively for the time of presentation and to memorize them as pairs. The entire list of 20 pairs was presented five times.

In order to reduce primacy and recency effects, pairs were presented in a random order. After the fifth presentation, participants were tested on the syllables and given feedback (feedback test, not depicted in Figure 33). The time of the feedback test was not limited. After each answer, visual feedback ("correct" or "incorrect") was given and the correct pair was presented on the monitor. After all pairs were tested once, only those pairs that had been answered incorrectly were tested again, in a different order than before. Once at least 80% (= 16) of the pairs were remembered correctly the feedback test was complete.

Then, the test was performed once again without feedback during the immediate test (Figure 33 "Test"). Two forms existed for both the feedback and immediate test: cued recall and recognition. Participants of groups I and III were tested via cued recall, while participants of group II were tested via recognition. For cued recall, the 20 syllables of list A were presented one by one. Each A-syllable served as a cue (Figure 33, Test for Group I as well as Test for Group III). The participant had to complete the A-B pair by remembering the correct syllable of list B. Subjects typed their answers using a standard German keyboard.

For the recognition test, the cue syllable (from list A) was presented in the middle of the screen and the subject had to choose the correct answer among four given syllables, as in a multiple choice test (Figure 33, Test for Group II). There was only one correct answer, the syllable from list B. The three wrong answer choices were syllables that were not part of lists A, B or C. For the immediate test, the score of correct answers was recorded.

Participants of the wake condition then stayed awake throughout the day, while participants of the sleep condition went home and slept for 7-8 hours during the night. Twelve hours after the learning session, participants returned to the laboratory to learn an interference list in the interference-learning session (I-Learning). The goal of the interference-learning session was to learn 20 pairs of syllables that interfered with the previous list. The syllables were from lists A and C and presented as A-C pair (Figure 33, I-Learning). Thus, the syllable presented on the left was old and had been learned during the previous learning session. The syllable on the right was new. The procedure of interference-learning was the same as during the previous learning session. After the presentation of all 20 syllable pairs, subjects were given a feedback test on the learned A-C pairs. The learning criterion was 80% correct answers.

Once subjects had reached the learning criterion, they were tested on the A-C pairs without feedback in the interference test (I-Test). Participants of groups I and III were tested via cued recall, while participants of group II were tested via recognition for both the feedback and interference test

(Figure 33, I-Test). Answer scores of the interference test were recorded. Directly after the interference test, subjects were tested on the A-B pairs in the delayed test (Figure 33, Delayed Test). The A-B pairs had been learned twelve hours before. Here, syllable from list A was presented and the correct syllable from list B had to be retrieved. The types of retrieval test given to the three groups were the following: Group I received a cued recall test, whereas groups II and III underwent a recognition test. Participants did not receive feedback on their answers. The score of correct responses was recorded.

4.2.4 Analysis and Statistics

Study 3 is a within subject design in which two conditions (sleep and wake), each consisting of three scored tests (immediate-, interference- and delayed test) were compared. Data were analyzed using IBM SPSS Statistics (version 17.0), Matlab (version R2010, The MathWorks, Inc.), Microsoft Excel 2007 and SigmaPlot (versions 11.0) for graphs. Averages of results were calculated as arithmetic mean and standard error (mean ± S.E.) or standard deviation (mean ± S.D.). For a comparison between the means of the sleep versus wake conditions, paired two sample t-tests were applied. Repeated measures ANOVA were calculated and two factors were used: The type of retrieval test (recall / recognition) and the brain state (sleep /daytime wakefulness). The probability of guessing in the recognition test was 25% (1 of 4 options). However, when comparing recognition performance within the group, no correction for guessing was necessary, as guessing rates were the same for both conditions. Results were considered statistically significant when p-values were below 0.05.

4.3 Study 3 – Results

Table 9 shows that in the immediate test (Figure 33, Test), which took place after having successfully memorized at least 16 of 20 non-sense syllable pairs and before sleeping or staying awake, subjects of group I tended (p = 0.05 paired samples t-test) to recall more syllables in the wake condition than in the sleep condition. (The high rate of forgetting between learning and testing is discussed in the discussion section of this study.) Moreover, when the immediate test was performed in the morning, subjects of group I performed better than when the immediate test took place in the evening (Table 9, group I). In groups II and III, the immediate test showed no significant difference for the time of day (p = 0.21 and p = 0.45 respectively, both paired samples t-tests) when comparing sleep and wake conditions within a group.

Table 9: Study 3 – Results – Immediate test
Participants of groups I and III performed a cued recall test and those of group II performed a recognition test. The table lists the mean number and standard error of correctly remembered syllable pairs during the immediate test. The tables shows absolute values. N = 12 per group.

	Group I recall	Group II recognition	Group III recall
Immediate Test (sleep condition, evening)	6.83 ± 0.94	12.08 ± 1.03	8.33 ± 1.31
Immediate Test (wake condition, morning)	8.92 ± 1.30	13.08 ± 0.80	9.33 ± 1.72
p-value	0.05	0.21	0.45

Comparing night- and day- interference, there were no significant differences between memory performances in the morning compared to evening sessions between groups as interference test results show (Table 10). P-values of paired samples t-tests are as follows: 0.09 (group I), 0.82 (group II) and 0.36 (group III).

Table 10: Study 3 – Results – Interference test
Subjects of groups I and III underwent a cued recall test and those of group II a recognition test. The table lists the mean number of syllable pairs (± S.E.) remembered correctly during the interference test. N = 12 for each group.

	Group I recall	Group II recognition	Group III recall
Interference Test (sleep condition, morning)	8.08 ± 1.35	12.75 ± 1.11	9.45 ± 1.26
Interference Test (wake condition, evening)	6.58 ± 1.36	13.00 ± 0.72	8.58 ± 1.43
p-value	0.09	0.82	0.36

Values of the delayed test (Table 11) of group I show that subjects remembered significantly more syllable pairs after a period of sleep than after wakefulness ($p = 0.02$, paired samples t-test). No significant differences between the sleep and wake conditions could be found during delayed test in group II ($p = 0.36$). Contrary to group I, in group III participants tended to recognize more syllables after a period of wakefulness than after a period of sleep ($p = 0.07$).

Table 11: Study 3 – Results – Delayed test
Participants of group I performed a cued recall test whereas subjects of group II and III performed a recognition test. The table lists the mean and standard error of correctly remembered syllable pairs during the delayed test. N = 12 per group.

	Group I recall	Group II recognition	Group III recognition
Delayed Test (sleep condition, morning)	4.08 ± 1.28	10.58 ± 1.18	16.17 ± 0.89
Delayed Test (wake condition, evening)	2.33 ± 0.83	11.42 ± 0.66	14.92 ± 0.85
p-value	0.02	0.36	0.07

In contrast to Table 10 and Table 11, each bar in Figure 34 displays the mean of performance change (mean of percentage decrease). In the delayed test of the sleep condition (Figure 34), subjects of group I correctly recalled about half of the syllables they had recalled during the immediate test (51.14% ± 8.14 S.E). In the wake condition, subjects recalled significantly less: only 22.26% (± 5.77 S.E.) of the syllables were remembered in the immediate test. Subjects of group II recognized 87.56% ± 8.13 (S.E.) of the matching syllables after sleep and 88.90% ± 4.70 (S.E.) after a period of wakefulness. In group III which performed a cued recall as immediate test and a recognition type of test for the delayed test, participants recognized 241.88% ± 31.94 S.E. (sleep condition) and 313.17% ± 103.99 S.E. (wake condition).

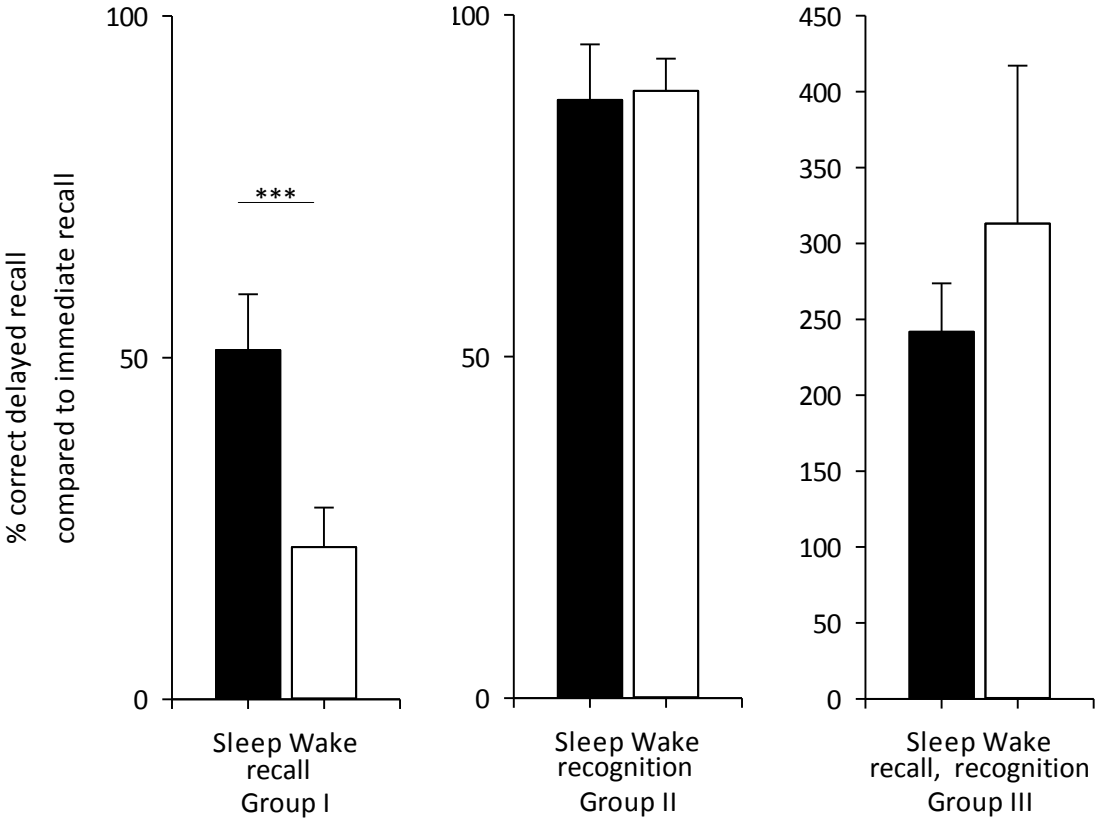


Figure 34: Study 3 – Results – Performance at delayed testing
Percentage of correct syllable pairs remembered during delayed testing, setting results of immediate test scores to 100%. Subjects of group I performed significantly better ($p < 0.001$) during the sleep (51.14% recalled correctly) compared to the wake condition (22.26% recalled correctly). In both groups II ($p > 0.87$) and group III ($p > 0.45$) subjects performed equally well during the sleep condition, compared to the wake condition. Error bars represent S.E. Pairwise comparisons between the two conditions, sleep and wake, using two sampled t-tests, were performed. * indicates significance under p = 0.001.**

When examining the scores of the test, which occurred before undergoing sleep or wake, a session effect was seen in groups II and III but not in group I. As expected, no pre-conditional differences were found in group I: In the immediate test ("Test", Figure 33), subjects of group I correctly recalled

8.00 ± 1.19 syllables (mean ± S.E.) on the first day of the study and 7.75 ± 1.16 syllables (mean ± S.E.) on the second day. Thus, when comparing the two days of the study, there was no significant difference found in the recall *before* sleeping or staying awake in group I ($p = 0.83$, $T = 2.20$, paired samples t-test). However, pre-conditional effects were found in the other groups: In groups II and III the mean recall score differed between the two examination days (group II: $p = 0.05$, and group III: $p = 0.002$; both $T = 2.20$, paired samples t-test). Subjects of both groups showed improved learning performance on the second day of examination. On the first day, subjects of group II correctly remembered 11.83 ± 0.78 (mean ± S.E.) or 59% ± 4% (mean ± S.E.) in the immediate test ("Test", Figure 33). On the second day the mean recall score was 13.33 ± 1.02 (mean ± S.E.) or 67% ± 5% (mean ± S.E.). In group III, subjects correctly remembered 7.17 ± 1.45 (mean ± S.E.) or 36% ± 7% (mean ± S.E.) syllable pairs on the first day and 10.50 ± 1.46 (mean ± S.E.) or 53% ± 7% (mean ± S.E.) pairs on the second day of examination in the immediate test ("Test", Figure 33). Thus, a session-effect was visible in groups II and III. In all groups immediate test scores were well below the learning criterion (80%) that needed to be reached just before taking the immediate test. The effect sizes (d') of the groups were 0.47 (group I), -0.25 (group II) and 0.42 (group III).

Overall, a beneficial effect of sleep on memory retrieval after learning (more syllables were recalled in the sleep compared to the wake condition) occurred only in group I. In group I the immediate and delayed tests were both cued recall tests. In two of the three groups a circadian influence was present when comparing morning performance (immediate test wake condition + interference test sleep condition + delayed test sleep condition) to evening performance (immediate test sleep condition + interference test wake condition + delayed test wake condition): In group I the analysis of circadian influence resulted in $p < 0.01$. Results of group III also tended to be influenced by the time of day when testing took place ($p = 0.08$). In both groups (I and III), subjects achieved better scores during retrieval in the morning than in the evening. Only in group II, where no cued recall tests were given, results did not seem to be influenced by circadian rhythm effects ($p= 0.95$).

4.4 Study 3 – Discussion

When newly and thus fragile memory is consolidated, it is converted into a more stable state, making it more robust against interference. This consolidation and thus better performance of declarative memory at retrieval, has been attributed to the function of sleep (Benson and Feinberg, 1975; Diekelmann et al., 2011; Ellenbogen et al., 2009; Lahl et al., 2008; Nesca and Koulack, 1994). Aim of study 3 was to see whether the *type* of retrieval is crucial for an effect of sleep on the human declarative memory. Examined were two types of retrieval: cued recall and recognition (see section 10 for definition). A within subject analysis was performed for each of three groups. Group I was tested via cued recall before and after the sleep/wake phase. Group II was tested via recognition before and after the sleep/wake phase. Group III was a mixture of both: Subjects were tested via cued recall before the sleep/wake phase and via recognition after the sleep/wake phase.

A beneficial effect of sleep on memory retrieval occurred when the learned non-sense syllables were tested via cued recall *only* (group I). In group I memory recall was significantly better after a night of sleep than after a phase of wakefulness. However, results of group I do not reliably corroborate the idea that an effect of sleep on declarative memory is supported by cued recall, in comparison to recognition (Ellenbogen et al., 2006). Neither can these results clearly show that the type of retrieval is crucial for an effect of sleep on declarative memory. The reason is that the results of study 3 show circadian rhythm effects with better test scores in the morning than in the evening.

The cause of the visible, higher rate of forgetting in group I after a day of wakefulness cannot be disentangled: It cannot be ruled out that the significant difference of the two conditions in group I was due to a circadian effect, negatively influencing retrieval in the evening sessions, instead of a sleep benefit. Results showing that the circadian rhythm effect was more pronounced in the groups tested through cued recall, suggest that the effect could be specific to the type of recall. This, however, would have to be scrutinized in further studies. Whether the influence of the experience during wakefulness throughout the day is negligible and thus data after a period of wakefulness during the day are comparable to those after sleep deprivation (and recovery) is still a matter of debate. Whereas some authors argue that there is no time-of-day effect on declarative memory retrieval (Ellenbogen et al., 2006), the activity during the day has been shown to influence sleep patterns and homeostasis (Horne and Minard, 1985; Horne and Walmsley, 1976; Rattenborg et al., 2009).

Due to the non-imaginability and thus high difficulty of the learning material in combination with the more difficult type of retrieval test in group I, results indicate a floor effect. This makes discriminations among subjects with low retrieval scores more difficult. The design used involved the trade-off between motivation and performance: The duration of the learning phase in group I was

approximately 45 minutes. More syllables could have led to a higher total amount of syllables remembered, but could have led to less motivation and worse retrieval performance. Astonishingly, after having reached the learning criterion of 80%, subjects forgot almost half of the pairs immediately, as the scores of the immediate test show. According to Ebbinghaus who recorded the rate of forgetting of non-sense syllables (Ebbinghaus, 1983), around 40% of non-sense syllables are forgotten after a retention period of 20 minutes. In the present study, slightly more syllables were forgotten in the immediate test, which succeeded the last feedback test immediately. One reason for the higher amount of forgetting could be the high difficulty of the task. The retrieval test of study 3 was cued recall whereas free recall was applied in the study by Ebbinghaus. Cued recall is likely to be more difficult. In study 3, syllables had been learned in pairs. Generating a matching non-sense syllable in a cued recall paradigm is likely to be more difficult than recalling non-sense syllables freely, as it was done in the study by Ebbinghaus. A further reason for the deviation from Ebbinghaus' curve of forgetting is likely to be the different sample size in study 3 compared to the study by Ebbinghaus: Ebbinghaus had a sample size of only one, in study 3 the sample size was 12 per group. Ebbinghaus developed the curve after conducting the study on himself. Being investigator and subject at once, he was probably highly motivated to forget as little as possible. In study 3 subjects did not have personal profits from results. Results from the subjects participating in study 3 can therefore be considered far less biased than the one-person result that led to Ebbinghaus' curve of forgetting.

Effect sizes of all groups were low and in group II results even indicated that sleep had a negative effect on recognition, compared to the wake condition. For cued recall, the key study (Ellenbogen et al., 2006) used for study 3 had shown a large effect size (d' = 3.07 comparing wake and sleep groups) but only for the experiment with interference. Effect size for the experiment without interference was comparatively low (d' = 0.92).

In study 3, subjects learned the material using no other strategy than rote repetition and vocalization of the non-sense syllable pairs. In order to restrict possible influence of learning strategies on the mnemonic process, subjects had been asked *not* to associate the non-sense syllables with similar sounding existing words. Learning strategies such as imagery is a more effortful and vivid learning processes and would have altered the learning process, leading to a dramatically improved storage of memory (Byrne, 2003). In contrast, mere repetition only slightly improves learning performance. The trade-off of rote repetition, however, is the low efficiency (Glenberg et al., 1977; Rundus, 1977). Material with high imagery content has a higher chance of being remembered than one of low imagery content (D. Schmidt et al., 2002). It is possible that a beneficial effect of sleep could not be detected due to the suppression of autobiographical association.

It is possible that a beneficial effect of sleep becomes detectable only when memory is coupled and triggered at retrieval testing, for instance when memory is associated with an odor (Rasch et al., 2007) or a tone (Geisler, 2011). In the present study, circadian effects obscure a possibly positive effect of nocturnal sleep on memory, thus the question of whether a sleep benefit exists and whether it depends on the type of retrieval remains. Therefore, a study design was implemented (see study 4) which tested performance at the *same* time of day for both conditions, that is for sleep and wake. This was possible with a *nap* sleep design.

5 Study 4 – Declarative Memory Retrieval after a Nap versus Wakefulness: Testing Cued Recall and Recognition of unrelated Word Pairs

5.1 Study 4 – Introduction

In study 3, several possible areas for confounds existed which could have impeded a beneficial effect of sleep on memory and which shall be improved in study 4: Circadian rhythm, retention period and the learning material. Regarding circadian rhythm, in the previous study encoding and retrieval processes had taken place at different times of the circadian cycle. In study 3 circadian rhythm effects obscured results. This is improved in study 4: The nap design allows for encoding and retrieval at the same time of day, in the afternoon. Furthermore, in study 3 subjects experienced different brain states before the learning period. This needed to be done in order to avoid that subjects had to stay awake at night for the wake condition in study 3. Thus, in the wake condition, learning in the morning was preceded by a period of sleep. In contrast, in the sleep condition, learning in the evening was preceded by a period of wakefulness. There have even been suggestions that sleep prior to encoding can cause detrimental effects on learning (Grosvenor and Lack, 1984). Other authors argue that a period of sleep before learning is necessary in order to prepare the brain for initial memory formation (Yoo et al., 2007). This confounding factor is controlled by choosing a nap sleep paradigm, in which the encoding periods for the wake as well as the sleep condition take place at the same time of day (in the afternoon). Study 4 uses a nap design with two learning phases, one before and one after the period of nap sleep / wake. Results of study 3 contradict Grosvenor and Lack's finding that sleep before learning is detrimental on learning.

In study 4, initial learning is preceded by a period of wakefulness and a period of sleep precedes a second learning session. Furthermore, sleep in this study follows initial learning immediately, in contrast to study 1, in which sleep was not guaranteed to follow encoding immediately. Talamini et al. (2008) find that if sleep follows shortly, that is within a few hours, after learning, deterioration of the memory trace does not occur. For example, when sleep follows within 3 hours after learning, its benefit is greater than when sleep onset is delayed by half a day (Benson and Feinberg, 1977; Gais et al., 2006). The design of study 4 allows for immediate sleep after learning.

In the current study, subjects are allotted a period of 60 minutes of sleep, which is before a person enters REM sleep. The purpose of the cut-off after 60 minutes is to avoid influences from REM sleep. Effects of sleep can thus be clearly directed to slow wave sleep only. Hippocampus-declarative memories are said to profit more from NREM sleep than from REM sleep (Gais and Born, 2004; Peigneux et al., 2004; Plihal and Born, 1997; Tucker and Fishbein, 2008; Yaroush et al., 1971). Often

times the benefit of sleep is attributed to certain stages, yet literature is far from accordance: On one hand, the beneficial effect of sleep is attributed to stages that make up slow wave sleep, stages three and four (Drosopoulos et al., 2007a; Ellenbogen et al., 2006; Gais et al., 2007; Rasch et al., 2007), on the other hand, it is attributed to sleep spindles (generated by the thalamus) and sharp-wave-ripples (SWR, generated by the hippocampus) which can occur during all NREM sleep stages. Then again the beneficial effect of sleep is attributed to sleep stage two (Genzel et al., 2009). In study 4, the use of EEG during nap sleep allows for the recording of sleep stages and possible correlations between sleep stages and sleep benefit.

Regarding the learning material and retention period, the design of study 4, nap sleep, allows for another advantage in regards to subject performance. In study 3 results of the only group showing a significant difference between sleep and wake conditions (group I) indicated a “floor effect”. Such low test scores most likely arose due to high difficulty of the learning material, non-sense syllables, in combination with the difficult type of retrieval test, cued recall, and the long retention period of 12 hours. Since cued recall is at the scope of this study in investigating whether a sleep benefit is specific to a certain retrieval type, only the other two variables, retention period and learning material, can be adapted in study 4. Compared to a night of sleep, a short nap greatly decreases the retention period, which in turn decreases the time (from 12 hours to one hour) left for the natural process of forgetting. Concrete nouns instead of non-sense syllables are used in study 4 to decrease task difficulty. Concrete nouns are highly imaginable words, which are better remembered (concreteness effect) than non-imaginable material (Byrne, 2003).

In study 3 a second list of non-sense syllables which served as interference was learned after sleep (and also after wake). The question of whether sleep protects memory from retroactive interference could not be clarified from the results of study 3. Yet, it has been suggested in several studies that a beneficial effect of sleep is sensitive towards interference, such that a significant beneficial effect of sleep is visible on the behavioral level compared to declarative memory tasks without interference (Drosopoulos et al., 2007a; Ellenbogen et al., 2006). Thus, the design of study 4 will include a list of interference after the nap / wake period. Most paradigms involving interference memory after sleep test retention after a period of night time sleep. For procedural memory, Korman (2007) investigated the influence of nap sleep on procedural memory and found that subjects who spent 90 minutes asleep immediately after learning a motor sequence task were less susceptible towards interference that took place two hours after initial learning.

As mentioned in previous paragraphs, the type of retrieval is important to this study in order to see whether sleep supports specific memory traces. In study 3, declarative material had been tested after a period of sleep using two different types of retrieval, recall and recognition. The former

procedure demanded that the subject retrieved the material in the lack of the learned stimulus under the process of recollection, while the latter demanded that the subject recognized the stimulus among a set of given answers, underlying the process of familiarity. The dual-process model of recall and recognition (Jacoby, 1991; Mandler, 1980; Tulving, 1985) states that memory retrieval can be executed by two independent processes: recollection and familiarity (Ciaramelli et al., 2008; Yonelinas, 1994, 2002). Recollection involves vividly re-experiencing the episode in which the stimulus was encoded in its contextual details (Tulving, 1985). Familiarity lacks the subjective spatio-temporal memory trace, leading to a sense of "knowing" that the stimulus was presented, without the presence of memories of contextual details (Yonelinas, 1994). In the past, theories on episodic memory have been concentrating increasingly on retrieval mechanisms (Greve et al., 2010). When retrieving personally experienced information learned before a period of sleep, the type of retrieval test seems to determine whether a sleep benefit is exposed on the behavioral level (Diekelmann et al., 2009). Whereas numerous studies report a positive effect of sleep on the process of recollection (Barrett and Ekstrand, 1972; Fowler et al., 1973; Grosvenor and Lack, 1984; Yaroush et al., 1971), the process of familiarity oftentimes remains less (U. Wagner et al., 2007) or even unaffected by sleep loss (Atienza and Cantero, 2008; Drosopoulos et al., 2005; Hu et al., 2006; Rauchs et al., 2004). Administration of benzopiazapines, drugs treating sleep disorders, at the encoding phase have detrimental effects on recollection but not familiarity (Curran et al., 1993). Back to the question in the beginning if sleep supports specific memory traces: Sleep might support memory traces activated during recall but not during recognition. Results of study 3 could not clearly disprove the hypothesis that a beneficial effect of sleep is revealed equally well for recall and recognition on the behavioral level. Thus both types of retrieval are again applied in study 4.

The aim of this study is to clarify whether the exposure of a behavioral sleep benefit is critical to the *type* of retrieval test whilst controlling for side effects from circadian rhythm and high task difficulty. Despite containing less time of sleep, nap sleep has been found to be comparable to nocturnal sleep in terms of a benefit on memory. Benefit from nap sleep has been reported to be as good as a full night of sleep (S. Mednick et al., 2003; Stickgold et al., 2000a; Stickgold et al., 2000b). Therefore, results of study 4 can be expected to give reliable conclusions on the effect of sleep on declarative memory.

5.2 Study 4 – Materials and Methods

The study was approved by the ethics committee of the department of psychology of the LMU and was conducted in accordance with the principles described in the Declaration of Helsinki.

5.2.1 Subjects

Sixteen native German speakers participated in the study for monetary compensation. One subject was excluded from analysis due to intake of medication known to influence sleep, on one of the two experimental days. The remaining subjects (n = 15, 5 female and 10 male university students, mean age 22 ± 3 years (mean ± S.D.)) reported to be free of psychoactive medication, were non-smokers and free of sleep disorders. Subjects had not engaged in activities resulting in a disruption of the regular sleep cycle (i.e. shift- or night-time work; overseas flight) 6 weeks prior to the study. On the two days of the experiment, subjects abstained from caffeinated drinks and food. A prerequisite for participation was to be able to take a nap in the afternoon. In order to control for individual daytime wakefulness among subjects, further prerequisites were a bedtime between 10:30 p.m. and 12:30 a.m. and a regular sleep pattern of 7 to 8 hours of sleep per night. Each subject kept a sleep log (see Appendix) documenting the times students went to be, fell asleep, woke up and left the bed, in order to control for equal amounts of sleep before the start of the learning session of each day. The sleep log was started 5 days prior to the first day of the study and ended when the study was completed. On each of the 2 days of the experiment, participants rose 1 hour before their regular wake-up time in order to allow for a nap in the afternoon.

5.2.2 Learning Materials

The learning material was composed of concrete German nouns from the CELEX lexical database created by the Instituut voor Nederlandse Lexicografie (INL) and the Max-Planck-Institute, Nijmegen (Baayen et al., 1995). From a pool of 1080 nouns, 18 lists of 60 German concrete nouns, e.g. “Zucker” (engl.: sugar) were created such that the mean frequencies of the lists, according to values given by the CELEX lexical database, were as similar as possible. For each subject, 9 lists were randomly assigned to the sleep condition, the other 9 to the wake condition. For each condition, letters A through I were assigned to the lists. Words were presented to the subjects on a TFT-LCD screen with a diagonal length of 23 inches. Words were presented in white letters of the font Courier New (size 45) on a black background using Matlab (version R2010, The MathWorks, Inc.) and the Cogent 2000 toolbox (UCL, UK). Subjects sat in a dark and quiet room at a comfortable viewing distance of approximately half a meter from screen.

5.2.3 Materials for EEG Recordings

For polysomnographic electroencephalographic (EEG) recordings, software and hardware by *Brain Products Inc.* were used. The EEG amplifier *BrainAmp* and software *Vision Recorder* served for data

acquisition. A sampling rate of 250 Hz was applied. During the recording, no filter was used. Ten Ag-AgCl-electrodes electrodes were attached to the subject's head and face, according to the International 10–20 System (Jasper, 1958), to record the following: Central electroencephalography (C3 and C4), electro-oculography (EOG) from the outer canthus of the left and right eye, and two electrodes for an electromyography (EMG) on the chin. An electrode on the nose served as a reference and another on the forehead served as the ground electrode.

5.2.4 Procedure

The experiment was a within-subject design. Each subject underwent two conditions on two different days, a wake condition and a sleep condition. The order of the starting condition was distributed equally and randomly[18] among subjects. On the days of the experiment, subjects arrived at the sleep laboratory at 1:30 p.m. (Figure 35). Each subject was seated in a separate room equipped with a PC and a bed. The instructor asked the subjects for their consent to participate in the study and verified the subjects' fulfillment of the prerequisites. Subjects then filled out the Stanford Sleepiness Scale (see Appendix). Instructions for the learning session were presented on a monitor and read aloud to the subject by the instructor. To avoid misunderstandings, the subject was asked to repeat the instructions in his/her own words. Information as to whether the subject would spend the retention time napping or awake was not revealed until after the learning session.

	1:30 pm-2:00 pm	2:00 pm-2:05 pm		4:00pm-4:30 pm	4:30 pm-4:35 pm	4:35 pm-4:40 pm	4:40pm-4:50pm
Nap Condition	Learning	Test	60 min Nap	I-Learning	I-Test	Break	Delayed Test
One Week Later							
Wake Condition	Learning	Test	Wake	I-Learning	I-Test	Break	Delayed Test

Figure 35: Study 4 – Study design
Subjects took part in a nap and a wake condition. In the afternoon, subjects learned 60 word pairs (Learning) and were immediately tested (Test). During the two-hour retention phase, subjects either took a 60 min nap or stayed awake. Thereafter, subjects learned (I-Learning) and were tested (I-Test) on an interference list. After a short break, subjects were tested (Delayed Test) on the word pairs learned before the nap / wake period.

For learning, words of lists A and B were randomly paired to eliminate semantic relationships between them. Words from list A served as stimulus words. All 60 pairs were presented once for four seconds per word pair. The inter-stimulus interval was one second. Both words of a pair were presented horizontally, separated by a hyphen (Figure 36 panel "Learning"). Subjects had been instructed to visualize the words as objects, but to restrain from creating a story or motion picture. One word of each of the lists D, E and F was randomly paired to each of the 60 existing word pairs

[18] No mathematical function was used to create randomness, instead, randomness was created to the best ability of the experimenters.

from lists A and B. The immediate test (Test) was a *recognition* type of test: A word from list A was randomly picked and displayed in the middle of the monitor in the color pink. The four words from lists B, D, E and F that had been previously paired, were displayed in white, surrounding the word from list A (Figure 36, panel "Test"). The position of the four words was changed randomly for each of the sixty words from list A. Answer choices were displayed for 3 seconds, in which the subject needed to *recognize* the correct word learned during the original encoding phase (word from list B). This short duration of 3 seconds was chosen in order to force recognition and to avoid free recall. Subjects could either choose one of the four presented answer choices, or, if they did not know the answer, tell the instructor to continue.

The instructor recorded the subject's answer choice, but did not give feedback about the correctness of the choice. A test criterion of 50 percent correctness needed to be fulfilled to move on. If subjects scored below the criterion, learning and the immediate test were repeated. Only those word pairs which had been answered incorrectly were re-learned and tested. During a repetition, the A-B word pairs as well as the assigned words from lists D, E and F stayed constant. The order of presentation during learning was chosen at random for each repetition. The location of the four answer choices in the immediate test was also randomized for each repetition. Once subjects scored at least 50 percent correct answers during the immediate test, they were informed about the condition of the retention period (nap or wake). The learning and immediate test phase ended shortly after 2 p.m.

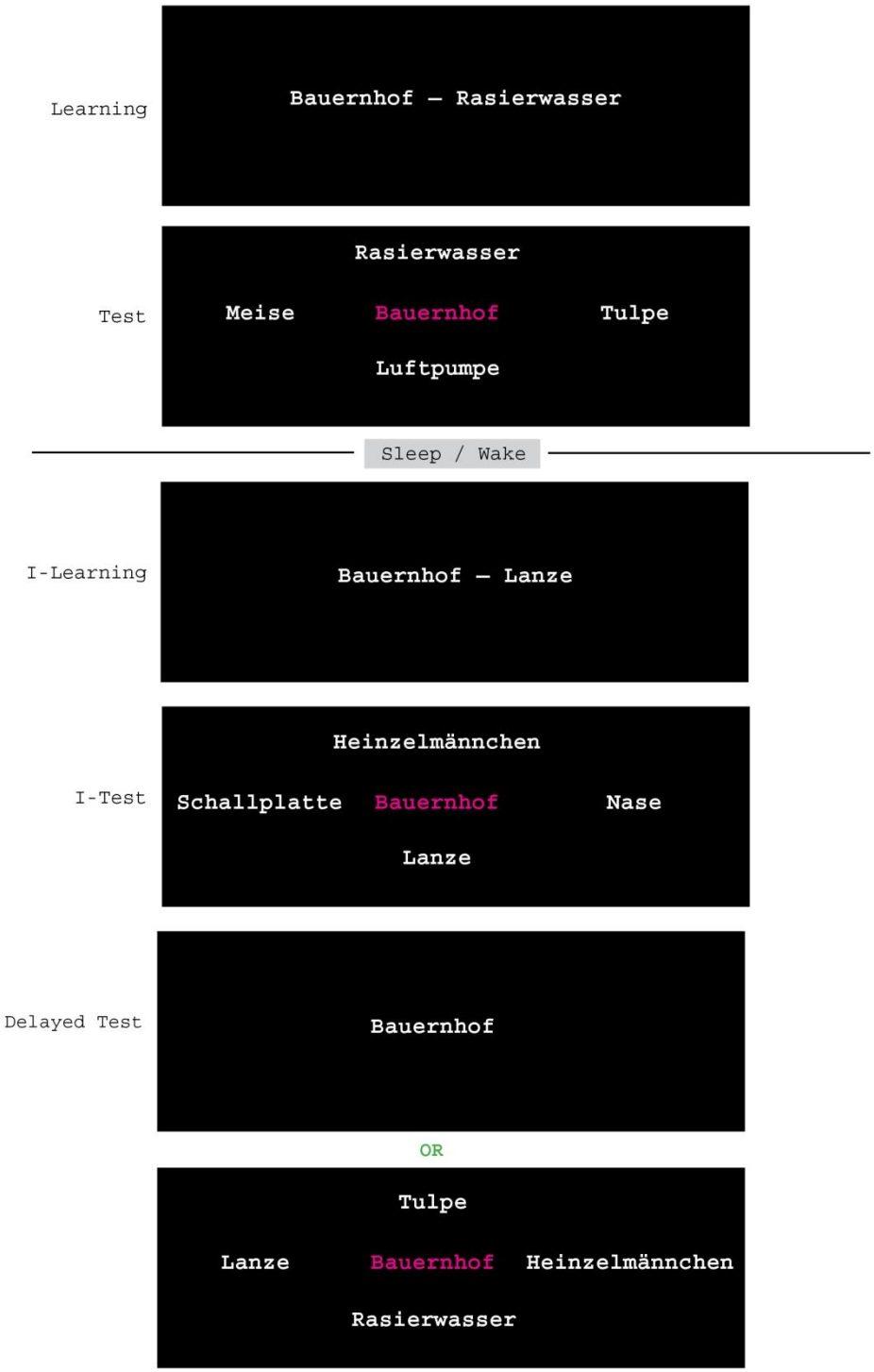


Figure 36: Study 4 – Method – Learning and testing
Panels display how words were presented to participants. Sessions occurred in the order in which they are presented in this figure. The sessions were: Learning of word pairs (Learning), immediate test (Test), interference-learning (I-Learning), interference test (I-Test), and delayed test (Delayed Test). Test and I-Test were recognition tests. In the delayed test, half of the words were tested using a cued recall test (second to last panel), the rest by using a recognition test (last panel).

Subjects then either spent two hours awake or took a 60 minute nap. When awake, subjects engaged in playing simple board games, such as ludo or nine men's morris. Before napping, electrodes were applied to record sleep (see section "Materials"). Sleep onset was declared as the appearance of a clear spindle on the polysomnograph. Sixty minutes later the experimenter woke the subject who

then filled out a questionnaire (see Appendix) about the nap and electrodes were taken off. After the retention period, at approximately 4 p.m., subjects filled out a second Stanford Sleepiness Scale (see Appendix).

Thereafter, interference learning began. Here, words of list A were randomly paired with words from list C to eliminate semantic relationships between them. Words from list A were known to the subject, words from list C were new. The procedure of this phase was the same as that of the learning phase before the retention period. The newly learned word associations were then tested immediately. One word of each of the lists G, H and I were randomly paired to each of the 60 existing word pairs from lists A and C. Except for the different lists used, the procedure of this test was the same as that of the immediate test before the retention period. Once subjects scored at least 50 percent correct answers during the interference test, they took a break (5 min) and proceeded with the last test. Subjects were tested (delayed test) on the words learned before the retention period (which took place approximately three hours before). Subjects had not been informed about this test until this stage.

During delayed testing, subjects were presented all stimulus words (from list A) in a random order, and needed to remember the correct word (from list B). Here two types of tests were performed, recognition and cued recall. The order and the type of test for each word pair were chosen at random and balanced across the 60 word pairs. Half of the word pairs (chosen at random) were tested using recognition, the other half using cued recall. During *recognition* testing, the correct word had to be recognized in the same fashion as during the immediate and interference tests. This time, the four answer choices were displayed in the following fashion: The correct word from list B, another word from list B (but incorrect), and two words from list C (both incorrect). One of the words from list C had been the correct answer in the interference test. The locations of the answer choices were randomized. The time limit for recognition was the same as during immediate and interference tests. The remaining half (30) of the word pairs were tested using *cued recall*. Here, for each of the 30 word pairs, the stimulus word was shown, serving as a cue. Subjects had to verbally recall the correct word (from list B) to the instructor. There was no time limit for the cued recall. If subjects did not know the answer, they were asked to tell the instructor and the next stimulus word was presented. Subjects were not given feedback about their performance.

5.2.5 Analysis and Statistics

The present study included two conditions, one sleep and one wake condition. In the delayed test each subject underwent a cued recall *and* a recognition test. The within-subject design was used to avoid inter-individual differences. Analysis of behavioral data was performed using IBM SPSS Statistics (version 17.0), Matlab (version R2010, The MathWorks, Inc.), Microsoft Excel 2007,

G*power (version 3 (Faul et al., 2007)) for power analysis and SigmaPlot (versions 11.0) for graphs. Averages are calculated as arithmetic mean and standard error (mean ± S.E.) or standard deviation (mean ± S.D.). Paired two sample t-tests were used in order to compare means of the two conditions.

Repeated measures ANOVA were calculated with conditions (sleep vs. wake) and tests as factors. Guessing rate in the recognition test of the delayed test was 1:4 or 25%. No corrections for guessing were necessary when comparing recognition performance in the sleep and wake condition, since guessing rates were the same for both conditions. Correlations were calculated using Pearson's r. The significance level for all statistical tests was $p < 0.05$.

EEG signals from the ten electrodes (as described in the part "procedure") were filtered at 50 Hz (Notch-filter) and a 10 Hz high-cutoff filter was applied to EOG and a 25 Hz low-cutoff filter to EMG signals. The EEG signals from C3 and C4 were bandpassed between 0.5 and 30 Hz using Vision-Analyser (*Brain Products*). For offline identification of sleep stages, EEG data were manually scored offline in 30 second epochs according to standard criteria by Rechtschaffen and Kales (1968). The stages wake, sleep stages 1 to 4 and rapid-eye-movement (REM) sleep were identified. Sleep stages 3 and 4 were defined as slow wave sleep. For one of the 15 subjects (female) EEG sleep data could not be scored due to technical malfunctioning during electrode recording. Reported are sleep data from 14 subjects and behavioral data of all 15 subjects. Correlations between behavioral performance and sleep were performed for 14 subjects.

One male and one female subject entered REM sleep for 6 and 6.5 minutes, respectively. Since p-values calculated without these two subjects did not alter results, the two subjects who entered REM were included in the analysis.

5.3 Study 4 – Results

The amount of words remembered in the three tests (immediate, interference and delayed tests) did not differ significantly between the sleep and wake condition (Figure 37): Subjects did not perform significantly better in the delayed test after sleep compared to wakefulness ($p = 0.50$, $t_{14} = 1.76$). When comparing test results of the delayed tests with those of the immediate tests for both conditions, no interaction was found (test x condition, ANOVA, $F_{1,14} = 2.45$, $p = 0.14$). There was no difference between the two conditions in the test immediately following learning ($p= 0.41$, $t_{14} = 2.14$, immediate test). Similarly, word pairs from the interference list were remembered equally well after a period of sleep compared to wakefulness ($p = 0.45$, $t_{14} = 2.14$, interference test). No interaction between the two tests (immediate test and interference test) and the two conditions was found (ANOVA, $F_{1,14} = 0.02$; $p = 0.90$).

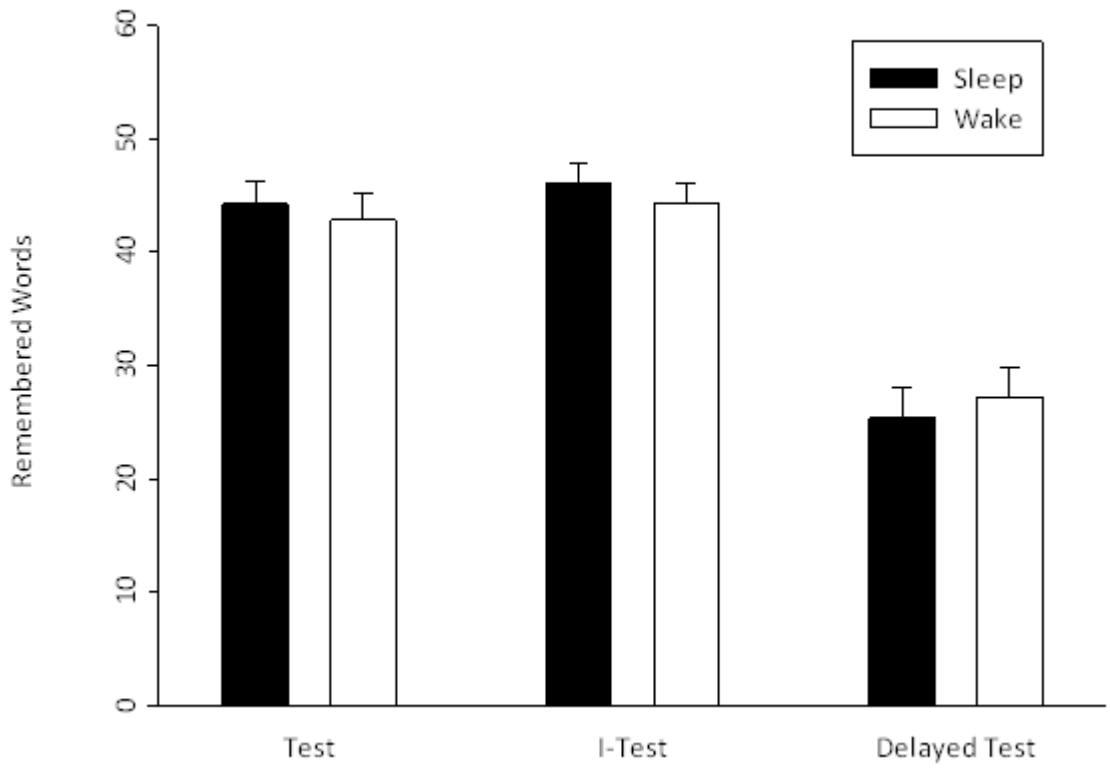


Figure 37: Study 4 – Results – Remembered words
In the sleep condition, subjects remembered 44.20 ± 2.03 (mean ± S.E.) of a maximum of 60 word pairs in the immediate test before taking a nap in the afternoon. In the wake condition, subjects remembered 42.08 ± 2.30 (mean ± S.E.) of a maximum of 60 word pairs in the immediate test before staying awake for about two hours. After sleep, subjects remembered 46.00 ± 1.85 (mean ± S.E.) of the 60 interference word pairs in the interference test. After wakefulness, subjects remembered 44.27 ± 1.83 (mean ± S.E.) of the 60 interference word pairs in the interference test. Of the word pairs learned before the nap, subjects remembered 25.40 ± 2.61 (mean ± S.E.) of a total of 60 word pairs in the delayed test. Of the word pairs learned before the wake retention period, subjects remembered 27.20 ± 2.55 (mean ± S.E.) of a total of 60 word pairs in the delayed test. N = 15.

When learning, subjects had the tendency to learn the interference word pairs faster after a period of sleep than after wakefulness, needing less word pair presentations until the criterion of at least 50% correctness was reached (ANOVA, $F_{1,14} = 4.38$; $p = 0.06$).

Results of the tests can be separated into words tested via cued recall and those tested via recognition in the delayed test (Figure 38).

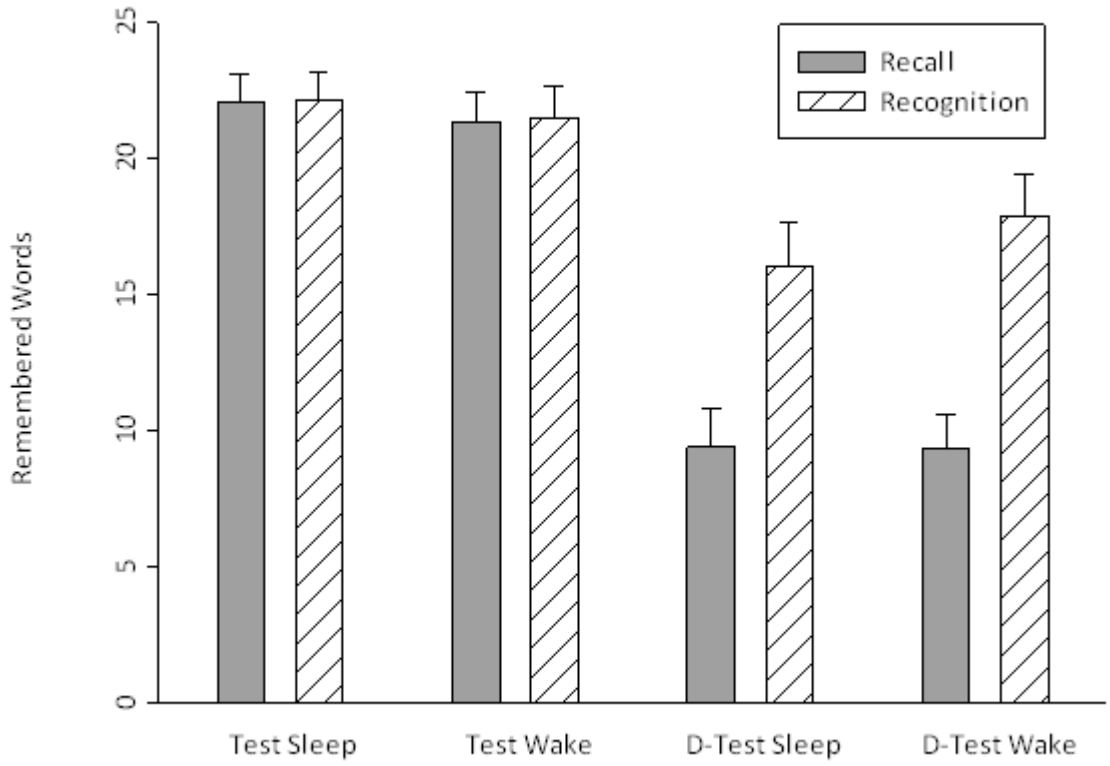


Figure 38: Study 4 – Results – Cued recall vs. recognition
Results can be separated into words tested via cued recall (grey bars) and words tested via recognition (striped bars) as of the delayed test. Test Sleep: In the immediate test, before entering the sleep condition subjects remembered 22.08 ± 1.09 (mean ± S.E.) words (later tested via cued recall) correctly and remembered 22.13 ± 1.02 (mean ± S.E.) words (later tested via recognition) correctly each of a total of 30 words. Test Wake: On the day of the wake condition, in the immediate test before staying awake, subjects remembered 21.33 ± 1.12 (mean ± S.E. for) words (later tested via cued recall) correctly and remembered 21.47 ± 1.19 (mean ± S.E.) words (later tested via cued recognition) correctly. D-Test Sleep: In the delayed test of the sleep condition subjects recalled 9.40 ± 1.38 (mean ± S.E.) words correctly and recognized 16.00 ± 1.65 (mean ± S.E.) words correctly. D-Test Wake: In the delayed test of the wake condition, subjects recalled 9.33 ± 1.25 (mean ± S.E. for) words correctly and recognized 17.87 ± 1.51 (mean ± S.E. for after wakefulness) words correctly. N = 15.

Thirty words had been chosen at random for each of the two types of retrieval in the delayed test. There were no prior learning effects between recall and recognition: Word pairs that were remembered via cued recall in the delayed test and words tested via recognition had been learned equally well ($p=0.77$, $t_{14} = 2.14$ sleep condition; $p = 0.50$, $t_{14} = 2.14$ wake condition). Sleep, however did not significantly improve the results achieved in the delayed test compared to scores of wakefulness ($p = 0.96$, $t_{14} = 2.14$ cued recall; $p = 0.24$, $t_{14} = 2.14$ recognition, (Figure 38). Effect sizes were small (for cued recall Cohen's $d = 0.01$, for recognition Cohen's $d = -0.32$, corrected for

dependence between means, using Morris and DeShon's (2002) equation 8). Post-hoc power analyses using the G*power test (Faul et al., 2007) showed that the sample size needed in order to achieve a significant difference between sleep and wake for cued recall is at least 129,935 subjects (power value: 0.95) and for recognition at least 116 subjects (power value: 0.95).

When setting the amount of word pairs chosen correctly during the immediate test to 100% (Figure 39) subjects did not perform significantly better after a period of napping compared to a period of wakefulness ($p = 0.98$, $t_{14} = 2.14$ for cued recall and $p = 0.12$, $t_{14} = 2.14$ for recognition).

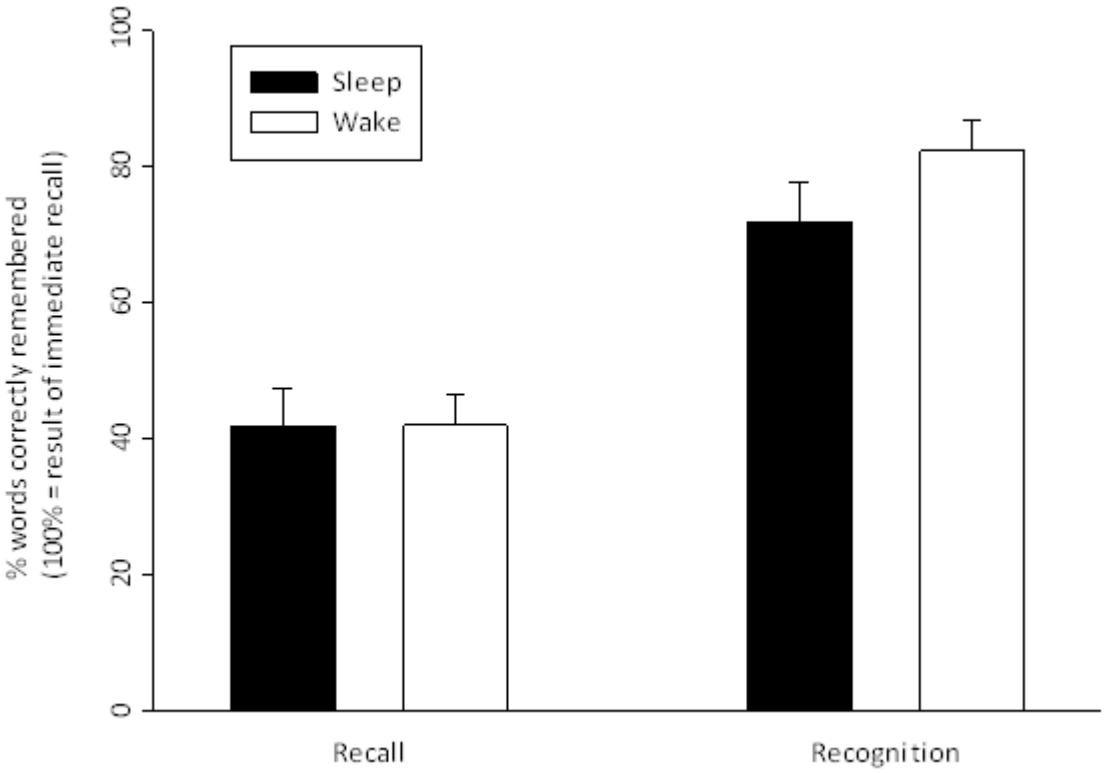


Figure 39: Study 4 – Results – Delayed test
When comparing results of the delayed test to the immediate test, subjects answered 41.81 ± 5.63 % (mean ± S.E.) correctly after a period of nap sleep and when words were tested with cued recall. After having spent two hours awake, subjects scored 41.92 ± 4.45 % (mean ± S.E.) correct. When testing words via recognition, subjects correctly chose 71.75 ± 5.89 % (mean ± S.E.) of the words after a nap and 82.31 ± 4.40 % (mean ± S.E.) after a period of wakefulness. N = 15.

The starting condition did not influence results of the delayed test ($p = 0.48$, $t_7 = 2.36$) when words were generated in cued recall nor when words were tested using recognition ($p = 0.63$, $t_8 = 2.36$). No significant differences in scores of the Stanford Sleepiness Scale were observed.

All electrophysiological recordings (polysomnographs) were scored according to (Rechtschaffen and Kales, 1968) and resulted in hypnograms, which were used for statistical analyses. Figure 40 shows an example of the result of scoring a polysomnograph.

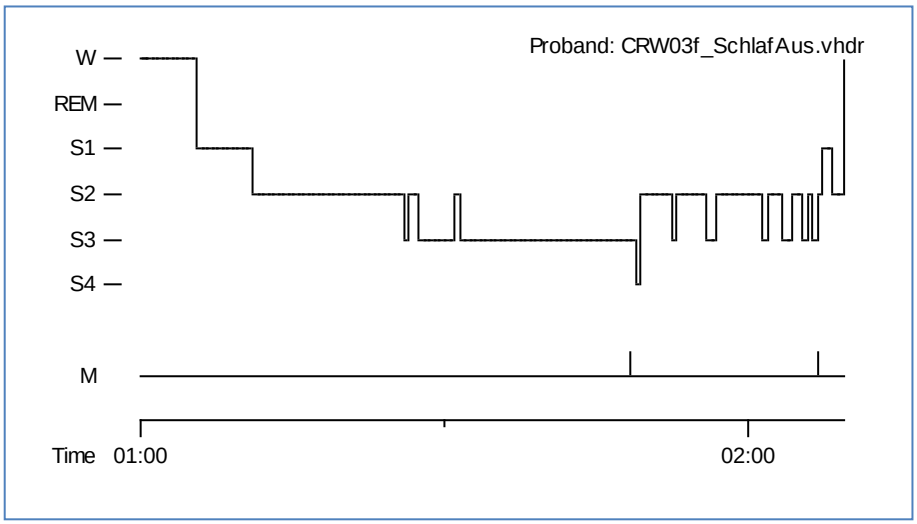


Figure 40: Study 4 – Results – Hypnogram of a representative nap sleeper
The hypnogram represents the subject's sleep stages: W = wake, REM = REM sleep; non-REM stages: S1 = sleep stage 1, S2 = sleep stage 2, S3 = sleep stage 3, S4 = sleep stage 4; M = movement (artifact). The hypnogram was created using the program "SchlafAus", created by Prof. Dr. Steffen Gais. The time of the hypnogram was set to 01:00 at the start of recording and 02:00 indicates the sleep stage 60 minutes after recording onset. The subject was woken by the experimenter from stage 2 sleep.

Latency to fall asleep of the 14 subjects whose data were scored was 14.68 ± 8.18 min (mean ± S.D.). Nap time (the time spent asleep after sleep onset, including arousal and movement time) averaged 56.93 ± 11.80 min (mean ± S.D.). Nap time does not equal 60 minutes since time spent awake (if no further sleep followed), is not counted as arousal. Total sleep time (stages S1, S2, S3, S4 and REM) averaged 45.36 ± 16.06 min (mean ± S.D.). Taking a closer look at the sleep condition, subjects spent the majority of the sleep time in stage 2 (Figure 41).

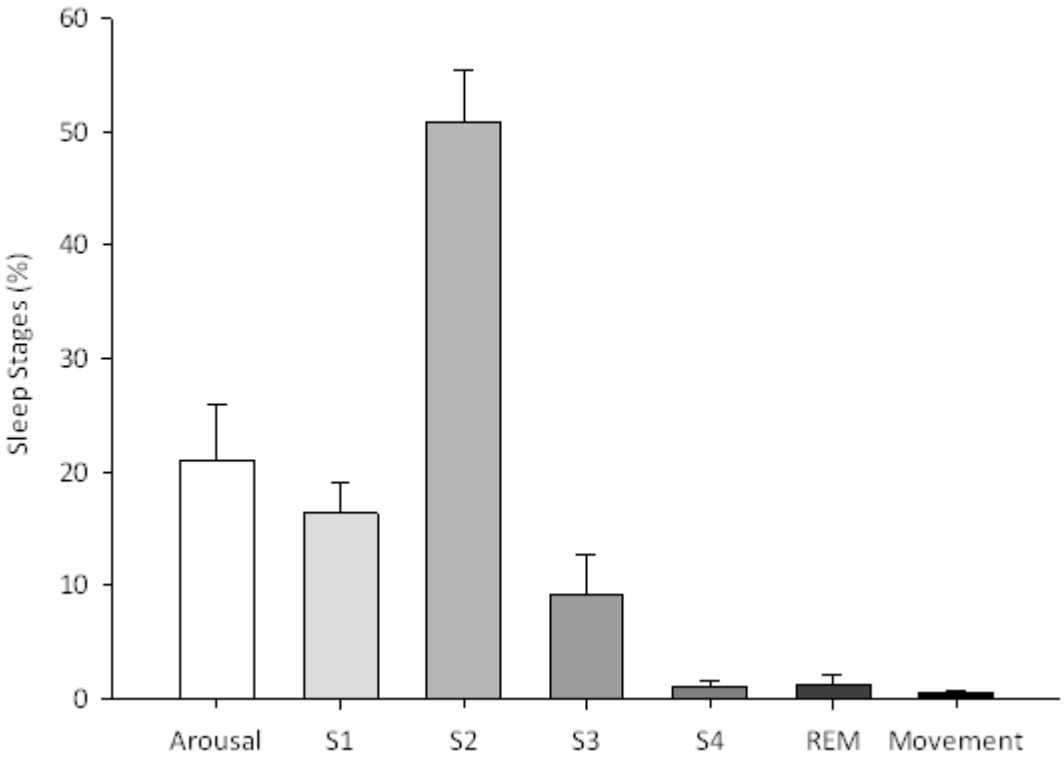


Figure 41: Study 4 – Results – Sleep stages
Distribution of sleep stages: 20.95 ± 4.94 % of the nap was counted as arousal, 16.37 ± 2.76 % were spent in stage 1 sleep, 50.84 ± 4.50 % in stage 2, 9.09 ± 3.58 % in stage 3, 0.99 ± 0.60 % in stage 4 and 1.27 ± 0.86 % in REM sleep. 0.49 ± 0.17 % of the time had to be counted as movement. Sleep stages are abbreviated: S1 = stage 1; S2 = stage 2, etc. Values are presented as mean ± S.E.; N = 14.

The cued recall performance in the delayed test correlated negatively (Pearson's coefficient, r = -0.61, p = 0.02, two-tailed, n = 14) with sleep stage two. A positive correlation with delayed recall scores was found for arousal (Pearson's coefficient, r = 0.57, p = 0.04, two-tailed, n = 14). For both correlations, the percentage of the word pairs recalled correctly at the delayed test compared to the immediate test was calculated and correlated with the according percentage of the nap time spent in stage two or arousal. There were no significant correlations between sleep stages and the recognition test.

5.4 Study 4 – Discussion

The present study investigated whether declarative memory profits from sleep after learning, when sleep comes in form of a midday nap. This study further examined whether a sleep benefit on the behavioral level is specific to the *type* of retrieval test. As in study 3, two types of retrieval tests were examined, cued recall and recognition. According to the dual-process models memory retrieval can be performed by using one of two independent processes, recollection or familiarity (Jacoby, 1991; Tulving, 1985). The cued recall procedure demanded that the subject retrieved the material under the process of recollection, while recognition demanded that the subject retrieved the material under the process of familiarity. In brief, a memory benefit after a 60 minute midday nap could not be demonstrated for the processes of either recollection or familiarity. Familiarity is said to be more sensitive towards time and the resulting forgetting than recollection (Rugg and Yonelinas, 2003). The amount of words forgotten (tested via cued recall) within the two hour retention period of this study is in accordance with the amount of forgetting established by Ebbinghaus (1983) for both the sleep and the wake condition. This study contradicts the findings that sleep benefits recollection (Drosopoulos et al., 2005; Ekstrand, 1967; Fowler et al., 1973; Tali Gorfine et al., 2007; Grosvenor and Lack, 1984; Yaroush et al., 1971).

In our study, sleep in comparison to wakefulness tended to improve the rate of post-sleep learning, but not retrieval performance. Yoo et al. (2007) suggest that sleep before learning prepares the brain for the formation of new memories. This contradicts the theory by Grosvenor and Lack (1984) who argued that pre-encoding sleep compared to wakefulness has a detrimental effect on learning word pairs, leading to more forgetting, but not influencing the rate of learning. The authors did not, however, test 60 min midday sleep but based their theory on four hours of night-time sleep.

Familiarity is a quick process whereas recollection is a slow process (Rugg and Yonelinas, 2003). The time allotted for displaying answer choices when testing via recognition was three seconds in order to avoid too much time for recollection to be started (re-living the situation). However, three seconds were enough time to capture the words displayed on the screen. In the test and interference test no feedback had been given (see procedure). Feedback strengthens memory representations (Lewis et al., 2011a). In study 4, the avoidance of additional strengthening of memory representations aimed to have equal strength of memory for all words, or at least as equal as possible. In study 3 feedback had been given immediately after learning non-sense syllables. Furthermore, syllables not paired correctly were re-presented and tested with feedback until a criterion of 80% correct answers was achieved. This might have led to unequal strengths of memory representations among the set of non-sense syllables learned in study 3. This could have jeopardized results, not showing whether sleep had a beneficial effect on memory per se. From study 4, no such

conclusions can be drawn. As an outlook, at least one additional group of subjects who experiences testing *with* feedback under both sleep and wake conditions would need to be investigated.

Testing the role of sleep in relation to interference, results showed that nap sleep, compared to wakefulness, did not significantly hinder the forgetting of word pairs. This is in accordance with recent findings from a nap study by Diekelmann (2011). Similar to this study, subjects after a period of nap sleep or wakefulness encountered a declarative interference task (visuo-spatial association), and delayed test results show no significant sleep benefit. Only when sleep was coupled with an odor did subjects achieve significantly better results (Diekelmann et al., 2011). Backhaus and Junghanns (2006) who tested related word pairs also could not find a positive effect of nap sleep (45 minutes) on declarative memory in contrast to restful waking. It is possible that memory consolidation took place immediately after the first test (immediate test). In the sleep condition, subjects needed to stay awake for at least half an hour after the immediate test since electrodes needed to be attached to head and face. Already in 1968 Postman and colleagues (Postman et al., 1968) suggested spontaneous recovery from interference through a retention period of 20 minutes of wakefulness and thus Ekstrand et al. (1971) related their subjects' improvements of retrieval performance found in the earlier study (Ekstrand, 1967) not to sleep but to time.

The topic of whether night time sleep is directly comparable to nap sleep is still under debate. At night time, the level of cortisol is lower and the level of melatonin is ten times higher than during midday. When sleeping at daytime, a suppression of cortisol does not occur, which could be a factor hindering memory consolidation. In the evening, low concentrations of cortisol are proposed to have positive effects on memory consolidation (Lupien et al., 2002). When administered during post-learning sleep, cortisol has been found to impair consolidation of word pairs (Plihal and Born, 1999; Plihal et al., 1999). In rats, injections of cortisol following training immediately enhanced memory consolidation while high levels of cortisol at retrieval led to an impairment of water-maze spatial performance (Roozendaal, 2002). However, the same lab that previously mentioned to find a detrimental effect of cortisol (Wilhelm et al., 2011b) saw that administration of cortisol in the evening had no effect on the retrieval (of emotional or neutral texts) in the sleep or wake condition. Thus, memory consolidation could be less effective during midday sleep than at later hours due to the high levels of cortisol.

Melatonin, which is secreted by the pineal gland, has also been proposed to affect the declarative memory system, improving cognitive activity (Furio et al., 2007) and, more specifically, promoting verbal memory retrieval (H. M. Chang et al., 2009; Tali Gorfine et al., 2007; T. Gorfine and Zisapel, 2009). In humans, melatonin receptors have been found in the hippocampus (Mazzucchelli et al., 1996; Savaskan et al., 2005). Animal studies confirmed that the hormone affects synaptic plasticity in

the hippocampus (Baydas et al., 2002) and when given to humans at midday, exogenous melatonin leads to a decreased activation of the left parahippocampus (Tali Gorfine et al., 2007).

In a study with similar learning material (unrelated word pairs), length of retention time (two hours) and time of day for the experiment (1 – 4 p.m.), authors found a tendency ($p = 0.052$) of better performance after nap sleep compared to wakefulness. Authors had tested 20 subjects and recorded the number of correctly recalled word pairs after a one hour nap. An increase in sample size resulted in a more significant difference in performance after nap sleep compared to wakefulness (Tali Gorfine et al., 2007). In contrast, as strong results of the G*power test in study 4 showed, an increase in sample size (even to 100) would not have led to a significant effect of sleep on declarative memory retrieval.

During the 60 minutes of sleep in this study, subjects spent most of the time in stages one and two which is typical for midday naps (Lahl et al., 2008). The short time spent in slow wave sleep could be explained by the low amount of melatonin which affects the circadian rhythm and induces slow wave sleep (H. M. Chang et al., 2009). No positive correlations between sleep stages (one to four and REM) and retrieval results were found and the correlation of stage two sleep with cued recall ability at delayed testing was negative. Although some authors have proposed that stage two sleep, with sleep spindles as its defining feature, is related to consolidation of declarative memories (Schabus et al., 2004; C. Schmidt et al., 2006), memory consolidation cannot be assigned to a specific sleep stage (Diekelmann and Born, 2010). Correlations in the current study show that declarative memory performance, when tested via cued recall, becomes moderately worse with increasing time spent in sleep stage two and moderately better the more of the time allowed for a nap was spent in arousal. Activities of spindles and delta waves during sleep have been found to be influenced by the amount of previous wakefulness and the circadian rhythm (Dijk and Czeisler, 1995).

From this study, it can be suggested that a short period of midday sleep does not improve memory retrieval of unrelated word pairs after interference, but tends to enhance the learning ability of semantic material.

6 Study 5 – Declarative and Procedural Memory Retrieval after 12, 72 and 144 hours of nocturnal Sleep, Sleep Deprivation or Diurnal Wakefulness

6.1 Study 5 – Introduction

In none of the two sleep studies (studies 1 and 4) and neither in study 3 groups II and III, a beneficial effect of sleep on declarative memory could be demonstrated. This entails the question of whether an overt post-learning beneficial effect of sleep on hippocampus dependent memories relies upon a critical time period. Already in 1963, Richardson and Gough found no post-learning beneficial effect of sleep for the intervals of 24 or 48 hours after learning (Richardson and Gough, 1963). Yet, for the interval of 144 hours (6 days), Richardson and Gough detected a significant sleep benefit. In studies 1, 3 and 4 described in this thesis, retention of declarative material was either tested after a relatively short interval (between 12 and 72 hours) or a long retention time (two and a half months). Even earlier than Richardson and Gough, Graves (1937) had published that she could find no positive beneficial effect of sleep before the interval of 72 hours. Thus, the thought arises that the critical period in which a sleep benefit is detectable on the behavioral level, could have been missed in studies 1, 3 and 4. Furthermore, none of the behavioral studies (studies 3 and 4) tested total sleep deprivation at night. Sleep deprivation effects on cognitive performance, including non-sense syllables, were first tested over a century ago by Patrick and Gilbert (1896) and sleep deprivation has been an important part of sleep research ever since. Sleep loss has been found to induce cognitive impairment, although results seem clearer for the effects on task acquisition in case of deprivation *before* learning, than on task retrieval, in case of sleep deprivation *after* learning (for a review on sleep-dependent memory processing see Walker (2008a)).

Albeit the minority of the sleep literature, some sleep deprivation studies examine the role of sleep *before* learning. Sleep loss before encoding can perturb cognitive abilities, such as temporal memory, which is the ability to discriminate the recency of previously learned items (Harrison and Horne, 2000; G. O. Morris et al., 1960). Studying the neuronal effects of 35 hours of sleep deprivation, Drummond et al. (1999) show that during a verbal learning task, the medial temporal lobe fails to function normally, whereas parietal areas try to compensate the dysfunction, and the parietal lobe becomes significantly more active than is necessary under normal circadian circumstances. In a long-term study by Grosvenor and Lack (1984) it was not the mnemonic state *after* learning (post-learning conditional), but whether subjects had slept or stayed awake *before* learning (prior learning conditional) that determined retrieval performance. During retrieval testing 144 hours after learning,

subjects who had been awake before learning, performed significantly better than subjects who had slept before learning. Interestingly, there was no post-conditional effect after 144 hours.

The fact that sleep *after* learning is important for newly acquired memories dates back to Jenkins and Dallenbach (1924) who discovered that subjects who slept after learning had achieved a higher retention rate during a recall than subjects who spent an equal amount of time awake (Jenkins and Dallenbach, 1924). Jenkins and Dallenbach used their findings as support for the interference theory of forgetting, which states that memory traces are only changed during periods of wakefulness, whereas during sleep, traces remain unchanged (Keppel, 1968; Nesca and Koulack, 1994). Thus, sleep was given the role of protection from degradation. Considering the consolidation process, several studies propose that the role of sleep be the opportunity for replay of novel memory traces (Gutwein et al., 1980; Hebb, 1949; Ribeiro, 2012). For example, during slow wave sleep pyramidal neurons of the hippocampus (pace cells) replay activities previously encoded during wakefulness (Girardeau and Zugaro, 2011). However, the beneficial role of sleep has sometimes been questioned. For instance, memory replay has also been found during states of quiet wakefulness in animals (Kudrimoti et al., 1999). Likewise, Gottselig et al. (2004) found that quiet wakefulness led to the same improvements in an auditory sequence task as sleep did.

The benefit of sleep on procedural memory, such as motor skills, has been found to be much more robust than the beneficial effect of sleep on declarative memory. Motor skills can be divided into two classes, motor adaptation and motor sequence learning. Speed and accuracy of motor sequence tasks have been shown to improve significantly after a night of sleep (Walker et al., 2003a; Walker et al., 2002) compared to an equal amount of wakefulness. Whereas some authors in favor of a procedural sleep benefit believe that it correlates with the amount of stage 2 NREM sleep (Walker et al., 2003b), others find a reactivation during REM sleep (Maquet et al., 2000; Tucker and Fishbein, 2009). Others dismiss the enhancement of procedural skills through nocturnal sleep, regardless of the sleep phase (Donchin et al., 2002; R. P. Vertes and Siegel, 2005).

The inconclusive results in the sleep literature as well as those of studies 1, 3 and 4 give rise to study 5, whose aim is to test whether a sleep benefit occurs between a critical period of 12 to 144 hours post learning, compared to wakefulness. Extending the study by Richardson and Gough, study 5 uses a third condition: daytime wakefulness. In contrast to studies 1, 3 and 4, the current study tests memory retrieval under two conditions of wakefulness, daytime *and* nighttime wakefulness. This allows for proper conclusions about circadian rhythm effects. Furthermore, with study 5, the hypothesis stating that sleep after learning has a positive effect on procedural memory shall be tested, by investigating performance on a motor sequence (finger tapping) task, which has been commonly used in sleep research.

6.2 Study 5 – Materials and Methods

The study was performed in accordance with the Declaration of Helsinki (World Medical Association, 2000) and was approved by the ethics committee of the department of psychology of the LMU.

6.2.1 Subjects

Thirty-six right-handed native German speakers (27 females, nine males, mean age ± S.D.: 23 ± 3 years) took part in the experiment. Subjects abstained from drinks and food containing caffeine or alcohol on the day of participation. Starting 5 days prior to the experiment, subjects filled out a questionnaire and a sleep log (see Appendix) for each day until the end of the experiment, documenting their sleep schedule. Participants were free of sleep disorders, did not take psychoactive drugs or medication influencing sleep, and followed a normal sleep pattern of 7 to 8 hours of sleep per night. Further exclusion criteria were transmeridian travel or activities disrupting the sleep-wake cycle (such as shift work) within six weeks prior to study onset.

6.2.2 Learning Materials

6.2.2.1 Declarative Learning Materials

Using Matlab (version R2010, The MathWorks, Inc.), all possible combinations of 3-letter syllables were generated. From the result, only those in the order consonant-vowel-consonant were chosen. Syllables that were words in German, English or French, or which were abbreviations in German, were excluded. From the remaining 711 syllables, 77 syllables were chosen at random and rated by seven test subjects. Test subjects were asked to rate each syllable on its level of difficulty to be remembered. There were three levels of difficulty: easy (= 3), intermediate (=2) and difficult (=1). No further instructions were given. Thirty (30) syllables with a rating between 1.6 and 2.4 (neither too difficult nor too easy) were chosen for the experiment. For each of the 36 subjects, three lists of 10 syllables were created randomly and prior to the first learning session (see section 6.2.3). Subjects learned a different list during each of the three learning sessions. Syllables were presented in white letters of the font “Courier New” in size 120. Instructions were presented in the font “Arial”. Syllables and instructional text were presented in white color on a black background on a 19 inch monitor. Subjects sat in a dark and quiet room at a comfortable viewing distance of approximately half a meter from the screen.

6.2.2.2 Procedural Learning Materials (Finger tapping task)

Six different finger movement sequences (finger tapping sequences) were learned by all subjects taking part in the experiment. Sequences were made up of five digits, each between 1 and 4. The first digit was the same as the last one. Each number referred to a finger of the subject’s non-dominant left hand: 1 referred to the little finger, 2 to the ring finger, 3 to the middle finger and 4 to

the index finger. The thumb was never used. Finger tapping sequences were learned using the four number keys 1 to 4 (row above the letters, not the number pad) of a standard German computer keyboard. The following 6 sequences were used in the experiment: 4-2-3-1-4, 2-4-1-3-2, 2-3-1-4-2, 3-1-4-2-3, 1-3-2-4-1, 1-4-2-3-1. The first 3 sequences will be referred to as “main sequences”, the others as “new sequences”. Stimuli were presented in white characters of the font type Arial (size 60) on a black background.

6.2.3 Procedure

The experiment was a design using one between-subject factor and two within subject factors. All subjects underwent three conditions: Sleep at night, sleep deprivation at night or wakefulness during the day. Each condition included two sessions, a learning session and a retrieval session. Thus, each subject took part in six sessions throughout the entire study. In order to avoid unwanted intrusions from prior sessions, conditions were separated by five days. Subjects came to the laboratory for learning sessions, then either went home to sleep, stayed awake in the sleep laboratory at the LMU or stayed awake during the day, going about their typical daily chores. When awake at night, subjects watched PG-rated videos or played board games from 11 p.m. to 8 a.m. After the sleep deprivation, subjects were allowed to go to bed after 8 p.m. Actimeters (ActiSleep Sleep Monitor, *ActiGraph LLC.*, Pensacola, USA), worn on the left arm, recorded subjects' motor activity. The actimeter served to detect possible periods of sleep and was worn during the night in which subjects were deprived of sleep. In order to ensure that subjects did not take a nap in the time window between the end of the learning session and the beginning of the night of sleep deprivation, subjects wore the actimeters starting immediately after the learning session.

After a given time following the learning session, subjects returned for a retrieval session. Three groups of 12 subjects were formed according to the retention time. Retention time was defined as the time between learning and retrieval in one condition. Retention times were 12 hours, 72 hours (3 days) or 144 hours (6 days). Each subject was assigned to a retention time and it was held constant over his/her three conditions (Figure 42 and Figure 43). The three groups will be referred to as 12h group, 72h group and 144h group.

SLEEP CONDITION			Five Days Later	SLEEP DEPRIVATION CONDITION			Five Days Later	WAKE CONDITION		
Evening	Night	Morning		Evening	Night	Morning		Morning	Day	Evening
Learning	Sleep	Retrieval		Learning	Wake	Retrieval		Learning	Wake	Retrieval

Figure 42: Study 5 – Study design for the 12h group
All subjects underwent the same three conditions: 1) Sleep condition, in which subjects slept at night (black); 2) Sleep deprivation condition in which subjects stayed awake at night (dark grey) or 3) Wake condition in which subjects stayed awake during the day (white). Conditions were separated by five days. In the sleep condition (black) and in the sleep deprivation condition (grey), subjects learned in the evening and returned 12 hours later in the morning for the retrieval session. In the wake condition (white) subjects learned in the morning and returned 12h later in the evening for the retrieval session. During learning, subjects first performed a procedural task, then a declarative task. At retrieval subjects were first tested on the declarative, then the procedural task. The order of the conditions was randomized and balanced across subjects of a group.

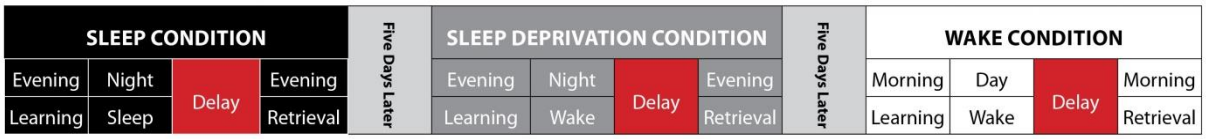


Figure 43: Study 5 – Study design for the 72h and 144h groups
Since the study design of both groups differed only in the delay (interval between learning and retrieval, depicted in red), one design is depicted for both groups. All subjects of both groups underwent the same three conditions: 1) Sleep condition, in which subjects slept at night (black); 2) Sleep deprivation condition in which subjects stayed awake for a full night (dark grey) and 3) Wake condition in which subjects stayed awake during the day (white). Conditions were separated by five days. The order of the conditions was randomized and balanced across subjects of a group. In the sleep condition (black) and in the Sleep Deprivation Condition (grey), subjects learned in the evening and returned after a delay (of 72 or 144 hours) for the retrieval session. For example, for the sleep condition subjects of the 72h group learned in the evening, slept for a full night and returned to the laboratory 72 hours later for retrieval in the evening. In the wake condition (white) subjects learned in the morning and returned after a delay (of 72 or 144 hours) in the morning for the retrieval session. During learning, subjects first performed a procedural task, then a declarative task. At retrieval subjects were first tested on the declarative, then the procedural task.

The order of the conditions was randomized and balanced across subjects of a group. Learning and retrieval sessions took place at the Department of Psychology, LMU, Munich. Subjects learned and were tested in groups with a maximum size of five subjects[19]. As starting time of a session, subjects could choose among three time slots: For sessions in the morning, subjects started at 8 a.m., 9 a.m. or 10 a.m. For sessions in the evening, subjects started at 8 p.m., 9 p.m. or 10 p.m. Each subject kept the chosen time constant for all conditions. In the sleep condition and sleep deprivation condition, subjects learned in the evening. The learning session of the wake condition took place in the morning. In the sleep and sleep deprivation conditions, retrieval was performed in the morning. In the wake condition, the retrieval session took place in the evening.

All subjects gave consent to participate in the study. After a general introduction about the experiment, subjects were seated at a desk with a computer monitor. At the first session, subjects started with two tests of intelligence, the German verbal Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B) and the non-verbal Zahlen-Verbindungs-Test (ZVT). The order of the two tests was randomized and balanced across subjects. The test was instructed according to the guidelines of the test manuals for the MWT-B (Lehrl, 2005) and ZVT (Oswald and Roth, 1987). In all other sessions, the IQ tests were not repeated. In all sessions, subjects engaged in two learning tasks, one procedural and one declarative task. Instructions were presented on a monitor and read aloud to each subject by the instructor. Each subject repeated the information to the instructor to demonstrate comprehension.

[19] In order to avoid human bias, only the computer (see Learning Materials) was used for learning and testing of the finger movement sequences and the syllables.

6.2.3.1 Learning session

Subjects learned two tasks. First, subjects learned a finger movement sequence (procedural task) afterwards they learned syllables (declarative task).

Procedural task: Before starting with the actual task, subjects practiced finger movements using the trial sequence 1-1-2-3-4. When subjects felt comfortable with the trial sequence, they started with the main sequence: One of the three main sequences was chosen at random. The stimulus, e.g. 2-4-1-3-2 was displayed on the monitor for 30 seconds and the subject had to type the sequence as fast and as accurately as possible. A pause of 30 seconds followed and feedback about the amount of sequences typed (correct or incorrect) as well as the amount of correct sequences typed, was given. Subjects completed 12 rounds of finger tapping and pauses (12 minutes total), using the same sequence. In each condition, subjects learned a different main sequence. The order of the sequences was randomized across conditions and subjects.

Declarative task: Ten non-sense syllables were presented serially for 1.6 seconds and an inter-stimulus interval of 0.8 seconds. Thereafter, a free recall started, during which subjects had to type as many of the ten syllables as they remembered, independent of the order of presentation. No feedback was given. After each recall, the presentation of the list of ten syllables was repeated. The order of syllables stayed constant for all repetitions. The criterion of this session was to recall 100% of the syllables correctly in three consecutive recalls. The number of rounds needed to reach the criterion was recorded and will be referred to in later sections as the learning rounds. Once subjects reached the criterion, the learning session was considered complete. Concerning the learning sessions in the evening, information as to whether the session was followed by sleep or sleep deprivation was revealed after the learning session.

6.2.3.2 Retrieval session

Subjects first performed a declarative, then a procedural task.

Declarative task: First, subjects underwent one round of free recall in which they needed to type as many syllables as they remembered from the previous learning session. Rounds of presentation of all ten syllables and their free recall followed in the same manner as during the learning session. The number of rounds needed to reach the criterion (100% correctly recalled syllables in three consecutive recalls, including the first round) was recorded. This number will be referred to in later sections as the recall rounds. Thereafter, subjects continued with the procedural task.

Procedural task: As in the learning session, subjects tested comprehension of the task using the trial sequence 1-1-2-3-4. Thereafter, subjects started with the actual task: The goal of the task was to repeat the sequence which the subject had acquired during the previous learning session. The

sequence was displayed on the monitor for 30 seconds and the subject had to type the sequence as fast and as accurately as possible, as during the learning session. A pause of 30 seconds followed and feedback about the amount of sequences typed (correct or incorrect) as well as the amount of correct sequences typed were recorded and displayed during each pause. Subjects completed three rounds of finger tapping and pauses (three minutes total). Afterwards, one of the new sequences e.g. 1-4-2-3-1 was learned. A new sequence was tested in order to find out whether an improvement in accuracy is specific for a certain condition or if procedural improvement occurs in general, i.e. after sleep finger tapping ability is improved, regardless of whether the sequence was learned before sleep.

The new sequence was treated in the same manner as the main sequence: Subjects completed three rounds of finger tapping and pauses (three minutes total). Thereafter, the retrieval session was complete. During each of the three retrieval sessions of the experiment (one per condition), subjects learned a different new sequence. The order of the sequences was randomized across conditions and subjects.

6.2.4 Analysis and Statistics

One subject was excluded from analyses because she did not participate in the wake condition. Sample sizes used for analyses were 12 for the 12h and 72 h groups, and 11 for the 144h group. Data were recorded using Matlab (version R2010, The MathWorks, Inc.), analyzed using IBM SPSS Statistics (version 17.0), Matlab (version R2010, The MathWorks, Inc.) and Microsoft Excel 2007 and graphed using SigmaPlot (versions 11.0). Repeated measures ANOVA and paired student t-tests (two tailed) were performed. For ANOVA, the within-subject factor was the condition, which had three levels: sleep, sleep deprivation and wake. The between-subject factor was the retention time: 12h, 72h and 144h.

As a performance measure of the declarative task, the *savings score* (in percent) was calculated: Savings Score = (LR - RR) / LR x 100%, where LR = learning rounds; RR = recall rounds. (See descriptions of the declarative task for the definition of the scores).

As a performance measure of the procedural task, subjects' accuracy was used since it best represents the performance of the subjects, incorporating also the speed and the errors made. The accuracy represents the mean number of correctly typed sequences. Since subjects performed 12 rounds of the main sequence during the learning session and three rounds during retrieval, the mean of the last three (of 12) rounds was used as accuracy measure during the learning session. As a measure of speed, the mean of the total number of sequences typed was calculated. For the new sequence, the mean of all three rounds was calculated. If the outcome of Mauchly's test of sphericity

was significant, such that the assumption of homogeneous variances and covariances had to be rejected, the Greenhouse-Geisser values of the ANOVA were chosen.

6.3 Study 5 – Results

6.3.1 Declarative Task

In the declarative part of this study, free recall performance of ten non-sense syllables that had been learned either 12 hours, 72 hours or 144 hours (each by a separate group of subjects) before, was analyzed for the three post-learning conditions: sleep, sleep deprivation and wake.

As expected, no effects during the learning session, pre-conditional effects, were found: A group by condition interaction yielded no significant results (ANOVA, $F(2,2) = 0.34$, $p = 0.85$), (Figure 44). There were no within-subject effects during the learning session (ANOVA, 12h group: $F(2,2) = 3.44$, $p = 0.59$; 72h group: $F(2,2) = 1.5$, $p = 0.26$; 144h group: $F(2,2) = 0.83$, $p = 0.44$).

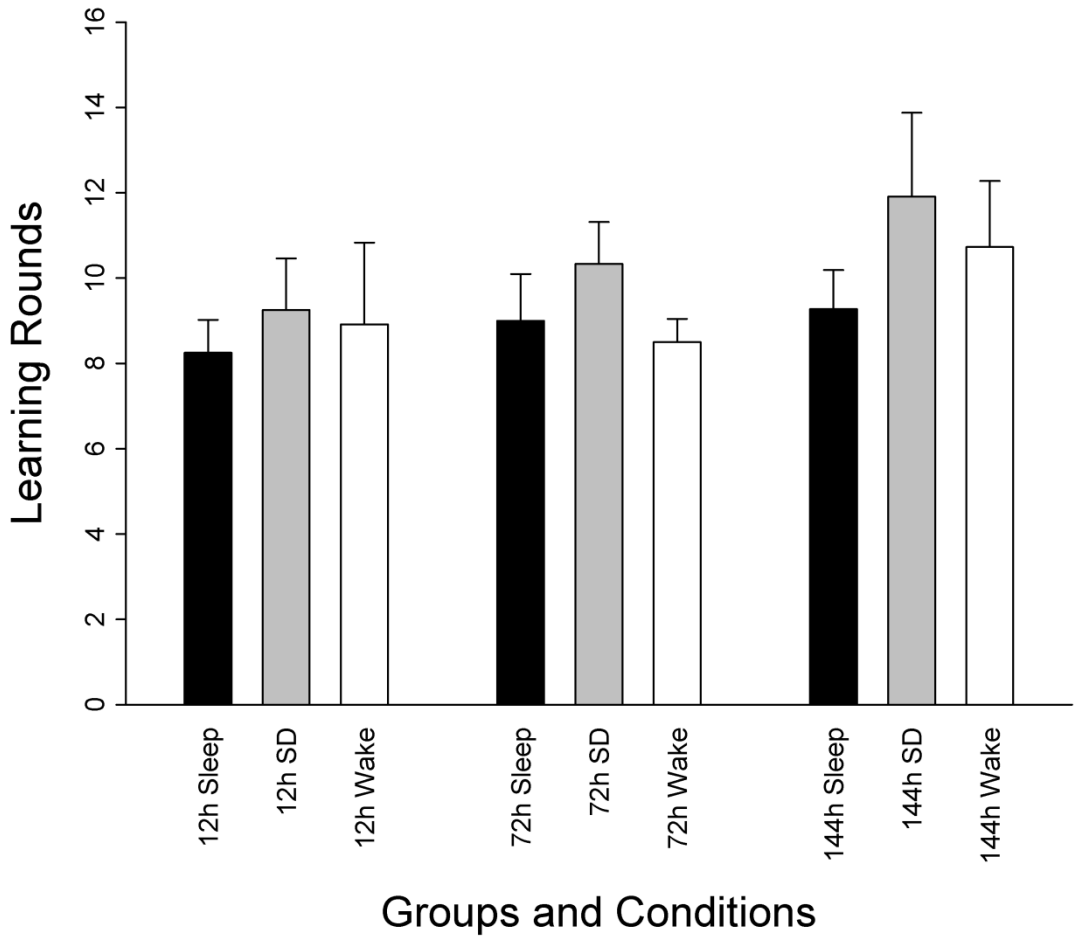


Figure 44: Study 5 – Results – Declarative task – Learning rounds
Number of rounds subjects needed in order to reach the learning criterion of 100% correctly recalled (free recall) non-sense syllables. Presented are the mean values and standard error of groups, for each condition (Sleep, SD = sleep deprivation, and wake). N = 12 for the 12h and 72h groups and n = 11 for the 144h group.

6.3.1.1 Declarative Recall

A group by condition interaction yielded no significant results (ANOVA, F (2,2) = 0.54, p = 0.71). The factor condition (sleep, sleep deprivation or wake) did not influence declarative recall results (Figure 45), (ANOVA, F (2,2) = 1.03, p = 0.15). The factor retention time, tended to influence declarative recall results (ANOVA, F(2,2) = 0.06). A Tukey's post-hoc test was not significant.

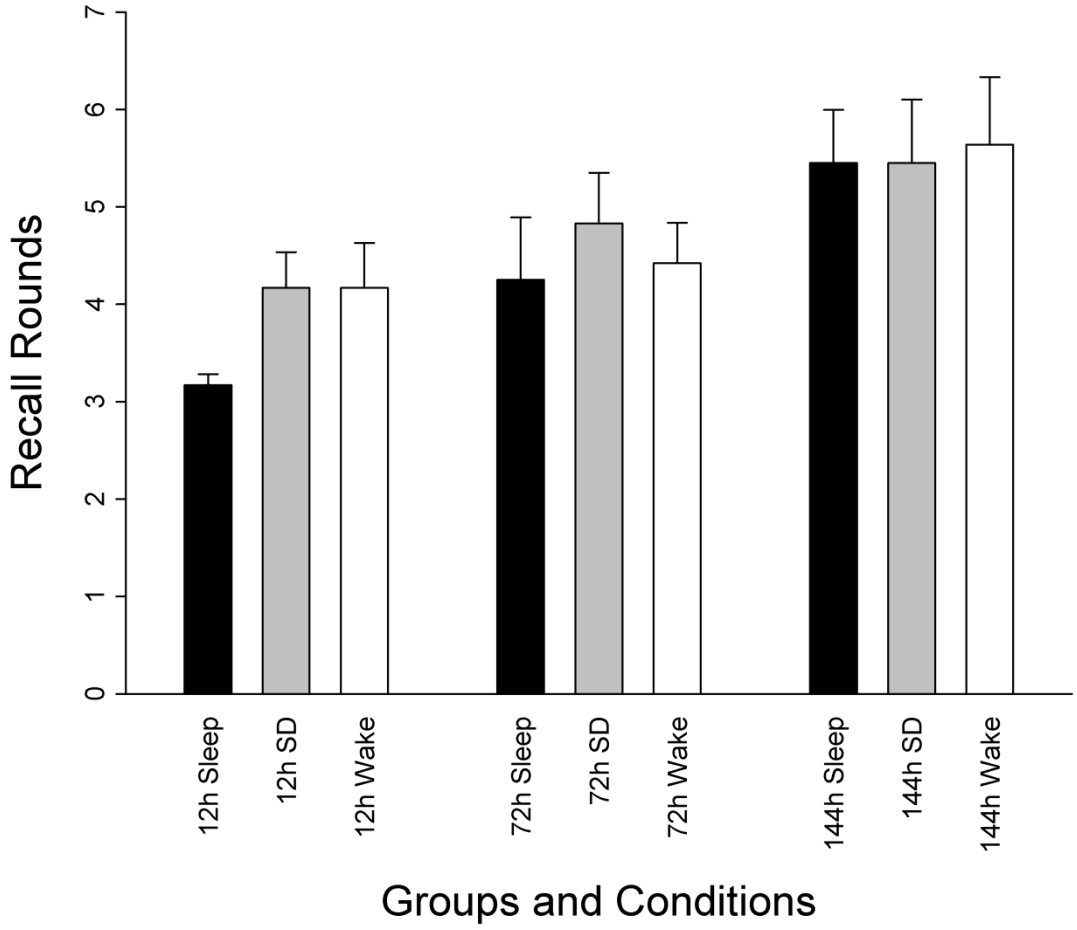


Figure 45: Study 5 – Results – Declarative task – Recall rounds
Free recall of declarative material: Depicted are the mean and standard error of the recall rounds (see methods for calculation of this score) of all groups, for all three conditions (sleep, SD = sleep deprivation and wake). N = 12 for the 12h group and the 72h group. N = 11 for the 144h group.

Likewise, recall rounds did not differ significantly across the three conditions (Figure 45), (ANOVA F (2,2) = 1.37, p = 0.26). Yet, recall rounds differed across groups and the between-subject effect (retention time) was significant: F(2,2) = 4.93, p = 0.01 (not indicated). A Tukey's post-hoc test revealed that the difference between the means (of the recall rounds) of the 12h and 144h groups was significant (Tukey HSD: p = 0.01). During free recall, subjects needed significantly more rounds to remember 100% of the learned non-sense syllables after a retention time of 144 hours than after 12 hours.

A group by condition interaction did not yield significant results (ANOVA, F (2,2) = 0.53, p = 0.72). The savings score (Figure 46) serves as a measure of declarative recall performance. This score did not differ significantly across the three conditions, (ANOVA F (2,2) = 0.17, p = 0.84). Savings scores also did not differ across groups: F(2,2) = 1.10, p = 0.35.

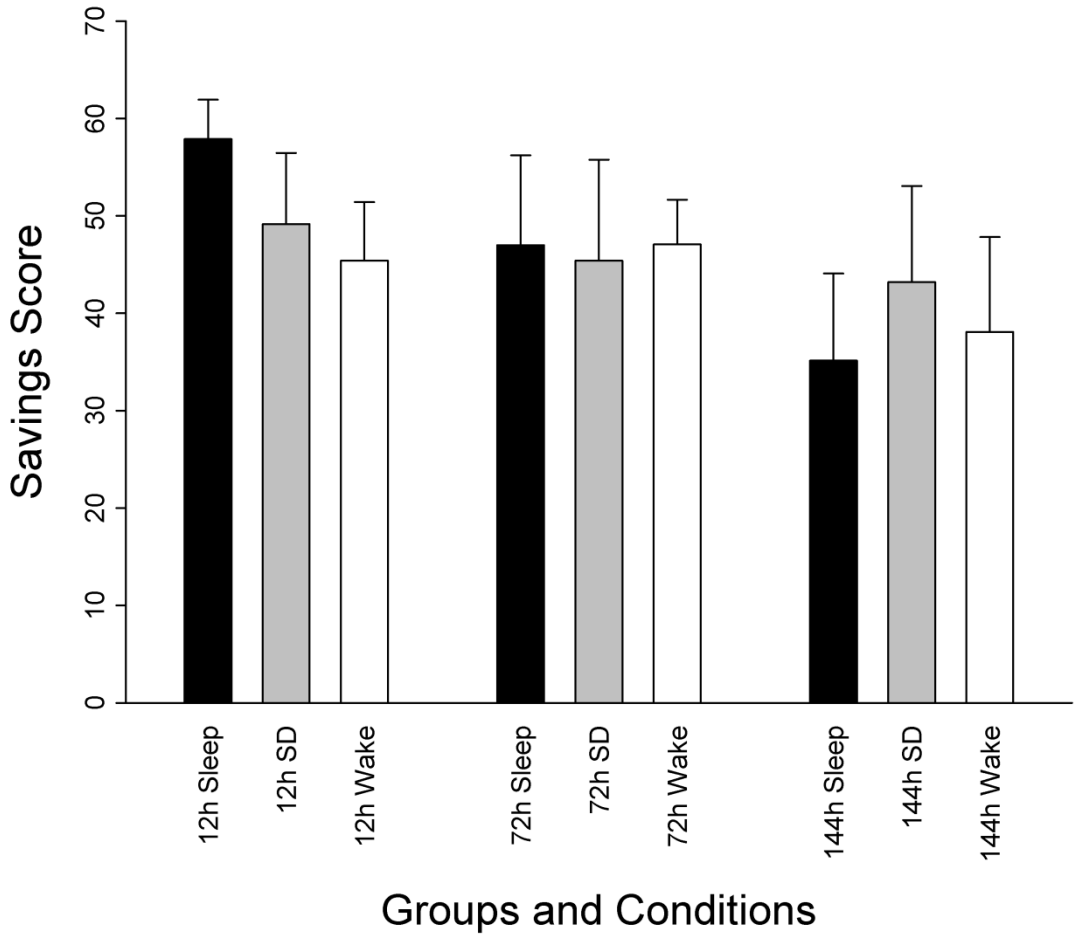


Figure 46: Study 5 – Results – Declarative task – Savings score
Savings score of declarative material: Depicted are the mean group savings score (in percent) and standard error (see 6.2.4 for calculation of this score) for all three conditions (sleep, SD = sleep deprivation and wake). N = 12 for the 12h group and the 72h group. N = 11 for the 144h group.

6.3.2 Procedural Results

6.3.2.1 Accuracy

In each group, the accuracy of the procedural task was measured in each condition (sleep, sleep deprivation and wake) for all three groups (12h group, 72h group and 144h group): Accuracy and speed of a main sequence were tested before the retention time (*pre*), a second time after the retention time (*post*), and a third time for a new sequence (*new*) after the retention time.

The difference between post- and pre-accuracy results (improvement of accuracy) was calculated (Figure 47). In all conditions, subjects improved over the interval. However, accuracy of the procedural task was not significantly improved after a period of sleep, compared to results of the sleep deprivation and wakefulness conditions: The interaction between the factor condition and the factor retention time was not significant (ANOVA, $F(2,2) = 2.10$, $p = 0.11$). No significant within-subjects effect was found, (ANOVA, $F(2,2) = 0.37$, $p = 0.69$). A new sequence had been tested in case significant effects were found. However, the case did not occur. (The new sequence would have helped to find out whether an improvement in accuracy is specific to a certain condition or if procedural improvement occurs in general, i.e. after sleep finger tapping ability is improved, regardless of whether the sequence was learned before sleep. Since subjects improved over the interval in *all* conditions, results of the new sequence are not helpful.)

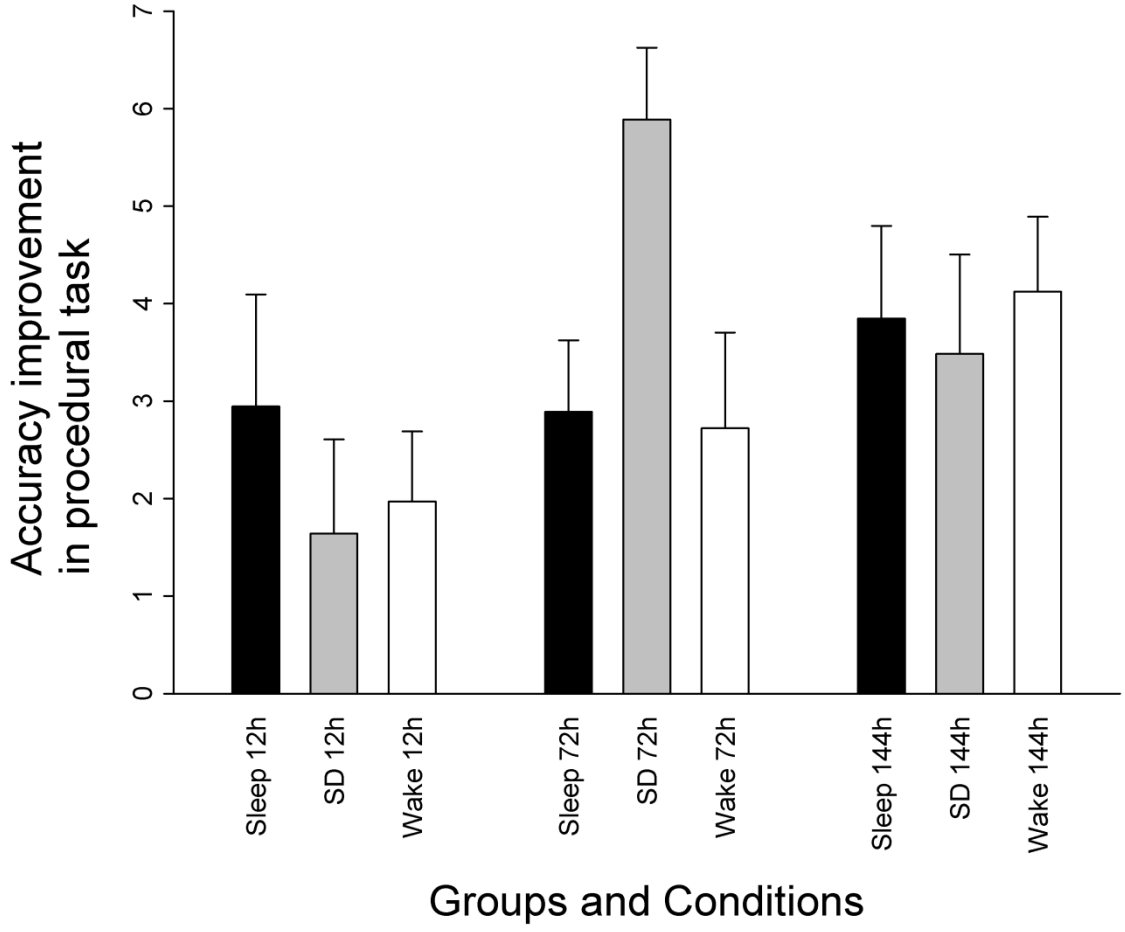


Figure 47: Study 5 – Results – Procedural task – Accuracy improvement
Improvement of accuracy in the procedural task. Graphs present the difference between accuracy results of the main sequence over the retention time (accuracy after the retention period minus accuracy before the retention period). Depicted are means and standard error of means for the improvement of correctly typed main sequences (differences between post- and pre-results). Graphs present means for the three groups (12h, 72h and 144h group) and each condition (sleep, SD = sleep deprivation and wake). Sample size (N) of the 12h group is 12; N for the 72h group is 12; and N for the 144h group is 11.

6.3.2.2 Speed

In general, subjects improved in speed over the retention time (Figure 48). Considering speed improvement (comparing *post* to *pre* speed) of the main sequence, a group by condition interaction yielded a significant difference ANOVA, F (2,2) = 3.63, p = 0.01. A one-way ANOVA showed significant results in the improvement of sleep for the sleep deprivation condition (ANOVA, F (2, 32) = 3.7, p = 0.04). For the sleep deprivation condition, a Tukey's post-hoc test revealed a significant difference between the 12h and 72h groups. When testing speed immediately after a night of sleep deprivation (12h group), its improvement was significantly lower than when testing was performed after two recovery nights (72h group), (Tukey, HSD: p = 0.04).

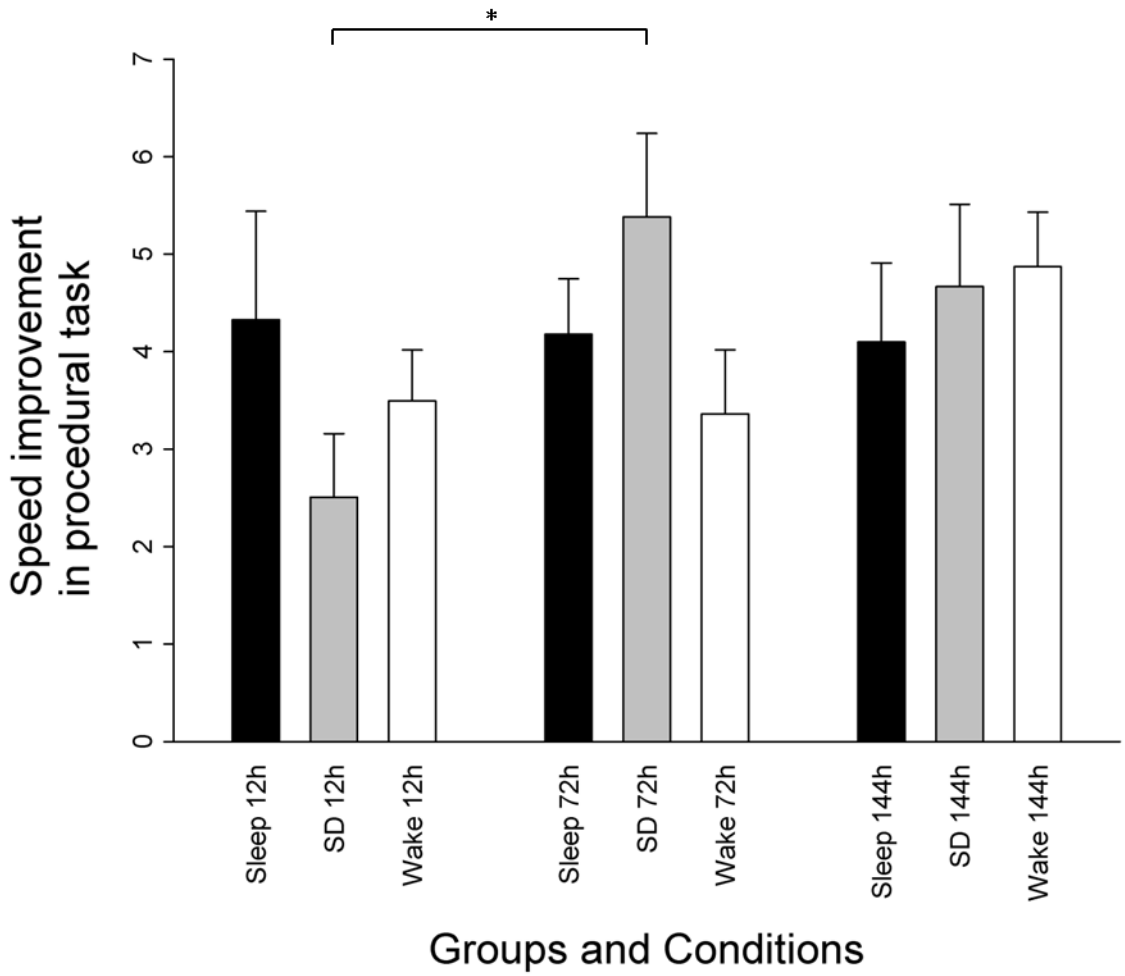


Figure 48: Study 5 – Results – Procedural task - Speed
Improvement of speed in the procedural task. Graphs present the difference between speed results of the main sequence over the retention time (speed after the retention period minus speed before the retention period). Depicted are means and standard error of means for the improvement of correctly typed main sequences (differences between post- and pre-results). Graphs present means for the three groups (12h, 72h and 144h group) and each condition (sleep, SD = sleep deprivation and wake). Sample size (N) of the 12h group is 12; N for the 72h group is 12; and N for the 144h group is 11.

A group by condition interaction for the variable speed of the *new* sequence yielded no significant difference (ANOVA, F (2,2) = 1.24, p = 0.305). The condition (sleep, sleep deprivation or wake) did not

have an effect on the speed of the new sequence in any of the three groups. Post-hoc t-test showed no significant difference between the conditions sleep and sleep deprivation of the 12h group ($p = 0.09$). For detailed values of accuracy and speed of main and new motor sequences in study 5 see appendix section 10.7.

6.3.2.3 Correlations

No significant correlation was found between accuracy improvement and MWT or ZVT scores. ZVT scores correlated moderately and positively with accuracy improvement after sleep in the 12h group (Pearson's coefficient, $r = 0.57$, $p = 0.06$, two-tailed, $n = 12$). There was no significant correlation between MWT and ZVT scores (Pearson's coefficient, $r = -0.22$, $p = 0.19$, two-tailed, $n = 35$).

6.4 Study 5 – Discussion

Consolidation is the post-acquisitional strengthening of labile memories. Study 5 investigated whether nocturnal sleep (in comparison to total sleep deprivation or daytime wakefulness after acquisition) supports consolidation, leading to improvements in declarative and / or procedural task performance. Memory performances after the critical time intervals of 12, 72 or 144 hours, without further practice between learning and retrieval, were tested. Overall, no beneficial post-learning effect of sleep could be detected in the declarative and procedural tasks over the retention interval of up to six days. Decay of declarative memory, also found in other declarative memory studies (Kopasz et al., 2010) increased over time in study 5, but did not differ between the three post-learning conditions. Pilcher and Huffcutt (1996) who conducted a meta-analysis on 19 studies in the field of sleep research, note that sleep deprivation has a much smaller effect on cognitive performance and motor functions than on fatigue and mood. In accordance with the results of study 5, several studies (most of them published in the 1960's and 1970's) report no difference in verbal memory performance after post-training REM sleep deprivation (Chernik, 1972; Meienberg, 1977). Furthermore, when depriving subjects of REM sleep, their performance to recall paired associates was as good as that of control subjects who received no sleep deprivation (Chernik, 1972).

In study 5, declarative learning was preceded by a procedural task. In contrast to the study by Brown and Robertson (2007), sleep did not lead to a difference in performance, compared to sleep deprivation or wakefulness. In the study by Brown and Robertson (2007) subjects learned a declarative task (single words) and then proceeded with a serial reaction time task (containing a 12-item sequence). The authors show that subjects' performance at the procedural task, when followed by a declarative task, improved significantly after sleep but not after a period of wakefulness (when re-tested 12 hours after learning). The authors argue that declarative learning interferes with the consolidation of motor skills, and that sleep – in contrast to wakefulness – retroactively disinhibits motor skill improvement. Furthermore, Brown and Robertson (2007) assume that sleep functionally disconnects declarative and procedural systems, so that they can operate independently.

The only significant finding of study 5 was found in the speed improvement of the motor task, in which subjects improved significantly less when tested immediately after a night of sleep deprivation. Since the accuracy of the motor task was not affected, it is likely that slower finger movements were a result of tiredness. According to Yoo et al. (2007), sleep deprivation is associated with stress and whether results achieved after a night of sleep deprivation can be assigned to the lack of sleep or the resulting stress is controversial.

The results of study 5 do not support the view that overnight sleep leads to a stable improvement of motor skills. On one hand, this is surprising because especially motor skills seem to be sensitive

towards a beneficial effect of sleep (Robertson et al., 2004; Walker et al., 2003b). On the other hand, results of study 5 are in congruence with recent studies, such as that of Geisler (2011) who assessed procedural memory using a similar design to that of Brown and Robertson (2007), and could not find a *general* sleep-dependent improvement at re-testing of motor sequences. This was surprising to the author since procedural memory is considered sensitive towards the effect of sleep. For example Walker writes that the enhancement of memory consolidation depends on sleep (Walker et al., 2003a). In their study Walker et al. (2002) found that participants performed a certain motor sequence task 20 percent faster after a night of sleep.

In their recently published article Schönauer et al. (2014) agree that sleep facilitates procedural memory consolidation. Beneficial effects of sleep could be detected when procedural memory learning was coupled with auditory signals. No such effect could be demonstrated *without* auditory coupling (Geisler, 2011; Schönauer et al., 2014). Subjects learned a serial reaction time task (Robertson, 2007) on a keyboard. Similar to playing the piano, each element of the presented sequences was linked to a tone. During subsequent sleep or wakefulness, half of the tone sequence was re-played (via ear plugs or loud speakers) for two hours. Subjects were then tested on the "sequence-halves" that had been reactivated and those that had not. Results showed that subjects who slept were significantly better during the recall of reactivated sequence-halves than subjects who stayed awake. However, subjects who stayed awake performed equally well in the test of sequence-halves that had not been reactivated, as subjects who slept. This is in congruence with the findings of procedural tests (study 5).

Other studies on procedural memory, not testing finger sequences, report the same lack of sleep enhancement. For instance, Donchin et al. (Donchin et al., 2002) tested the effect of pharmacological agents on motor memory. Subjects received training of certain arm movements (reaching movements in velocity-dependent force fields) and were tested 24 hours later. Amongst their six groups of subjects, two groups did not receive pharmacological drugs: a sleep deprived group and a control group (sleep group) whose subjects slept normally. Both the sleep deprived and sleep groups performed equally well at testing.

In their article Yoo et al. (2007) examine whether sleep *before* learning is essential in preparing the brain for encoding. In study 5, subjects either learned in the morning or in the evening. Those who learned in the morning had spent the night before asleep, and for learning sessions in the evening, subjects had spent the day awake. In this study, sleep before learning declarative material did not affect the number of repetitions (rounds) needed to learn ten non-sense syllables. Likewise, animal studies investigating hippocampus dependent learning, support the notion that sleep deprivation prior to learning does not always disrupt acquisition (Hicks et al., 1973; Plumer et al., 1974).

Whereas Yoo et al. (2007) investigated the effect of pre-training sleep on declarative memory *acquisition*, Grosvenor and Lack (1984) focused on the effect of sleep on memory *retrieval*. Grosvenor and Lack (1984) proposed that sleep before learning had a detrimental effect on retention. In their study, subjects were divided into four groups, using the four possible combinations that can be created from the prior-learning condition (sleep or wakefulness) and the post-learning condition (sleep or wakefulness). Subjects learned word pairs during the night and recalled them four hours later (initial test) and again after six days (retest). Grosvenor and Lack (1984) found a significant prior learning effect: Those subjects who had slept before learning performed *worse* during the initial test and retest than subjects who had stayed awake. Regarding the long-term effect of post-learning sleep, there was no difference in performance between subjects who had stayed awake and those who had slept after learning, which is in congruence with the findings of study 5. Several studies in the 70s demonstrated a detrimental effect of sleep prior to learning on long-term recall, although sleep before learning had no effect on learning rates or immediate recall of items. (Shearer, 1973; Stones, 1973). Since study 5 did not aim at testing prior learning conditional effects on long term retention, it is possible that a prior learning effect could have been found, if the study had excluded circadian influences due to different times of learning sessions: Subjects in study 5 who slept before learning, had their learning session in the morning whereas subjects who stayed awake before learning, had their learning session in the evening. G. Ficca et al. (2000) postulate that the prior-learning condition disturbs recall of declarative material only when the sleep cycle is not intact. This contrasts the belief that sleep can be simply split into two halves, the first half dominated by slow wave sleep, promoting declarative memory consolidation and the second half dominated by REM sleep, promoting procedural memory consolidation (Plihal and Born, 1997; Plihal et al., 1999). According to Peigneux et al. (2001), all stages of sleep are involved in learning and memory consolidation, and declarative memory systems cannot be clearly attributed to slow wave sleep only, and nondeclarative systems to REM sleep functions.

Thus, procedural results of study 5 support the assumption drawn from the declarative results of this and the other two behavioral studies (studies 3 and 4), that behavioral memory results do not overtly reflect a sleep related enhancement.

7 General Discussion

The inconsistency of published results in studies examining memory consolidation through sleep gave rise to this thesis. Whereas a multitude of studies give evidence for a positive effect of sleep on declarative memory and procedural memory [for a review see Diekelmann and Born (2010) and (Diekelmann et al., 2009)], others show that a period of sleep is not superior to a period of wakefulness (R. P. Vertes, 2004; R. P. Vertes and Siegel, 2005). From recent findings reported in sleep literature it can be concluded that the benefit from sleep on memory seems to be selective (Schönauer et al., 2014) and the exact factors leading to a benefit of sleep are still unclear.

An important finding was drawn from the only study not involving the topic of sleep (study 2). This study investigated in more detail the role of the hippocampus in episodic memory, tackling the ongoing debate of the diverse functions which have been attributed to this brain structure. The hippocampus has been argued to be crucial for spatial information processing and storage (O'Keefe and Nadel, 1978), remembering object relations (Cohen, 1993) and autobiographical memory (Nadel and Moscovitch, 1997). From study 2 it can be inferred that declarative memory is rapidly consolidated and spatial memory becomes independent of the hippocampus within half an hour after encoding. Interestingly, recall of autobiographical but not sequential or spatial memory tasks required the hippocampus thirty minutes post memory acquisition.

This thesis comprises five memory studies (studies 1 to 5), all of which are based on behavioral data acquired during the retrieval of declarative (all studies) and procedural (study 5) memory after different time intervals. Furthermore, two studies offer neuroimaging results acquired during declarative memory recall (studies 1 and 2). This thesis provides one of only a few investigations studying the effect of sleep in declarative memory recall that involve *all* of the following elements: fMRI, sleep deprivation and a time span of over two and a half months: The studies here offer results of declarative memory tests as early as 30 minutes and as late as two and a half months after encoding. It shall be noted that the two neuroimaging studies of this thesis were among the first to successfully apply an event related overt recall procedure for fMRI acquisition (for more details see sections 2.2.3.2 and 3.2.3.3).

With the exception of study 2, central to all studies is the relationship between sleep and memory. The memory tested in all studies was declarative memory and thus sleep and declarative memory, specifically episodic memory, will be the main point of this general discussion.

7.1 The role of the Hippocampus in Episodic memory retrieval

Episodic memory is the part of declarative memory which provides contextual information of when, what and where an episode was learned. Each time we acquire new information, regardless of its nature (pictures, words, routes, etc.), episodic memory of the event is formed and stored. The neuroimaging results of this thesis showed that the hippocampus is not accessed equally for all types of episodic memory recall. Instead, it acts more specifically, and was found to be required for an autobiographic but not a spatial or purely associative (sequential) memory task: Results of study 1 suggest that remote episodic memories do not become completely independent of temporal lobe structures. Study 2 further narrowed the role of the hippocampus to the recall of autobiographical associations. Together, this thesis supports the multiple trace theory, established by Nadel and Moscovitch (1997), who proposed that personally experienced episodes stay hippocampus dependent, whereas semanticized memories (see section 1.1) become hippocampus independent over time. It was further demonstrated (through study 2) that consolidation is a rapid process in which non-autobiographic information achieves hippocampal independence within half an hour after acquisition, contradicting the theory of O'Keefe et al. (1978) who proposed that the hippocampus is permanently accessed for spatial memory retrieval.

According to Eichenbaum (2000), the hippocampus binds new information coupled with an episode into a network of existing memory traces. If the hippocampus were required for the retrieval of any declarative memory association, it should have been activated in all three tasks tested in study 2. However, this was not the case. Hippocampal activation could not be found for the recall of associations in which subjects made mere word associations(sequential task), mentally connecting words with other words learned through rote repetition and without bringing the words into relation with another context, such as spatial or autobiographic information. Thus, the view that the hippocampus is required for any associations (Eichenbaum, 2000) also during recall is contradicted by the results in study 2. However, this study does not deny that the hippocampus holds an associative function in the memory process as a whole.

Interestingly, the recall of spatial associations in study 2 did not require the hippocampus: The spatial task required subjects to mentally navigate among the routes which they had chosen. Recall activated the medial temporal gyrus, but not the hippocampus (for exact areas of activation see 3.3.1). At a first sight, advocates of the theory that the hippocampus is required for spatial memory contradict the results of this study. However, when the materials and methods applied are critically considered, conclusions about the function of the hippocampus cannot be generalized. For example, several studies make conclusions about the function of the hippocampus based on the encoding process (Henke, 2010; Schacter and Wagner, 1999; C. Schmidt et al., 2006). Conclusions from the studies of this thesis are restricted to the mnemonic *retrieval* process in humans. Thus, conclusions

about brain activations during other memory processes, such as encoding, cannot be given through the studies of this thesis. Baumann et al. (2010) conducted a virtual navigation study and found, using an event related fMRI design, that poor navigators showed significantly more activity in the hippocampus than good navigators. It is possible that no significant hippocampal activity was detected for the spatial task of study 2 because subjects knew the route very well and spatial information had been consolidated rapidly, becoming independent of the hippocampus. Subjects could thus be considered good navigators. However, the hypothesis formed by O'Keefe and Nadel (1978) is based on results from animal navigation studies. The function of the hippocampus cannot always be generalized across species. Especially the *verbal* spatial memory task, as the one used in this thesis, does not directly relate to any task performed by animals. Thus, an interpretation about the function of an animal's brain structure cannot be directly applied to the human brain.

In describing the function of the hippocampus, conclusions are sometimes drawn from human lesion studies. For example, Goodrich-Hunsaker et al. (2009) based their conclusions on results with patients suffering from hippocampal damage. Hippocampal damage was found to lead to deficits in solving a spatial task and odor-place associations. Patients with hippocampal damage were presented six odors, which were paired with six different target locations. In an immediate test following the learning session, subjects were given an odor and needed to identify its correct position (on a blackboard). In contrast to normal control subjects, patients showed severe behavioral difficulties and made more incorrect odor-place pairs. When the odor was tested alone, without an association to a spatial location, patients performed as well as control subjects. In a spatial location test (without odors), patients again showed impairment. Thus, authors could conclude that the hippocampus is required in the recognition process of associations (odor-location) as well as the retrieval of spatial memories (but not odor memory itself).

Results of patient studies are not directly comparable to results of healthy subjects. The size of the lesion cannot be controlled and often multiple brain areas area affected, and the deficits are the result of the *sum* of affected areas. Brain areas are widely interconnected, interact with many other areas and, depending on the age of the damage, brain plasticity can establish alternative solutions or routes to solve a cognitive task. Thus, lesions studies cannot directly give conclusions about the function of normal brain processes. Therefore, results of this thesis do not entirely contradict the findings that the hippocampus is important for spatial navigation or memory associations in general. Overall, one must be careful in drawing conclusions about the function of a brain area.

7.2 Declarative Memory Retrieval

7.2.1 Autobiographical Memories and the Hippocampus

From both fMRI studies of this thesis it can be inferred that the retrieval of context-rich declarative memories requires the medial temporal lobe. Whether shortly after encoding, after three days or after two and a half months, the retrieval of autobiographical memories activates the medial temporal lobe. Furthermore, study 2 supports the findings by Harand et al. (2012) that the engagement of the hippocampus decreases for semantic memories. Furthermore, an analysis of correlation between the age of autobiographical memories (from study 2) and BOLD responses during retrieval showed that the age of autobiographical memories does not influence brain activity (Roselli, 2010). In study 1, no FWE corrected differences could be seen for autobiographical memories whether the episodes were retrieved immediately, after several days or months when regular sleep follows learning. Harand et al. (2012) clearly found that consistently episodic memory depends on the hippocampus up to at least three months after encoding. From study 1 of this thesis, it can be extended that for the retrieval of remote context-rich episodic memories, a network consisting of the precuneus, occipital gyrus, lingual gyrus and also the angular gyrus is activated (study 1, conjunction analysis of autobiographical recall for all 3 test time points).

Harand et al. (2012) investigated brain regions involved in the retrieval of recent and remote memories after three days and three months, using the remember-know procedure (Gardiner et al., 1998) for emotional and neutral pictures (see section 5.1). Authors differentiated between episodic and semantic memories, the latter being retrieved without details of the learning episode and without re-experiencing the episode in which the information was initially learned: After three days subjects were tested in the MRT, and rated pictures either as previously learned items (old) or new. If old, subjects chose between “remembering” the picture, thus remembering in detail having seen the picture, or “knowing” that they had seen the picture, but not remembering details about the learning episode. The same test procedure was applied after three months. The qualitative change of remote memories over time was scrutinized using fMRI. For those pictures that were “remembered” after three days as well as after three months, authors declared the memory as consistently episodic. For retrieval of consistently episodic information, the posterior hippocampus remained necessary. (For fMRI analysis, correct rejections, being new pictures, were used as baseline). Pictures which subjects “remembered” after three days, but rated to only “know” them after three months, were declared as semantic memories. From fMRI analyses, hippocampal disengagement was concluded for semantic memories. For consistently episodic retrieval, the precuneus and the parietal lobule, were also activated during both recalls after three days and three months.

7.2.2 The role of the Precuneus in Episodic Memory retrieval

A brain region that plays an important role in the retrieval of episodic memory is the precuneus. Anatomically, the precuneus is located in the medial parietal lobe of the brain near the cingulated cortex. It has extensive connections to other brain regions, i.e. it connects to the frontal, temporal, parietal and occipital cortices. It is a highly developed structure. (It exhibits the most complex columnar cortical organization of the brain and, compared to animals and non-human primates, it covers a proportionally larger volume in humans (Cavanna and Trimble, 2006)). Supporting evidence for the role of the precuneus in episodic memory retrieval comes from recent and past fMRI and PET studies. In a recent fMRI study, Kwok et al. (2012) focused on the mnemonic processes involved in the retrieval of television episodes. Subjects viewed a sequence of scenes and were asked to make temporal, spatial and object related decisions. For instance, when asked to make a temporal decision, subjects were shown a scene and needed to choose the correct scene that occurred before the presented scene. Temporal order decisions activated the precuneus bilaterally. Furthermore, Krause et al. (1999) conducted a PET study in which they detected involvement of the precuneus in the retrieval of abstract, as well as highly imaginable words. The structure is involved in the retrieval of words, independent of the presentation modality: whether words had been acquired through an auditory or a visual procedure, activation of the precuneus occurred. Interestingly, the precuneus does not seem to be important for the retrieval of non-sense words, pseudo-words that do not exist in the (German) dictionary (Krause et al., 1999).

The results of this thesis support the role of the precuneus and angular gyrus in episodic memory retrieval. In studies 1 and 2 functional magnetic resonance imaging was used to detect the cerebral regions involved in the retrieval of episodic information both before (studies 1 and 2) and after periods of sleep (study 1). When recalling episodic information, regardless of the brain state following the acquisition phase (sleep or wakefulness), the precuneus was involved. One specific part of the precuneus was repeatedly found active during episodic memory retrieval as in the study by Krause et al. (1999): A conjunction analyses (FWE corrected) revealed that Brodmann area 7 was the common area of the precuneus activated in both sleep and sleep deprivation groups during both the autobiographical and spatial task on all three days of study 1. Furthermore, retrieval of episodic information after several months still depended on the precuneus (study 1). It can be inferred that the precuneus is involved in memory recall regardless of the interval between learning and retrieval. Since the precuneus was found active in all three tasks tested in study 2 (sequential, spatial and autobiographic tasks), it can be inferred that its involvement is not reduced to certain tasks but it is rather a structure generally involved in episodic recall of imaginable words.

7.3 Procedural Memory Retrieval

Albeit a minor part of this thesis, procedural memory retrieval was investigated in one of the five studies (study 5) in order to clarify whether the lack of significant differences between performance after sleep versus wakefulness found in previous studies is specific to declarative tasks. Results showed that sleep after learning does not support retrieval of procedural memory more than wakefulness. Interestingly, results of this study contradict, to a certain extent, the theory of Brown and Robertson (2007) who state that consolidation of motor skills is hindered when followed by declarative learning, and that a period of sleep – in contrast to wakefulness – retroactively disinhibits motor skill improvement. In study 5 the procedural memory task was not studied separately, but in combination with a declarative task. In study 5, subjects learned a procedural task, then a declarative task and were tested in reverse order. Results of study 5 do not point at sleep-related disinhibition of motor skill improvement. Study 5 merely demonstrated that sleep after learning did not lead to better performance of motor skills than wakefulness after learning. It is possible that the mechanisms of consolidation of the declarative and procedural tasks do not interfere with each other and that sleep is not necessary to solve such an impediment.

7.4 Factors influencing Retrieval Success

Memory consolidation is a sensitive operation. Several factors influence test results and confound data, rendering conclusions about the effect of sleep on declarative memory difficult.

7.4.1 Type of Material

According to the literature on declarative memory and sleep, one factor found to influence retrieval success is the type of learning material [for a review see Diekelmann et al. (2009)]. Commonly, different types of material for declarative memory tasks are employed: non-sense syllables, words, pictures (including faces and objects) and spatial routes. Studies with verbal or lexical material often use words, e.g. pairs of nouns, which can either be related (e.g. table and chair) or unrelated (e.g. table and car), but combinations, such as pairs consisting of one noun and one adjective have also been applied. Often, single words are learned, commonly nouns (concrete or abstract) and adjectives.

In this thesis, the types of learning material used for the declarative memory tasks were single nouns (studies 1 and 2), pairs of non-sense syllables (study 3), pairs of unrelated nouns (study 4), single non-sense syllables (study 5). When nouns were learned, they were nouns of concrete objects and thus highly imaginable. Noun concreteness affects encoding (Marschark and Surian, 1992). For example, recall of imaginable words is much better than recall of abstract words. In a PET study by Krause et al. (1999) the retrieval success (immediate cued recall) of highly imaginable words was 94%, compared to 36% for abstract words.

In congruence with the findings of this thesis, several studies investigating declarative memory performance after sleep, using the same learning material as in this thesis, found that items are not always retained better after sleep compared to wakefulness.

7.4.1.1 Related vs. Unrelated Word Pairs

From declarative memory studies, it has been proposed that the nature of the learned material influences its post-sleep retrieval success (Byrne, 2003). Unrelated word pairs are assumed to depend more on newly built associations, mediated by the hippocampus, than related word pairs (Stickgold, 2004). Furthermore, the type of material possibly influences EEG oscillations during periods of consolidation while asleep. C. Schmidt et al. (2006) propose that learning tasks consisting of unrelated word pairs affect post-training sleep oscillations more than related word pairs. Therefore, in study 4, unrelated words (nouns) were used for the declarative learning task. Furthermore, C. Schmidt et al. (2006) saw increased synchronized EEG oscillations during non-REM nap sleep following learning of unrelated word pairs. Thus, electrophysiological activity during sleep is affected by aforegoing learning. In study 4, wakefulness after learning resulted in similar retrieval performance as nap sleep. In the study by C. Schmidt et al. (2006), neuronal changes in post-learning sleep were specific to the task difficulty. Task difficulty was determined by the concreteness of the words. Only difficult encoding affected post-training sleep parameters of spindles in the study by C. Schmidt et al. Thus, for study 4 it cannot be ruled out that sleep related changes would have been absent in a more difficult declarative learning task. Further investigations with different learning material are necessary for more concrete conclusions (see section Encoding Strength for more information on task difficulty).

Similarly, in a study by Tucker and Fishbein (2008) subjects learned and were tested on a list of unrelated word pairs (of common objects e.g. “alligator” and “cigar”), took a nap in the afternoon (subjects did not enter REM sleep), and were then re-tested on the word pairs. Not all subjects showed improved performance after sleep compared to wakefulness. The authors divided the group of subjects into “good” and “bad” performers, according to their training score during the test prior to the period of sleep or wakefulness. In comparison to good performers, bad performers showed no improvement during re-test after a period of sleep compared to a period of wakefulness.

The enhancing effect of sleep does not seem to be stable for related word pairs either. For example, Tucker et al. (2006) observed better recall in subjects who napped after learning pairs of related words (e.g. “clock” and “hands”) than those who did not sleep. On the contrary, Backhaus and Junghanns (2006) saw that recall performance of related nouns improved over time, regardless of the brain state (sleep or wake) after encoding. Further calculations even showed that memory recall worsened after a nap, compared to wakefulness, if the nap did not contain slow wave sleep.

7.4.1.2 Syllables

The comparison of the different learning materials (words versus non-words) gives further insight for the relation of sleep to memory retrieval. Non-sense syllables were learned in studies 3 and 5.

The advantage of non-sense syllables in comparison to existing lexical words is their lack of meaning. Thus, distracting influence from associations with the material to be learned and already existing associations is kept low. Furthermore, the question of a sleep effect due to relatedness of meaning does not arise because non-sense syllables simply do not have a meaning. Non-sense syllables are devoid of visual information, in contrast to concrete nouns of objects, which are immediately associated with the object they describe. A disadvantage of using non-sense syllables as learning material is their high level of difficulty for the subject and also the high level of forgetting. In study 3, this side effect was even increased through syllable pairs. Subjects not only had to keep in mind which syllables were learned, but also the correct pairs. This resulted in long learning sessions and low retrieval scores. Instead of increasing the amount of syllables to be remembered to achieve a greater amount of items to be recalled after sleep or wakefulness, the amount of syllables to be remembered was reduced to ten items in study 5. Murnane and Shiffrin (1991) describe a list-length effect, which occurs when more items are added to a list. Each new item that is added to a list to be memorized, causes interference for the memory of the other items on the list. Furthermore, it is trivial that the fewer items a list contains, the easier it is to remember them. No problems with low retrieval scores occurred in study 5.

In congruence with the findings of this thesis on non-sense syllables, Richardson and Gough (1963) detected that subjects who slept were not significantly better at remembering non-sense syllables when tested after a delay of up to two days. Overall, a comparison of studies 1, 3 and 5, elucidated that sleep is not specific to the type of declarative verbal material learned. (The type of retrieval test (free recall) was held constant for studies 1 and 5. Furthermore, the time between learning and testing is comparable for studies 1 and 5.)

7.4.1.2.1 The hippocampus and syllables

The hippocampus has been found to play a role in binding together information about syllable pairs. For example, Kroll et al. (1996) investigated how well patients with hippocampal damage could differentiate between new and old (already presented) syllables. New words consisted of two syllables, each of which had been presented in another two-syllable word before. For instance, subjects had learned the words “valley” and “barter” and were presented with the new two-syllable combination “barley”. Patients with hippocampal damage in the left hemisphere had significantly higher rates of false alarms (declaring the new word as old). Interestingly, patients with right hemispheric lesions performed as well as normal control subjects.

7.4.1.3 Emotional Material

It is known that emotional material is forgotten less easily than neutral material. Investigating the role of the amygdala, the prototypical emotion region, Dolcos et al. (2005) saw that subjects recalled more emotional pictures than neutral pictures one year after encoding. The consolidation of emotional memory has been found to be supported by sleep: In a study by (Nesca and Koulack, 1994) subjects associated an episode of an emotionally arousing film with words and were given a recognition test 24 hours later. Retention scores were significantly higher for those subjects who slept after the learning task than those subjects who stayed awake. Studying the neural mechanisms influencing the enhancement of recollection of emotional material, Dolcos et al. (2005) detected coactivity in the hippocampus and the amygdala during emotional recollection. The authors conclude that emotion enhances the activity of memory recollection in the hippocampus and recollection enhances emotion-related activity in the amygdala. It is possible that sleep specifically supports such synergistic mechanisms leading to behavioral improvement of retrieval after sleep.

7.4.2 Encoding Strength

Encoding strength possibly influences the effect of sleep on memory. In study 1, subjects did not forget significantly more words over the time of four days, whether they slept or stayed awake during the first night after encoding. Thus, it cannot be concluded that only sleep protects memories. Walker proposes that consolidation comprises two processes, stabilization and enhancement (Walker, 2008b; Walker et al., 2003a). The former occurs over time, thus also during wakefulness, whereas the latter depends on sleep. Results of this thesis contradict Walker's theory since retrieval performance after sleep was not superior to performance after a period of wakefulness, whether this wakefulness was filled with night- or daytime sleep. It is likely that a post-acquisitional process of stabilization, protecting memories from forgetting, occurred rapidly and thus was independent of the first night's brain state (sleep / wake). This assumption is supported by Peigneux et al. (2006) who gave evidence for post-learning memory consolidation after two hours of wakefulness and Kopasz et al. (2010) who proposed a sensitive period within a few hours after acquisition for a beneficial memory consolidating effect. However, the absence of a sleep benefit on declarative memory can also be the result of the encoding strength. The more often an item is presented, the better it is encoded. The higher the encoding strength of an item, the better it is retrieved over time. Encoding strength has been found to be related to the effect of sleep: Drosopoulos et al. (2007a) observed that free recall was only significantly better after a night of sleep if the encoding of word pairs had been weak. Encoding was divided into "weak" and "strong" depending on the learning criterion (60% vs. above 90%) and the duration of feedback in the test immediately following the presentation of word pairs. No sleep-associated benefit could be found for the strongly encoded items. It is therefore possible that a beneficial effect of sleep was missed in study 1 due to strong encoding. As a reminder,

in the first fMRI study (study 1), the associations with nouns needed to be learned very well in order to avoid risking a statistical "floor effect" in the analysis of retrieval scores in the last recall (recall 3) after two and a half months.

Furthermore, in all "night sleep" studies, thus studies 1, 3 and 5, the learning criterion (percentage of the amount of items that had to be achieved during immediate test) was high, and thus encoding was strong. In study 1, the criterion of the immediate retrieval test following learning was at 90%. Learning criterion in the behavioral studies was high as well: In the first behavioral study with non-sense syllables, study 3, the criterion was 80%. In the study on single non-sense syllables, study 5, the criterion was at 100% (in three consecutive recalls). Thus, all learning criteria were high and encoding procedures of all studies can be considered as "strong". If the hypothesis Drosopoulos et al. (2007a) holds true, then the strength of encoding possibly impeded a sleep benefit in the studies of this thesis.

However, sleep benefits despite high encoding strength have been found. For example, Benson and Feinberg (1975) demonstrated that memory retrieval after sleep is superior to retrieval after wakefulness despite strong encoding. In their study, Benson and Feinberg (1975) had a learning criterion of 75%. Procedures by Ellenbogen et al. (2006) relied on a learning criterion of 100%. Thus, if a beneficial effect of sleep is independent of the encoding strength, then the behavioral data of this thesis support the view that memory is consolidated equally well during sleep and wakefulness.

7.4.3 Circadian Rhythm and Hormones

Richter (1967) first introduced the concept of a biological clock and the resulting sleep-wake cycle, the circadian rhythm which is generated by the brain. Thus, sleep is a circadian phenomenon and changes related to sleep must be carefully considered in the light of the biological circadian rhythm. Circadian effects were found in study 3 of this thesis. Subjects of group I performed better in the morning than in the evening. Therefore, sleep could not be isolated as the phenomenon causing the observed memory improvement at retrieval after a night of sleep compared to a day of wakefulness. It is not clear whether sleep per se promotes the consolidation of memory or whether it is influenced by circadian rhythm. From their results, Nesca and Koulack (1994) suggest that a significantly better declarative memory performance following post-learning sleep may stem from circadian rhythm effects (instead of sleep). In their study, subjects learned words either in the morning or in the evening and slept at night. Retention was measured 24 hours later. When sleep followed learning immediately (after learning in the evening), retention was significantly better. The two groups equated in the amount of time spent awake, thus interference resulting from unequal amounts of wakefulness was eliminated as a factor influencing retention. A third group studied in the evening

and was sleep deprived. Here, no difference in comparison to the group that slept after the encoding phase could be detected.

The disadvantage of tests with night time sleep (learning in the evening before sleep and retrieval in the morning) is the different time of day in which learning and testing occurs. Several factors such as cortisol, growth hormone and alertness (Backhaus and Junghanns, 2006) can influence test results. The circadian pacemaker is the suprachiasmatic nucleus (SCN), which is located in the hypothalamus, and controls secretion of hormones, including cortisol. Cortisol is secreted by the adrenal glands. In the morning, shortly after awakening, cortisol levels are high. High levels of cortisol in the blood plasma inhibit memory consolidation because it inhibits protein synthesis (Idzikowski, 1984). In the evening, cortisol levels are low and sleep causes inhibition of secretion of cortisol. Thus, during most of the night, cortisol levels are low. High levels of cortisol can perturb memory retrieval. For example, in their PET study, de Quervain et al. (2003) administered cortisone one hour before memory retrieval. Subjects had learned two lists of unrelated word pairs (abstract nouns) and were now tested 24 hours after encoding. One list was tested in a cued recall procedure, the other in a recognition test. Cortisone was found to impair cued recall but not recognition. During cued recall, cerebral blood flow was significantly lower in the right medial temporal lobe when cortisone had been administered, compared to the placebo condition (administration of mannite). Recognition showed the same cerebral blood flow in the medial temporal lobe after cortisone intake as in the control condition. This incongruence of the two different retrieval tests is supported by study 3 of this thesis: Cued recall performance in the morning differed significantly from performance in the evening (groups I and III), but recognition seemed unaffected (group II). Thus, memory traces activated in cued recall procedures seem to be more volatile towards changes in cortisol levels.

7.4.4 Sleep Stages associated with Declarative and Procedural Memory Consolidation

In the nap study of this thesis, study 4, results of EEG showed that the majority of the nap was spent in stage 2 sleep. Typical for stage 2 are spindles. However, spindles also occur during other stages, e.g. in the transition between non-REM and REM sleep (see Introduction). Since performance levels at retrieval testing was not significantly different after the nap compared to wakefulness, study 4 does not support findings arguing that stage 2 sleep enhances declarative (Genzel et al., 2009) performance. In a nap study comparable to study 4 of this thesis (C. Schmidt et al., 2006), a positive correlation between recall performance (cued recall) of unrelated word pairs and spindle frequency during nap was found. Sleep spindle activity was not analyzed in study 4, yet spindle activity is often associated with the consolidation of memory, (Clemens et al., 2005; Gais et al., 2002; Schabus et al., 2008). Cortical sleep spindles are temporally related to hippocampal ripples (see 1.2.2). The

reactivation of hippocampal and cortical cells allows for synaptic plasticity (Poe et al., 2010) and is assumed to enable the embedding of initially hippocampus dependent associative memories in neocortical storage areas for the long term (Chrobak and Buzsaki, 1996).

Furthermore, stage 2 has been found to support procedural memory. For example, Walker et al. (2002) show that stage 2 sleep correlates positively with motor skill improvement. In study 4, motor skill was not tested. However, study 5, which tested motor skill, does not support Walker et al. (2002) who detected that subjects' performance increased significantly after a night of sleep but not over a period of wakefulness (during the day): Subjects were either trained on the motor task in the morning, stayed awake and were tested after 12 hours and again after a night of sleep, or they were trained in the evening, and tested after a night of sleep and again after 12 hours of wakefulness. Significant motor performance improvement was only observed in the tests following sleep. Other authors relate sleep dependent procedural improvement to other stages of sleep, such as slow wave sleep and REM sleep (Karni et al., 1994; Peigneux et al., 2003) or both (Stickgold et al., 2000a). However, in literature the stage to which sleep is related with a positive effect on memory performance is unclear. According to Stickgold et al. (2000a), both slow wave sleep, which is most abundant during the first half of the night, and REM sleep, which is most abundant during the second half, are indispensable for performance improvement and intermediate processes of memory consolidation between memory acquisition and permanent storage.

7.4.5 Type of Retrieval

From the results of this thesis it can be concluded that a positive effect of sleep on declarative memory is not specific to the type of retrieval test. Three different retrieval tests were studies in this thesis: free recall, recognition and cued recall. (Free recall was applied in studies 1, 2 and 5; recognition tests were used in studies 3 and 4; and cued recall was used in studies 3 and 4.) On the behavioral level, no significant difference, that was uninfluenced from possible effects of circadian rhythm, was observed when comparing the retrieval performance after a period of sleep to the retrieval performance after a period of wakefulness.

A sleep benefit is thought to depend on the declarative retrieval operation, depending on whether the subject *remembers* the information or *knows* the answer. According to the theory of recollection and familiarity (Jacoby, 1991; Tulving, 1985), remembering underlies the process of recollection, which means re-living the situation of encoding, including the "when" and "where" part of the encounter in which the memory was formed. Knowing underlies the process of familiarity, which lacks the vividness of the original episode in which the information was acquired. One knows the answer in a test, without qualitative information about the study episode. In "remember-know" procedures, recollection versus familiarity are tested.

Atienza and Cantero (2008) studied pictures with neutral or emotional content and concluded that sleep specifically influences recollection (remembering) but not familiarity (knowing). Subjects memorized over four hundred pictures, were either deprived of sleep for forty hours following the study phase or slept at night, and one week later were tested using the remember-know procedure. Subjects had not been informed about the test at the time of encoding. Performance accuracy for pictures that were remembered was significantly higher in the group that slept normally after learning. No such effect could be seen for pictures which subjects had evaluated as knowing.

In memory studies with lexical material (e.g. words), recollection can be tested through cued recall tests (e.g. when one item of a word pair is shown and the subjects need to generate the answer themselves) and familiarity can be tested with a recognition test (e.g. when several answers are offered and the subject needs to choose one immediately without pondering over the answer). The study in which each subject was tested with both procedures in the same session was study 4. Besides the trivial finding that subjects scored higher on the recognition tests than in cued recall tests, no sleep related differences could be detected on the behavioral level for the two retrieval types.

7.4.5.1 The type of retrieval and the hippocampus

Studies on patients with lesions in the hippocampus give evidence for the idea that the process of recollection requires the hippocampus, whereas the process of familiarity is independent of it. For example, Aggleton et al. (2005) compared free recall and recognition on a patient with a reduction of the hippocampus (of almost 50%) resulting in amnesia, versus healthy control subjects. Subjects memorized names, doors and shapes, and were tested immediately. The patient showed severe impairment on the free recall test, but not on the recognition test. Authors thus conclude that recollection and familiarity are dissociable retrieval mechanisms. They further conclude that recall is determined by the unrestricted function of the hippocampus, whereas familiarity-based recognition is not. Likewise, in study 2, in which episodic memory was tested in a free recall procedure, the hippocampus was involved. However, a second type of retrieval was not tested in study 2 and the probability for hippocampal involvement during free recall in the tested spatial and sequential tasks was below one percent. Thus, the hippocampus seems to be specifically involved during recollection of some types of declarative memory tasks, but not all. Furthermore, in study 4, in which recognition was tested in addition to cued recall, post-sleep memory retrieval was as good after a period of wakefulness as after a nap, whether memory traces were activated in order to recognize (recognition test) the answer or to generate it itself (cued recall test). Overall, this thesis cannot support the hypothesis that sleep specifically supports recollection such that memory traces activated through cued recall and free recall benefit from a period of sleep more than wakefulness.

7.4.6 Interference

In the behavioral studies 3 and 4 of this thesis the relation between sleep and interference was investigated. During wakefulness we engage in many tasks requiring the acquisition of new input. Several decades ago it was assumed that sleep offers a better ground for memory consolidation due to its lack of such interfering input (Grosvenor and Lack, 1984; Jenkins and Dallenbach, 1924). Benson and Feinberg (1977) addressed this issue and reported evidence that sleep offers a more active process than mere passive protection from interference. According to Wixted (2004), consolidation stabilizes memories, such that it is not overwritten by later acquired information. Interestingly, subjects in a study by Ellenbogen et al. (2006) showed no clear sleep benefit during retrieval testing 12 hours after encoding when no interfering information was learned succeeding the original learning phase. This is in congruence with the results of this thesis. In studies 1, 2 and 5 of this thesis subjects did not receive declarative material to learn that interfered with original encoding and showed no significant memory performance after sleep compared to wakefulness. Thus, the relation of sleep and retroactive interference was investigated in studies 3 and 4, the former investigating the effect of nocturnal sleep and the latter investigating a short period of daytime sleep. Results of this thesis contradict the proposition by Ellenbogen et al. (2006) that sleep offers a better milieu to counteract retroactive interference. According to the results of this thesis, our brain is able to counteract retroactive interference throughout the day. This is in accordance with our circadian rhythm, which keeps us awake throughout the day and increases tiredness in the evening, allowing us to sleep for several hours. If our declarative memory system needed periods of sleep after each acquisition phase in order to protect it from acquiring competing information before stabilization – we learn not only in the evening – it would be crucial for humans to sleep more often throughout the day.

7.4.7 Multimodal Tasks

A multisensory experience requires neurons in multiple regions to store information (for a review see Squire and Wixted (2011). Each region stores only certain aspects of an event. Simply stated, from a single event, looks of the scene are stored in visual brain areas, sounds are stored in auditory areas, the smells are stored in olfactory areas, etc. It is therefore plausible that the act of remembering this event reactivates the regions formerly involved in the process of multimodal memory storage.

It is possible that declarative and procedural memory performances are better when more modalities are explicitly engaged in the learning process. Two examples will be discussed: One using the addition of olfactory and the other using the addition of auditory material.

A relation between the hippocampus, odor and memory enhancement after sleep was found by Rasch et al. (2007). When hippocampus dependent declarative memory is acquired while an odor is

presented, and the odor is re-presented during slow wave sleep after learning, post-sleep memory performance is improved: The first group of subjects learned a spatial-location task in the evening. Simultaneously, subjects smelled an odor, thus the task was associated with the odor. During following slow wave sleep, the same odor was delivered (by an apparatus and in a certain delivery sequence to avoid habituation). Several control experiments were run. One control group learned the same task with the odor presentation during encoding, but received no odor during subsequent slow wave sleep and performed significantly worse during retrieval compared to the first group of subjects. The authors concluded that the re-exposure of the odor during slow wave sleep induces the reactivation of memories (see general introduction), which increases memory consolidation and in turn leads to an improvement in memory retention after sleep. The same beneficial effect of sleep was found in a nap study by Diekelmann et al. (2011). Here, subjects learned the same spatial location task as in Rasch et al. (2007) while an odor was presented, then napped for 40 minutes (sleep odor group) in which subjects were re-exposed to the same odor during slow wave sleep, or stayed awake (wake odor group). Subjects then learned information that served as interference. In a final test, subjects who had slept remembered significantly more spatial locations than those who had stayed awake after encoding. Furthermore, fMRI showed that odor cues during slow wave sleep activated the left hippocampus (anterior and posterior). The authors believe that the odor-associated reactivation during slow wave sleep eases the integration of new memory representations from the hippocampus to the neocortex. Nap sleep was efficient to produce stable long-term memories that were unaffected by interference. Thus, a stabilization of declarative memories occurs rapidly after encoding, on the neuronal level (for more information, see section 1.2.3.1). Interestingly, in the study by Diekelmann et al. (2011) two control groups performed equally well: When no odor was presented (vehicle task), subjects who slept detected no more locations in the retrieval test than subjects who stayed awake. Thus, sleep after declarative memory encoding does not always lead to memory enhancement. This is in congruence with the findings of this thesis. It can be assumed, that declarative learning needs to be coupled to another stimulus, such as an odor, in order for a sleep enhancing effect to be visible at post-sleep retrieval testing.

Memory consolidation may not solely depend on sleep and a sleep benefit is not guaranteed for declarative or procedural tasks. A memory enhancing effect after sleep is more likely to be overtly detected on the behavioral level when learning and subsequent sleep are coupled to sensory information.

7.5 Conclusion to Contradictory Findings

No general concepts or rules can be formulated for the assurance of a positive effect of sleep on memory. The variables influencing sleep are abundant and seem unreliable: For example, it has been

argued that post-learning sleep improves recall (of unrelated word pairs if performers are trained well ("good" performers) but not if they learned poorly (bad performers) (Schabus et al., 2006; Tucker and Fishbein, 2008). Furthermore, it has been argued that the beneficial effect of sleep occurs when the material to be learned is difficult but not when it is easy, e.g. when words are of little concreteness, a beneficial effect of sleep occurs, but not if words are easy to remember (C. Schmidt et al., 2006), thus when the learning material is encoded weakly (Drosopoulos et al., 2007a). Tucker and Fishbein (2008) believe that sleep improves unrelated word pairs, if words are tested after learning and before sleep onset, but not if the material is not tested before sleep. On one hand, a brief period of sleep (under an hour) has been proposed to be sufficient for a beneficial effect of sleep, on the other hand, a beneficial effect of sleep is not measurable before a time delay of several days (Richardson and Gough, 1963).

8 Conclusion

From the results of the five studies of this thesis, it can be concluded that declarative and procedural memories are consolidated equally well over a period of wakefulness compared to a period of sleep. It can also be assumed that the human brain is capable of compensating a night of sleep deprivation without significant behavioral deficits during retrieval of verbal declarative and motor skill tasks, whether memory is tested shortly after encoding (a few hours), days or after months[20].

The question of whether memory benefits from sleep is a controversial issue, and results from sleep studies can easily be affected by several factors. The type of retrieval, circadian[21] rhythm, retention period, interference, and the type of material might all contribute to this set of variables influencing the benefit of sleep on memory. This thesis thus tested the above mentioned variables: From the behavioral results of this thesis it can be concluded, that regardless of the chosen type of retrieval, time point of test, time interval between learning and retrieval, in- or exclusion of interference or the tested material, forgetting is not higher when encoding is followed by a period of wakefulness than when a period of sleep ensues. Furthermore, daytime wakefulness does not significantly alter retrieval performance and can be substituted with night-time wakefulness (sleep deprivation).

The fMRI studies of this thesis visualized the brain areas activated in the retrieval of three different episodic memory tasks. Clearly, the precuneus is always involved in verbal free recall of spatial, sequential and autobiographical episodic memory, whether memory is retrieved shortly after learning or after several months. From study 1 of this thesis, it can be extended that for the retrieval of remote context-rich episodic memories, a network consisting of the precuneus, occipital gyrus, lingual gyrus and also the angular gyrus is activated.

The hippocampus on the other hand was found to be activated *specifically* in the recall of autobiographical associations. Especially from study 2 it can be concluded that declarative memory consolidation is a rapid process that takes place within half an hour after memory acquisition. Results challenge the view of the standard model of systems-level consolidation (Squire, 1986), inspiring the theory of rapid consolidation (Sharon et al., 2011). The results of study 2 challenge the view that the hippocampus is indispensable for spatial memory (R. G. Morris et al., 1982; O'Keefe and Dostrovsky, 1971) and support the multiple trace theory. According to the multiple trace theory (Nadel and Moscovitch, 1997), when memories lose contextual detail, as it is the case for semantic memories (see General Introduction), information retrieval is no longer hippocampus dependent. The results of

[20] "Months" refers only to declarative memory. In this thesis, procedural memory was tested up to six days, but not after several months.

[21] Circadian is derived from *circa diem* = about a day

this thesis only support a selective role for the hippocampus: It is necessary for the retrieval of consolidated autobiographical memory. Retrieval of mere spatial associations or word associations learned through rote repetition does not seem to depend on the hippocampus after a consolidation phase of half an hour. Thus, it can be assumed that as spatial memories become devoid of episodic context-specific details, they become independent of the hippocampus.

9 References

Abel, M., and Bauml, K. H. (2013). Sleep can reduce proactive interference. *Memory, 22*(4), 332-339.

Aggleton, J. P., and Pearce, J. M. (2001). Neural systems underlying episodic memory: insights from animal research. *Philos Trans R Soc Lond B Biol Sci, 356*(1413), 1467-1482.

Aggleton, J. P., Vann, S. D., Denby, C., Dix, S., Mayes, A. R., Roberts, N., and Yonelinas, A. P. (2005). Sparing of the familiarity component of recognition memory in a patient with hippocampal pathology. *Neuropsychologia, 43*(12), 1810-1823.

Albouy, G., Sterpenich, V., Balteau, E., Vandewalle, G., Desseilles, M., Dang-Vu, T., Darsaud, A., Ruby, P., Luppi, P. H., Degueldre, C., Peigneux, P., Luxen, A., and Maquet, P. (2008). Both the hippocampus and striatum are involved in consolidation of motor sequence memory. *Neuron, 58*(2), 261-272.

Alvarez, P., and Squire, L. R. (1994). Memory consolidation and the medial temporal lobe: a simple network model. *Proc Natl Acad Sci U S A, 91*(15), 7041-7045.

Ashburner, J., Flandin, G., Henson, R., Kiebel, S., Kilner, J., Mattout, J., Penny, W., Stephan, K., and Hutton, C. (2007). SPM5 Manual. In W. T. C. N. Functional Imaging Laboratory (Ed.) (pp. 1-268). London: Institute of Neurology, UCL.

Atienza, M., and Cantero, J. L. (2008). Modulatory effects of emotion and sleep on recollection and familiarity. *J Sleep Res, 17*(3), 285-294.

Atkinson, R. C., and Shiffrin, R. M. (1968). Human Memory: A proposed system and its control processes. In S. K.W. & S. J.T. (Eds.), *The psychology of learning and motivation* (pp. 89 - 195). New York: Academic Press.

Baayen, R. H., Piepenbrock, R., and Gulikers, L. (1995). The CELEX Lexical Database (CD-ROM). In L. D. Consortium (Ed.). Philadelphia: University of Pennsylvania.

Backhaus, J., and Junghanns, K. (2006). Daytime naps improve procedural motor memory. *Sleep Medicine, 7*(6), 508-512.

Barakat, M., Doyon, J., Debas, K., Vandewalle, G., Morin, A., Poirier, G., Martin, N., Lafortune, M., Karni, A., Ungerleider, L. G., Benali, H., and Carrier, J. (2011). Fast and slow spindle involvement in the consolidation of a new motor sequence. *Behav Brain Res, 217*(1), 117-121.

Barnes, J. M., and Underwood, B. J. (1959). Fate of first-list associations in transfer theory. *J Exp Psychol, 58*, 97-105.

Barrett, T. R., and Ekstrand, B. R. (1972). Effect of sleep on memory. III. Controlling for time-of-day effects. *J Exp Psychol, 96*(2), 321-327.

Bass, J. (2012). Circadian topology of metabolism. *Nature, 491*(7424), 348-356.

Baumann, O., Chan, E., and Mattingley, J. B. (2010). Dissociable neural circuits for encoding and retrieval of object locations during active navigation in humans. *NeuroImage, 49*(3), 2816-2825.

Baydas, G., Nedzvetsky, V. S., Nerush, P. A., Kirichenko, S. V., Demchenko, H. M., and Reiter, R. J. (2002). A novel role for melatonin: regulation of the expression of cell adhesion molecules in the rat hippocampus and cortex. *Neurosci Lett, 326*(2), 109-112.

Benson, K., and Feinberg, I. (1975). Sleep and memory: Retention 8 and 24 hours after initial learning. *Psychophysiology, 12*, 192-195.

Benson, K., and Feinberg, I. (1977). The beneficaial effect of sleep in an extended Jenkins and Dallenbach paradigm. *Psychophysiology, 14*(4), 375-384.

Bliss, T. V., and Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol, 232*(2), 331-356.

Bohbot, V., Otahal, P., Liu, Z., Nadel, L., and Bures, J. (1996). Electroconvulsive shock and lidocaine reveal rapid consolidation of spatial working memory in the water maze. *Proc Natl Acad Sci U S A, 93*(9), 4016-4019.

Bosshardt, S., Degonda, N., Schmidt, C. F., Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. (2005). One month of human memory consolidation enhances retrieval-related hippocampal activity. *Hippocampus, 15*(8), 1026-1040.

Brown, M. W., and Aggleton, J. P. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci, 2*(1), 51-61.

Brown, R. M., and Robertson, E. M. (2007). Off-line processing: reciprocal interactions between declarative and procedural memories. *J Neurosci, 27*(39), 10468-10475.

Brown, R. W. (1957). Linguistic determinism and the part of speech. *J Abnorm Psychol, 55*(1), 1-5.

Buhry, L., Azizi, A. H., and Cheng, S. (2011). Reactivation, replay, and preplay: how it might all fit together. *Neural Plast, 2011*, 203462.

Burgess, N., Maguire, E. A., and O'Keefe, J. (2002). The human hippocampus and spatial and episodic memory. *Neuron, 35*(4), 625-641.

Buzsaki, G. (1984). Long-term changes of hippocampal sharp-waves following high frequency afferent activation. *Brain Res, 300*(1), 179-182.

Byrne, J. (2003). *Learning and Memory* (2nd ed.). New York: Macmillan Reference USA.

Cabeza, R., Ciaramelli, E., Olson, I. R., and Moscovitch, M. (2008). The parietal cortex and episodic memory: an attentional account. *Nat Rev Neurosci, 9*(8), 613-625.

Caminiti, R., Ferraina, S., and Johnson, P. B. (1996). The Sources of Visual Information to the Primate Frontal Lobe: A Novel Role for the Superior Parietal Lobule. *Cerebral Cortex, 6*(3), 319-328.

Cavanna, A. E., and Trimble, M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain, 129*(Pt 3), 564-583.

Chang, H. M., Wu, U. I., and Lan, C. T. (2009). Melatonin preserves longevity protein (sirtuin 1) expression in the hippocampus of total sleep-deprived rats. *J Pineal Res, 47*(3), 211-220.

Chang, Q., and Gold, P. E. (2003). Intra-hippocampal lidocaine injections impair acquisition of a place task and facilitate acquisition of a response task in rats. *Behav Brain Res, 144*(1-2), 19-24.

Chernik, D. A. (1972). Effect of REM sleep deprivation on learning and recall by humans. *Percept Mot Skills, 34*(1), 283-294.

Chrobak, J. J., and Buzsaki, G. (1996). High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. *J Neurosci, 16*(9), 3056-3066.

Ciaramelli, E., Grady, C. L., and Moscovitch, M. (2008). Top-down and bottom-up attention to memory: a hypothesis (AtoM) on the role of the posterior parietal cortex in memory retrieval. *Neuropsychologia, 46*(7), 1828-1851.

Clemens, Z., Fabo, D., and Halasz, P. (2005). Overnight verbal memory retention correlates with the number of sleep spindles. *Neuroscience, 132*(2), 529-535.

Cohen, N. J. (1993). *Memory, amnesia, and the hippocampal system*. Cambridge: MIT Press.

Corkin, S. (1984). Lasting Consequences of Bilateral Medial Temporal Lobectomy: Clinical Course and Experimental Findings in H.M. *Seminars in Neurology, 4*(2), 249-259.

Crane, J., and Milner, B. (2005). What went where? Impaired object-location learning in patients with right hippocampal lesions. *Hippocampus, 15*(2), 216-231.

Curran, H. V., Gardiner, J. M., Java, R. I., and Allen, D. (1993). Effects of lorazepam upon recollective experience in recognition memory. *Psychopharmacology (Berl), 110*(3), 374-378.

Dang-Vu, T. T., Schabus, M., Desseilles, M., Sterpenich, V., Bonjean, M., and Maquet, P. (2010). Functional neuroimaging insights into the physiology of human sleep. *Sleep, 33*(12), 1589-1603.

de Quervain, D. J., Henke, K., Aerni, A., Treyer, V., McGaugh, J. L., Berthold, T., Nitsch, R. M., Buck, A., Roozendaal, B., and Hock, C. (2003). Glucocorticoid-induced impairment of declarative memory retrieval is associated with reduced blood flow in the medial temporal lobe. *Eur J Neurosci, 17*(6), 1296-1302.

Diekelmann, S., and Born, J. (2010). The memory function of sleep. *Nat Rev Neurosci, 11*(2), 114-126.

Diekelmann, S., Buchel, C., Born, J., and Rasch, B. (2011). Labile or stable: opposing consequences for memory when reactivated during waking and sleep. *Nat Neurosci,* 10.1038/nn.2744.

Diekelmann, S., Wilhelm, I., and Born, J. (2009). The whats and whens of sleep-dependent memory consolidation. *Sleep Med Rev, 13*(5), 309-321.

Dijk, D. J., and Czeisler, C. A. (1995). Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. *J Neurosci, 15*(5 Pt 1), 3526-3538.

Dolcos, F., LaBar, K. S., and Cabeza, R. (2005). Remembering one year later: role of the amygdala and the medial temporal lobe memory system in retrieving emotional memories. *Proc Natl Acad Sci U S A, 102*(7), 2626-2631.

Donchin, O., Sawaki, L., Madupu, G., Cohen, L. G., and Shadmehr, R. (2002). Mechanisms influencing acquisition and recall of motor memories. *J Neurophysiol, 88*(4), 2114-2123.

Drosopoulos, S., Schulze, C., Fischer, S., and Born, J. (2007a). Sleep's function in the spontaneous recovery and consolidation of memories. *Journal of Experimental Psychology: General, 136*(2), 169-183.

Drosopoulos, S., Wagner, U., and Born, J. (2005). Sleep enhances explicit recollection in recognition memory. *Learn Mem, 12*(1), 44-51.

Drosopoulos, S., Windau, E., Wagner, U., and Born, J. (2007b). Sleep Enforces the Temporal Order in Memory. *PLoS ONE, 2*(4), e376.

Drummond, S. P., Brown, G. G., Stricker, J. L., Buxton, R. B., Wong, E. C., and Gillin, J. C. (1999). Sleep deprivation-induced reduction in cortical functional response to serial subtraction. *Neuroreport, 10*(18), 3745-3748.

Dudai, Y. (2004). The neurobiology of consolidations, or, how stable is the engram? *Annu Rev Psychol, 55*, 51-86.

Durmer, J. S., and Dinges, D. F. (2005). Neurocognitive consequences of sleep deprivation. *Semin Neurol, 25*(1), 117-129.

Ebbinghaus, H. (1983). *Urmanuskript "Ueber das Gedächtniß" 1880*. Passau: Passavia Univ.-Verl.

Edmond, J. C., and Foroozan, R. (2006). Cortical visual impairment in children. *Curr Opin Ophthalmol, 17*(6), 509-512.

Eichenbaum, H. (2000). A cortical-hippocampal system for declarative memory. *Nat Rev Neurosci, 1*(1), 41-50.

Eichenbaum, H., Schoenbaum, G., Young, B., and Bunsey, M. (1996). Functional organization of the hippocampal memory system. *Proc Natl Acad Sci U S A, 93*(24), 13500-13507.

Ekstrand, B. R. (1967). Effect of sleep on memory. *Journal of Experimental Psychology, 75*, 64-72.

Ekstrand, B. R., Sullivan, M. J., Parker, D. F., and West, J. N. (1971). Spontaneous recovery and sleep. *J Exp Psychol, 88*(1), 142-144.

Ekstrom, A. D., Kahana, M. J., Caplan, J. B., Fields, T. A., Isham, E. A., Newman, E. L., and Fried, I. (2003). Cellular networks underlying human spatial navigation. *Nature, 425*(6954), 184-188.

Ellenbogen, J. M., Hulbert, J. C., Jiang, Y., and Stickgold, R. (2009). The Sleeping Brain's Influence on Verbal Memory: Boosting Resistance to Interference. *PLoS ONE, 4*(1), e4117.

Ellenbogen, J. M., Hulbert, J. C., Stickgold, R., Dinges, D. F., and Thompson-Schill, S. L. (2006). Interfering with theories of sleep and memory: sleep, declarative memory, and associative interference. *Current Biology, 16*(13), 1290-1294.

Empson, J. A., and Clarke, P. R. (1970). Rapid eye movements and remembering. *Nature, 227*(5255), 287-288.

Evans, A. C., Collins, D. L., Mills, S. R., Brown, E. D., Kelly, R. L., and Peters, T. M. (1993). 3D statistical neuroanatomical models from 305 MRI volumes. *Nuclear Science Symposium & Medical Imaging Conference, 1-3*, 1813-1817.

Faul, F., Erdfelder, E., Lang, A. G., and Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods, 39*, 175-191.

Ficca, G., Lombardo, P., Rossi, L., and Salzarulo, P. (2000). Morning recall of verbal material depends on prior sleep organization. *Behav Brain Res, 112*(1-2), 159-163.

Ficca, G., and Salzarulo, P. (2004). What in sleep is for memory. *Sleep Medicine, 5*(3), 225-230.

Fishbein, W., Schaumburg, H., and Weitzman, E. D. (1966). Rapid eye movements during sleep in dark-reared kittens. *J Nerv Ment Dis, 143*(3), 281-283.

Fletcher, P. C., Frith, C. D., Grasby, P. M., Shallice, T., Frackowiak, R. S., and Dolan, R. J. (1995). Brain systems for encoding and retrieval of auditory-verbal memory. An in vivo study in humans. *Brain, 118 (Pt 2)*, 401-416.

Fogel, S. M., and Smith, C. T. (2006). Learning-dependent changes in sleep spindles and Stage 2 sleep. *J Sleep Res, 15*(3), 250-255.

Fogel, S. M., and Smith, C. T. (2011). The function of the sleep spindle: a physiological index of intelligence and a mechanism for sleep-dependent memory consolidation. *Neurosci Biobehav Rev, 35*(5), 1154-1165.

Fowler, M. J., Sullivan, M. J., and Ekstrand, B. R. (1973). Sleep and memory. *Science, 179*(70), 302-304.

Fox, S. I. (2005). *Human Physiology* (9 ed.). Boston: McGraw-Hill Higher Education.

Frankland, P. W., and Bontempi, B. (2005). The organization of recent and remote memories. *Nat Rev Neurosci, 6*(2), 119-130.

Friston, K. J., and Penny, W. (2003). Posterior probability maps and SPMs. *NeuroImage, 19*(3), 1240-1249.

Friston, K. J., Penny, W., Phillips, C., Kiebel, S., Hinton, G., and Ashburner, J. (2002). Classical and Bayesian inference in neuroimaging: theory. *NeuroImage, 16*(2), 465-483.

Furio, A. M., Brusco, L. I., and Cardinali, D. P. (2007). Possible therapeutic value of melatonin in mild cognitive impairment: a retrospective study. *J Pineal Res, 43*(4), 404-409.

Gais, S., Albouy, G., Boly, M., Dang-Vu, T. T., Darsaud, A., Desseilles, M., Rauchs, G., Schabus, M., Sterpenich, V., Vandewalle, G., Maquet, P., and Peigneux, P. (2007). Sleep transforms the cerebral trace of declarative memories. *Proc Natl Acad Sci U S A, 104*(47), 18778-18783.

Gais, S., and Born, J. (2004). Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation. *Proc Natl Acad Sci U S A, 101*(7), 2140-2144.

Gais, S., Lucas, B., and Born, J. (2006). Sleep after learning aids memory recall. *Learning & Memory, 13*(3), 259-262.

Gais, S., Molle, M., Helms, K., and Born, J. (2002). Learning-dependent increases in sleep spindle density. *J Neurosci, 22*(15), 6830-6834.

Gardiner, J. M., Ramponi, C., and Richardson-Klavehn, A. (1998). Experiences of remembering, knowing, and guessing. *Conscious Cogn, 7*(1), 1-26.

Geisler, T. (2011). *Reaktivierung spezifischer Kontextreize einer prozeduralen Lernaufgabe im Schlaf und deren Bedeutung für die schlafabhängige Gedächtniskonsolidierung.* Unpublished Bachelor's thesis, Ludwig-Maximilians-University Munich, see Schönauer, M., Geisler, T., and Gais, S. (2014) J Cogn Neurosci, 26(1), 143-153.

Genzel, L., Dresler, M., Wehrle, R., Grözinger, M., and Steiger, A. (2009). Slow wave sleep and REM sleep awakenings do not affect sleep dependent memory consolidation. *Sleep, 32*(3), 302-310.

Gibbs, E. L., and Gibbs, F. A. (1962). Extreme spindles: correlation of electroencephalographic sleep pattern with mental retardation. *Science, 138*(3545), 1106-1107.

Gilbert, P. E., Pirogovsky, E., Ferdon, S., Brushfield, A. M., and Murphy, C. (2008). Differential effects of normal aging on memory for odor-place and object-place associations. *Exp Aging Res, 34*(4), 437-452.

Gilboa, A., Winocur, G., Grady, C. L., Hevenor, S. J., and Moscovitch, M. (2004). Remembering our past: functional neuroanatomy of recollection of recent and very remote personal events. *Cereb Cortex, 14*(11), 1214-1225.

Girardeau, G., and Zugaro, M. (2011). Hippocampal ripples and memory consolidation. *Curr Opin Neurobiol, 21*(3), 452-459.

Glenberg, A., Smith, S. M., and Green, C. (1977). Type I Rehersal: Maintenance and More. *Journal of Verbal Learning and Verbal Behavior, 16*, 339.

Goodrich-Hunsaker, N. J., Gilbert, P. E., and Hopkins, R. O. (2009). The role of the human hippocampus in odor-place associative memory. *Chem Senses, 34*(6), 513-521.

Gorfine, T., Yeshurun, Y., and Zisapel, N. (2007). Nap and melatonin-induced changes in hippocampal activation and their role in verbal memory consolidation. *J Pineal Res, 43*(4), 336-342.

Gorfine, T., and Zisapel, N. (2009). Late evening brain activation patterns and their relation to the internal biological time, melatonin, and homeostatic sleep debt. *Hum Brain Mapp, 30*(2), 541-552.

Gottselig, J. M., Hofer-Tinguely, G., Borbely, A. A., Regel, S. J., Landolt, H. P., Retey, J. V., and Achermann, P. (2004). Sleep and rest facilitate auditory learning. *Neuroscience, 127*(3), 557-561.

Graves, E. A. (1937). The effect of sleep on rentention. *Journal of Experimental Psychology, 19*, 316-322.

Greve, A., Donaldson, D. I., and van Rossum, M. C. (2010). A single-trace dual-process model of episodic memory: a novel computational account of familiarity and recollection. *Hippocampus, 20*(2), 235-251.

Grosvenor, A., and Lack, L. C. (1984). The effect of sleep before or after learning on memory. *Sleep, 7*(2), 155-167.

Gutwein, B. M., Shiromani, P. J., and Fishbein, W. (1980). Paradoxical sleep and memory: long-term disruptive effects of Anisomycin. *Pharmacol Biochem Behav, 12*(3), 377-384.

Hanlon, E. C., Vyazovskiy, V. V., Faraguna, U., Tononi, G., and Cirelli, C. (2011). Synaptic potentiation and sleep need: clues from molecular and electrophysiological studies. *Curr Top Med Chem, 11*(19), 2472-2482.

Harand, C., Bertran, F., La Joie, R., Landeau, B., Mezenge, F., Desgranges, B., Peigneux, P., Eustache, F., and Rauchs, G. (2012). The Hippocampus Remains Activated over the Long Term for the Retrieval of Truly Episodic Memories. *PLoS ONE, 7*(8), e43495.

Harrison, Y., and Horne, J. A. (2000). The impact of sleep deprivation on decision making: a review. *J Exp Psychol Appl, 6*(3), 236-249.

Hasselmo, M. E. (1999). Neuromodulation: acetylcholine and memory consolidation. *Trends Cogn Sci, 3*(9), 351-359.

Hebb, D. O. (1949). *The organization of behavior a neuropsychological theory*. New York [u.a.]: Wiley.

Henke, K. (2010). A model for memory systems based on processing modes rather than consciousness. *Nat Rev Neurosci, 11*(7), 523-532.

Hennevin, E., Hars, B., Maho, C., and Bloch, V. (1995). Processing of learned information in paradoxical sleep: relevance for memory. *Behavioural Brain Research, 69*(1–2), 125-135.

Henson, R. N., Cansino, S., Herron, J. E., Robb, W. G., and Rugg, M. D. (2003). A familiarity signal in human anterior medial temporal cortex? *Hippocampus, 13*(2), 301-304.

Hicks, R. A., Paulus, M. J., and Johnson, J. C. (1973). Effect of REM sleep deprivation on electric shock threshold in rats. *Psychol Rep, 32*(3), 1242.

Horne, J. A. (2000). REM sleep - by default? *Neurosci Biobehav Rev, 24*(8), 777-797.

Horne, J. A., and Minard, A. (1985). Sleep and sleepiness following a behaviourally 'active' day. *Ergonomics, 28*(3), 567-575.

Horne, J. A., and Walmsley, B. (1976). Daytime visual load and the effects upon human sleep. *Psychophysiology, 13*(2), 115-120.

Hoscheidt, S. M., Nadel, L., Payne, J., and Ryan, L. (2010). Hippocampal activation during retrieval of spatial context from episodic and semantic memory. *Behav Brain Res, 212*(2), 121-132.

Hu, P., Stylos-Allan, M., and Walker, M. P. (2006). Sleep facilitates consolidation of emotional declarative memory. *Psychol Sci, 17*(10), 891-898.

Huber, R., Ghilardi, M. F., Massimini, M., and Tononi, G. (2004). Local sleep and learning. *Nature, 430*(6995), 78-81.

Iber, C., Ancoli-Israel, S., Chesson, A., and Quan, S. F. (2007). *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications* (1 ed.). Westchester, II.

Idzikowski, C. (1984). Sleep and memory. *Br J Psychol, 75 (Pt 4)*, 439-449.

Jacoby, L. L. (1991). A process dissociation framework: Separating automatic from intentional uses of memory. *Journal of Memory and Language, 30*(5), 513-541.

Jasper, H. H. (1958). The ten-twenty electrode system of the International Federation. In: In: Report of the committee on methods of clinical examination in electroencephalography: 1957. *Electroencephalogr Clin Neurophysiol, 10*, 371-375.

Jenkins, J. G., and Dallenbach, K. M. (1924). Oblivescence during sleep and waking. *American Journal of Psychology, 35*, 605-612.

Ji, D., and Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nat Neurosci, 10*(1), 100-107.

Jo, Y. S., and Lee, I. (2010). Disconnection of the hippocampal-perirhinal cortical circuits severely disrupts object-place paired associative memory. *J Neurosci, 30*(29), 9850-9858.

Karni, A., Tanne, D., Rubenstein, B. S., Askenasy, J. J., and Sagi, D. (1994). Dependence on REM sleep of overnight improvement of a perceptual skill. *Science, 265*(5172), 679-682.

Keppel, G. (1968). Verbal learning and memory. *Annu Rev Psychol, 19*, 169-202.

Kesner, R. P., Gilbert, P. E., and Wallenstein, G. V. (2000). Testing neural network models of memory with behavioral experiments. *Curr Opin Neurobiol, 10*(2), 260-265.

Kokkinos, V., and Kostopoulos, G. K. (2011). Human non-rapid eye movement stage II sleep spindles are blocked upon spontaneous K-complex coincidence and resume as higher frequency spindles afterwards. *J Sleep Res, 20*(1 Pt 1), 57-72.

Kopasz, M., Loessl, B., Valerius, G., Koenig, E., Matthaeas, N., Hornyak, M., Kloepfer, C., Nissen, C., Riemann, D., and Voderholzer, U. (2010). No persisting effect of partial sleep curtailment on cognitive performance and declarative memory recall in adolescents. *J Sleep Res, 19*(1 Pt 1), 71-79.

Korman, M., Doyon, J., Doljansky, J., Carrier, J., Dagan, Y., and Karni, A. (2007). Daytime sleep condenses the time course of motor memory consolidation. *Nat Neurosci, 10*(9), 1206-1213.

Krause, B. J., Schmidt, D., Mottaghy, F. M., Taylor, J., Halsband, U., Herzog, H., Tellmann, L., and Muller-Gartner, H. W. (1999). Episodic retrieval activates the precuneus irrespective of the imagery content of word pair associates. A PET study. *Brain, 122 (Pt 2)*, 255-263.

Kroll, N. E. A., Knight, R. T., Metcalfe, J., Wolf, E. S., and Tulving, E. (1996). Cohesion failure as a source of memory illusions. *Journal of Memory and Language, 35*, 176-196.

Krueger, J. M., Obal, F., Jr., Kapas, L., and Fang, J. (1995). Brain organization and sleep function. *Behav Brain Res, 69*(1-2), 177-185.

Kudrimoti, H. S., Barnes, C. A., and McNaughton, B. L. (1999). Reactivation of hippocampal cell assemblies: effects of behavioral state, experience, and EEG dynamics. *J Neurosci, 19*(10), 4090-4101.

Kuriyama, K., Stickgold, R., and Walker, M. P. (2004). Sleep-dependent learning and motor-skill complexity. *Learn Mem, 11*(6), 705-713.

Kwok, S. C., Shallice, T., and Macaluso, E. (2012). Functional anatomy of temporal organisation and domain-specificity of episodic memory retrieval. *Neuropsychologia,* 10.1016/j.neuropsychologia.2012.07.025.

Lahl, O., Wispel, C., Willigens, B., and Pietrowsky, R. (2008). An ultra short episode of sleep is sufficient to promote declarative memory performance. *J Sleep Res, 17*(1), 3-10.

Laureys, S., Peigneux, P., Perrin, F., and Maquet, P. (2002). Sleep and Motor Skill Learning. *Neuron, 35*(1), 5-7.

Lee, A. K., and Wilson, M. A. (2002). Memory of sequential experience in the hippocampus during slow wave sleep. *Neuron, 36*(6), 1183-1194.

Lehrl, S. (2005). *Mehrfachwahl-Wortschatz-Intelligenztest MWT-B* (5th edition ed.). Balingen: Spitta Verlag.

Lepage, M., Habib, R., and Tulving, E. (1998). Hippocampal PET activations of memory encoding and retrieval: the HIPER model. *Hippocampus, 8*(4), 313-322.

Lewis, P. A., Cairney, S., Manning, L., and Critchley, H. D. (2011a). The impact of overnight consolidation upon memory for emotional and neutral encoding contexts. *Neuropsychologia, 49*(9), 2619-2629.

Lewis, P. A., Couch, T. J., and Walker, M. P. (2011b). Keeping time in your sleep: overnight consolidation of temporal rhythm. *Neuropsychologia, 49*(1), 115-123.

Lewis, P. A., and Durrant, S. J. (2011). Overlapping memory replay during sleep builds cognitive schemata. *Trends Cogn Sci, 15*(8), 343-351.

Loomis, A. L., Harvey, E. N., and Hobart, G. A. (1938). Distribution of disturbance patterns in the human electroencephalogram, with special reference to sleep. *J. Neurophysiol, 1*, 413-430.

Louie, K., and Wilson, M. A. (2001). Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. *Neuron, 29*(1), 145-156.

Lupien, S. J., Wilkinson, C. W., Briere, S., Menard, C., Ng Ying Kin, N. M., and Nair, N. P. (2002). The modulatory effects of corticosteroids on cognition: studies in young human populations. *Psychoneuroendocrinology, 27*(3), 401-416.

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., and Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage, 19*(3), 1233-1239.

Mandler, G. (1980). Recognizing: The judgment of previous occurrence. *Psychological Review, 87*(3), 252-271.

Maquet, P. (2001). The role of sleep in learning and memory. *Science, 294*(5544), 1048-1052.

Maquet, P., Laureys, S., Peigneux, P., Fuchs, S., Petiau, C., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., Meulemans, T., Luxen, A., Franck, G., Van Der Linden, M., Smith, C., and Cleeremans, A. (2000). Experience-dependent changes in cerebral activation during human REM sleep. *Nat Neurosci, 3*(8), 831-836.

Marr, D. (1970). A theory for cerebral neocortex. *Proc R Soc Lond B Biol Sci, 176*(43), 161-234.

Marr, D. (1971). Simple memory: a theory for archicortex. *Philos Trans R Soc Lond B Biol Sci, 262*(841), 23-81.

Marschark, M., and Surian, L. (1992). Concreteness effects in free recall: the roles of imaginal and relational processing. *Mem Cognit, 20*(6), 612-620.

Martin, S. J., de Hoz, L., and Morris, R. G. (2005). Retrograde amnesia: neither partial nor complete hippocampal lesions in rats result in preferential sparing of remote spatial memory, even after reminding. *Neuropsychologia, 43*(4), 609-624.

Massimini, M., Ferrarelli, F., Huber, R., Esser, S. K., Singh, H., and Tononi, G. (2005). Breakdown of cortical effective connectivity during sleep. *Science, 309*(5744), 2228-2232.

Mazzucchelli, C., Pannacci, M., Nonno, R., Lucini, V., Fraschini, F., and Stankov, B. M. (1996). The melatonin receptor in the human brain: cloning experiments and distribution studies. *Brain Res Mol Brain Res, 39*(1-2), 117-126.

McClelland, J. L., McNaughton, B. L., and O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev, 102*(3), 419-457.

McGaugh, J. L. (2000). Memory--a century of consolidation. *Science, 287*(5451), 248-251.

Mednick, S., Nakayama, K., and Stickgold, R. (2003). Sleep-dependent learning: a nap is as good as a night. *Nat Neurosci, 6*(7), 697-698.

Mednick, S. C., Drummond, S. P., Boynton, G. M., Awh, E., and Serences, J. (2008). Sleep-dependent learning and practice-dependent deterioration in an orientation discrimination task. *Behav Neurosci, 122*(2), 267-272.

Mednick, S. C., Nakayama, K., Cantero, J. L., Atienza, M., Levin, A. A., Pathak, N., and Stickgold, R. (2002). The restorative effect of naps on perceptual deterioration. *Nat Neurosci, 5*(7), 677-681.

Meienberg, P. (1977). The tonic aspects of human REM sleep during long-term intensive verbal learning. *Physiol Psychol, 5*(2), 250-256.

Miller, G. A. (1956). The magical number seven plus or minus two: some limits on our capacity for processing information. *Psychol Rev, 63*(2), 81-97.

Molle, M., and Born, J. (2011). Slow oscillations orchestrating fast oscillations and memory consolidation. *Prog Brain Res, 193*, 93-110.

Morris, G. O., Williams, H. L., and Lubin, A. (1960). Misperception and Disorientation During Sleep Deprivation. *Arch Gen Psychiatry, 2*(3), 247-254.

Morris, R. G., Garrud, P., Rawlins, J. N., and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297*(5868), 681-683.

Morris, S. B., and DeShon, R. P. (2002). Combining effect size estimates in meta-analysis with repeated measures and independent-groups designs. *Psychol Methods, 7*(1), 105-125.

Moscovitch, M., Nadel, L., Winocur, G., Gilboa, A., and Rosenbaum, R. S. (2006). The cognitive neuroscience of remote episodic, semantic and spatial memory. *Curr Opin Neurobiol, 16*(2), 179-190.

Moscovitch, M., Rosenbaum, R. S., Gilboa, A., Addis, D. R., Westmacott, R., Grady, C., McAndrews, M. P., Levine, B., Black, S., Winocur, G., and Nadel, L. (2005). Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. *J Anat, 207*(1), 35-66.

Müller, G. E., and Pilzecker, A. (1900). *Experimentelle Beiträge zur Lehre vom Gedächtnis*. Leipzig: Barth.

Murnane, K., and Shiffrin, R. M. (1991). Interference and the representation of events in memory. *J Exp Psychol Learn Mem Cogn, 17*(5), 855-874.

Nadel, L., and Moscovitch, M. (1997). Memory consolidation, retrograde amnesia and the hippocampal complex. *Curr Opin Neurobiol, 7*(2), 217-227.

Nelini, C., Bobbo, D., and Mascetti, G. G. (2010). Local sleep: a spatial learning task enhances sleep in the right hemisphere of domestic chicks (Gallus gallus). *Exp Brain Res, 205*(2), 195-204.

Nesca, M., and Koulack, D. (1994). Recognition memory, sleep and circadian rhythms. *Can J Exp Psychol, 48*(3), 359-379.

Nichols, T., Brett, M., Andersson, J., Wager, T., and Poline, J. B. (2005). Valid conjunction inference with the minimum statistic. *NeuroImage, 25*(3), 653-660.

Nishida, M., and Walker, M. P. (2007). Daytime naps, motor memory consolidation and regionally specific sleep spindles. *PLoS ONE, 2*(4), e341.

Nissen, M. J., and Bullemer, P. (1987). Attentional requirements of learning: evidence from performance measures. *Cognit. Psychol., 19*, 1-32.

Norman, K. A., and O'Reilly, R. C. (2003). Modeling hippocampal and neocortical contributions to recognition memory: a complementary-learning-systems approach. *Psychol Rev, 110*(4), 611-646.

O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res, 34*(1), 171-175.

O'Keefe, J., and Nadel, L. (1978). *The Hippocampus as a Cognitive Map*. Oxford: Oxford University Press.

Oldfield, R. C. (1971). *The Assesment and Analysis of Handedness: The Edinburgh Inventory* (Vol. 1).

Orban, G., Kiss, T., and Erdi, P. (2006). Intrinsic and synaptic mechanisms determining the timing of neuron population activity during hippocampal theta oscillation. *J Neurophysiol, 96*(6), 2889-2904.

Orban, P., Rauchs, G., Balteau, E., Degueldre, C., Luxen, A., Maquet, P., and Peigneux, P. (2006). Sleep after spatial learning promotes covert reorganization of brain activity. *Proc Natl Acad Sci U S A, 103*(18), 7124-7129.

Oswald, W. D., and Roth, E. (1987). *Der Zahlen-Verbindungs-Test (ZVT)* (2nd ed.). Göttingen: Hogrefe Verlag.

Oudiette, D., Antony, J. W., Creery, J. D., and Paller, K. A. (2013). The role of memory reactivation during wakefulness and sleep in determining which memories endure. *J Neurosci, 33*(15), 6672-6678.

Oztekin, I., Long, N. M., and Badre, D. (2010). Optimizing design efficiency of free recall events for FMRI. *J Cogn Neurosci, 22*(10), 2238-2250.

Packard, M. G., and Knowlton, B. J. (2002). Learning and memory functions of the Basal Ganglia. *Annu Rev Neurosci, 25*, 563-593.

Packard, M. G., and McGaugh, J. L. (1996). Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol Learn Mem, 65*(1), 65-72.

Pandya, D. N., and Barnes, C. L. (1987). Architecture and connections of the frontal lobes. In E. Perecman (Ed.), *The frontal lobes revisited* (pp. 41-72). New York: IRBN Press.

Patrick, G. T. W., and Gilbert, J. A. (1896). Studies from the psychological laboratory of the University of Iowa: On the effects of loss of sleep. *Psychological Review, 3*(5), 469-483.

Pavlides, C., and Winson, J. (1989). Influences of hippocampal place cell firing in the awake state on the activity of these cells during subsequent sleep episodes. *J Neurosci, 9*(8), 2907-2918.

Pavlov, I. P. (1927). *Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex* (Anat. 513 o). London: Oxford Univ. Press, Milford.

Payne, J. D., and Nadel, L. (2004). Sleep, dreams, and memory consolidation: the role of the stress hormone cortisol. *Learn Mem, 11*(6), 671-678.

Pearlman, C. A. (1981). Rat models of adaptive function of REM sleep. In W. Fishbein (Ed.), *Sleep, dreams and memory* (pp. 37-46). Lancaster: MTP Press.

Peigneux, P., Laureys, S., Delbeuck, X., and Maquet, P. (2001). Sleeping brain, learning brain. The role of sleep for memory systems. *Neuroreport, 12*(18), A111-124.

Peigneux, P., Laureys, S., Fuchs, S., Collette, F., Perrin, F., Reggers, J., Phillips, C., Degueldre, C., Del Fiore, G., Aerts, J., Luxen, A., and Maquet, P. (2004). Are spatial memories strengthened in the human hippocampus during slow wave sleep? *Neuron, 44*(3), 535-545.

Peigneux, P., Laureys, S., Fuchs, S., Destrebecqz, A., Collette, F., Delbeuck, X., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., Luxen, A., Cleeremans, A., and Maquet, P. (2003). Learned material content and acquisition level modulate cerebral reactivation during posttraining rapid-eye-movements sleep. *NeuroImage, 20*(1), 125-134.

Peigneux, P., Orban, P., Balteau, E., Degueldre, C., Luxen, A., Laureys, S., and Maquet, P. (2006). Offline persistence of memory-related cerebral activity during active wakefulness. *PLoS Biol, 4*(4), e100.

Peterson, L. R., and Peterson, M. J. (1959). Short-term retention of individual verbal items. *J Exp Psychol, 58*, 193-198.

Pilcher, J. J., and Huffcutt, A. I. (1996). Effects of sleep deprivation on performance: a meta-analysis. *Sleep, 19*(4), 318-326.

Piosczyk, H., Holz, J., Feige, B., Spiegelhalder, K., Weber, F., Landmann, N., Kuhn, M., Frase, L., Riemann, D., Voderholzer, U., and Nissen, C. (2013). The effect of sleep-specific brain activity versus reduced stimulus interference on declarative memory consolidation. *J Sleep Res, 22*(4), 406-413.

Plihal, W., and Born, J. (1997). Effects of early and late nocturnal sleep on declarative and procedural memory. *Journal of Cognitive Neuroscience, 9*(4), 534-547.

Plihal, W., and Born, J. (1999). Memory consolidation in human sleep depends on inhibition of glucocorticoid release. *Neuroreport, 10*(13), 2741-2747.

Plihal, W., Pietrowsky, R., and Born, J. (1999). Dexamethasone blocks sleep induced improvement of declarative memory. *Psychoneuroendocrinology, 24*(3), 313-331.

Plumer, S. I., Matthews, L., Tucker, M., and Cook, T. M. (1974). The water tank technique: avoidance conditioning as a function of water level and pedestal size. *Physiol Behav, 12*(2), 285-287.

Poe, G. R., Walsh, C. M., and Bjorness, T. E. (2010). Cognitive neuroscience of sleep. *Prog Brain Res, 185*, 1-19.

Poldrack, R. A., Clark, J., Pare-Blagoev, E. J., Shohamy, D., Creso Moyano, J., Myers, C., and Gluck, M. A. (2001). Interactive memory systems in the human brain. *Nature, 414*(6863), 546-550.

Poldrack, R. A., and Packard, M. G. (2003). Competition among multiple memory systems: converging evidence from animal and human brain studies. *Neuropsychologia, 41*(3), 245-251.

Postman, L., Stark, K., and Fraser, J. (1968). Temporal changes in interference. *Journal of Verbal Learning and Verbal Behavior, 1968*, 672-694.

Purdy, J. E., and Olmstead, K. M. (1984). New estimate for storage time in sensory memory. *Percept Mot Skills, 59*(3), 683-686.

Rasch, B., Buchel, C., Gais, S., and Born, J. (2007). Odor Cues During Slow-Wave Sleep Prompt Declarative Memory Consolidation. *Science, 315*(5817), 1426-1429.

Rasch, B., Pommer, J., Diekelmann, S., and Born, J. (2009). Pharmacological REM sleep suppression paradoxically improves rather than impairs skill memory. *Nat Neurosci, 12*(4), 396-397.

Rattenborg, N. C., Martinez-Gonzalez, D., and Lesku, J. A. (2009). Avian sleep homeostasis: convergent evolution of complex brains, cognition and sleep functions in mammals and birds. *Neurosci Biobehav Rev, 33*(3), 253-270.

Rauchs, G., Bertran, F., Guillery-Girard, B., Desgranges, B., Kerrouche, N., Denise, P., Foret, J., and Eustache, F. (2004). Consolidation of strictly episodic memories mainly requires rapid eye movement sleep. *Sleep, 27*(3), 395-401.

Rechtschaffen, A., and Kales, A. (1968). A manual of standardized terminology, techniques and scoring system of sleep stages of human subjects. *Bethesda, MD: United States Department of Health, Education, and Welfare, Public Health Service*, 1-59.

Ribeiro, S. (2012). Sleep and plasticity. *Pflugers Arch, 463*(1), 111-120.

Ribot, T. (1882). Diseases of the Memory: An Essay in the Positive Psychology (W. H. Smith, Trans.) *The international scientific series* (pp. 3 - 209). New York: D. Appleton and Company.

Richardson, A., and Gough, J. E. (1963). The long range effect of sleep on retention. *Australian Journal of Psychology, 15*, 37-41.

Richter, C. P. (1967). Psychopathology of periodic behavior in animals and man. *Proc Annu Meet Am Psychopathol Assoc, 55*, 205-227.

Rieth, C. A., Cai, D. J., McDevitt, E. A., and Mednick, S. C. (2010). The role of sleep and practice in implicit and explicit motor learning. *Behav Brain Res, 214*(2), 470-474.

Robertson, E. M. (2007). The serial reaction time task: implicit motor skill learning? *J Neurosci, 27*(38), 10073-10075.

Robertson, E. M., Pascual-Leone, A., and Press, D. Z. (2004). Awareness modifies the skill-learning benefits of sleep. *Curr Biol, 14*(3), 208-212.

Rolls, E. T., Xiang, J., and Franco, L. (2005). Object, space, and object-space representations in the primate hippocampus. *J Neurophysiol, 94*(1), 833-844.

Roozendaal, B. (2002). Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. *Neurobiol Learn Mem, 78*(3), 578-595.

Roselli, C. (2010). *Der differenzierte Beitrag verschiedener Hirnareale zur Speicherung von deklarativen Gedächtnisinhalten.* Unpublished Diploma thesis, Ludwig-Maximilians-University Munich, Munich.

Rosenbaum, R. S., Gao, F., Richards, B., Black, S. E., and Moscovitch, M. (2005). "Where to?" remote memory for spatial relations and landmark identity in former taxi drivers with Alzheimer's disease and encephalitis. *J Cogn Neurosci, 17*(3), 446-462.

Rosenbaum, R. S., Priselac, S., Kohler, S., Black, S. E., Gao, F., Nadel, L., and Moscovitch, M. (2000). Remote spatial memory in an amnesic person with extensive bilateral hippocampal lesions. *Nat Neurosci, 3*(10), 1044-1048.

Rudoy, J. D., Voss, J. L., Westerberg, C. E., and Paller, K. A. (2009). Strengthening individual memories by reactivating them during sleep. *Science, 326*(5956), 1079.

Rudy, J. W., and O'Reilly, R. C. (2001). Conjunctive representations, the hippocampus, and contextual fear conditioning. *Cogn Affect Behav Neurosci, 1*(1), 66-82.

Rugg, M. D., and Yonelinas, A. P. (2003). Human recognition memory: a cognitive neuroscience perspective. *Trends Cogn Sci, 7*(7), 313-319.

Rundus, D. (1977). Maintenance Rehearsal and Single-Level Processing. *Journal of Verbal Learning and Verbal Behavior, 16*, 665.

Sadowski, J. H., Jones, M. W., and Mellor, J. R. (2011). Ripples make waves: binding structured activity and plasticity in hippocampal networks. *Neural Plast, 2011*, 960389.

Savaskan, E., Ayoub, M. A., Ravid, R., Angeloni, D., Fraschini, F., Meier, F., Eckert, A., Muller-Spahn, F., and Jockers, R. (2005). Reduced hippocampal MT2 melatonin receptor expression in Alzheimer's disease. *J Pineal Res, 38*(1), 10-16.

Schabus, M., Gruber, G., Parapatics, S., Sauter, C., Klosch, G., Anderer, P., Klimesch, W., Saletu, B., and Zeitlhofer, J. (2004). Sleep spindles and their significance for declarative memory consolidation. *Sleep, 27*(8), 1479-1485.

Schabus, M., Hodlmoser, K., Gruber, G., Sauter, C., Anderer, P., Klosch, G., Parapatics, S., Saletu, B., Klimesch, W., and Zeitlhofer, J. (2006). Sleep spindle-related activity in the human EEG and its relation to general cognitive and learning abilities. *Eur J Neurosci, 23*(7), 1738-1746.

Schabus, M., Hoedlmoser, K., Pecherstorfer, T., Anderer, P., Gruber, G., Parapatics, S., Sauter, C., Kloesch, G., Klimesch, W., Saletu, B., and Zeitlhofer, J. (2008). Interindividual sleep spindle differences and their relation to learning-related enhancements. *Brain Res, 1191*, 127-135.

Schacter, D. L., and Wagner, A. D. (1999). Medial temporal lobe activations in fMRI and PET studies of episodic encoding and retrieval. *Hippocampus, 9*(1), 7-24.

Schendan, H. E., Searl, M. M., Melrose, R. J., and Stern, C. E. (2003). An FMRI study of the role of the medial temporal lobe in implicit and explicit sequence learning. *Neuron, 37*(6), 1013-1025.

Schenk, F., and Morris, R. G. (1985). Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. *Exp Brain Res, 58*(1), 11-28.

Schmidt, C., Peigneux, P., Muto, V., Schenkel, M., Knoblauch, V., Munch, M., de Quervain, D. J., Wirz-Justice, A., and Cajochen, C. (2006). Encoding difficulty promotes postlearning changes in sleep spindle activity during napping. *J Neurosci, 26*(35), 8976-8982.

Schmidt, D., Krause, B. J., Mottaghy, F. M., Halsband, U., Herzog, H., Tellmann, L., and Muller-Gartner, H. W. (2002). Brain systems engaged in encoding and retrieval of word-pair associates independent of their imagery content or presentation modalities. *Neuropsychologia, 40*(4), 457-470.

Schönauer, M., Geisler, T., and Gais, S. (2014). Strengthening procedural memories by reactivation in sleep. *J Cogn Neurosci, 26*(1), 143-153.

Scoville, W. B., and Milner, B. (2000). Loss of recent memory after bilateral hippocampal lesions. 1957. *J Neuropsychiatry Clin Neurosci, 12*(1), 103-113.

Sharon, T., Moscovitch, M., and Gilboa, A. (2011). Rapid neocortical acquisition of long-term arbitrary associations independent of the hippocampus. *Proc Natl Acad Sci U S A, 108*(3), 1146-1151.

Shearer, P. W. (1973). *The effect of first-half and second-half of the night sleep on single list retention, resistance to retroactive inhibition and spontaneous recovery of verbal material.* Ohio State University, Dissert Abst Int 1974;34:4097B-8B.

Siegel, J. M. (2003). Why we sleep. *Sci Am, 289*(5), 92-97.

Silber, M. H., Ancoli-Israel, S., Bonnet, M. H., Chokroverty, S., Grigg-Damberger, M. M., Hirshkowitz, M., Kapen, S., Keenan, S. A., Kryger, M. H., Penzel, T., Pressman, M. R., and Iber, C. (2007). The visual scoring of sleep in adults. *J Clin Sleep Med, 3*(2), 121-131.

Smith, C. (1995). Sleep states and memory processes. *Behav Brain Res, 69*(1-2), 137-145.

Smith, C. (2001). Sleep states and memory processes in humans: procedural versus declarative memory systems. *Sleep Med Rev, 5*(6), 491-506.

Smith, C., Kitahama, K., Valatx, J. L., and Jouvet, M. (1974). Increased paradoxical sleep in mice during acquisition of a shock avoidance task. *Brain Res, 77*(2), 221-230.

Smith, C., and MacNeill, C. (1994). Impaired motor memory for a pursuit rotor task following Stage 2 sleep loss in college students. *J Sleep Res, 3*(4), 206-213.

Squire, L. R. (1986). Mechanisms of memory. *Science, 232*(4758), 1612-1619.

Squire, L. R. (1987). *Memory and Brain*. New York: Oxford University Press.

Squire, L. R., and Alvarez, P. (1995). Retrograde amnesia and memory consolidation: a neurobiological perspective. *Curr Opin Neurobiol, 5*(2), 169-177.

Squire, L. R., and Wixted, J. T. (2011). The cognitive neuroscience of human memory since H.M. *Annu Rev Neurosci, 34*, 259-288.

Squire, L. R., and Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science, 253*(5026), 1380-1386.

Squire, L. R., and Zola, S. M. (1996). Structure and function of declarative and nondeclarative memory systems. *Proc Natl Acad Sci U S A, 93*(24), 13515-13522.

Staba, R. J., Wilson, C. L., Fried, I., and Engel, J., Jr. (2002). Single neuron burst firing in the human hippocampus during sleep. *Hippocampus, 12*(6), 724-734.

Stark, C. E., and Squire, L. R. (2001). When zero is not zero: the problem of ambiguous baseline conditions in fMRI. *Proc Natl Acad Sci U S A, 98*(22), 12760-12766.

Stephan, T., Böning, G., Cumming, P., Dera, T., Wängler, B., Petzschner, F., Wutte, M., Fraedrich, E. M., Schniepp, R., and Flanagin, V. L. (2010). Course: Introduction to functional Neuroimaging: fMRI and PET. February 1st to February 5th 2010. Neuroimaging Group. Klinikum der Universität München-Grosshadern.

Steriade, M., McCormick, D. A., and Sejnowski, T. J. (1993). Thalamocortical oscillations in the sleeping and aroused brain. *Science, 262*(5134), 679-685.

Stern, C. E., Corkin, S., Gonzalez, R. G., Guimaraes, A. R., Baker, J. R., Jennings, P. J., Carr, C. A., Sugiura, R. M., Vedantham, V., and Rosen, B. R. (1996). The hippocampal formation participates in novel picture encoding: evidence from functional magnetic resonance imaging. *Proc Natl Acad Sci U S A, 93*(16), 8660-8665.

Stickgold, R. (2004). Dissecting sleep-dependent learning and memory consolidation. Comment on Schabus M et al. Sleep spindles and their significance for declarative memory consolidation. Sleep 2004;27(8):1479-85. *Sleep, 27*(8), 1443-1445.

Stickgold, R., James, L., and Hobson, J. A. (2000a). Visual discrimination learning requires sleep after training. *Nat Neurosci, 3*(12), 1237-1238.

Stickgold, R., Whidbee, D., Schirmer, B., Patel, V., and Hobson, J. A. (2000b). Visual discrimination task improvement: A multi-step process occurring during sleep. *J Cogn Neurosci, 12*(2), 246-254.

Stones, M. J. (1973). The effect of prior sleep on rehearsal, recoding and memory. *Br J Psychol, 64*(4), 537-543.

Takashima, A., Petersson, K. M., Rutters, F., Tendolkar, I., Jensen, O., Zwarts, M. J., McNaughton, B. L., and Fernández, G. (2006). Declarative memory consolidation in humans: a prospective functional magnetic resonance imaging study. *Proc Natl Acad Sci USA, 103*(3), 756-761.

Talamini, L. M., Nieuwenhuis, I. L. C., Takashima, A., and Jensen, O. (2008). Sleep directly following learning benefits consolidation of spatial associative memory. *Learning & Memory, 15*(4), 233-237.

Tamaki, M., Matsuoka, T., Nittono, H., and Hori, T. (2009). Activation of fast sleep spindles at the premotor cortex and parietal areas contributes to motor learning: a study using sLORETA. *Clin Neurophysiol, 120*(5), 878-886.

Taylor, J. (2012). Limbic System. Retrieved 16.11.2012, 2012, from http://www.tayloredge.com/reference/Science/BiologySlides/LimbicSystem.gif

Thomas, M., Sing, H., Belenky, G., Holcomb, H., Mayberg, H., Dannals, R., Wagner, H., Thorne, D., Popp, K., Rowland, L., Welsh, A., Balwinski, S., and Redmond, D. (2000). Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. *J Sleep Res, 9*(4), 335-352.

Tilley, A. J., and Empson, J. A. (1978). REM sleep and memory consolidation. *Biol Psychol, 6*(4), 293-300.

Tononi, G., and Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Med Rev, 10*(1), 49-62.

Tucker, M., and Fishbein, W. (2008). Enhancement of declarative memory performance following a daytime nap is contingent on strength of initial task acquisition. *Sleep, 31*(2), 197-203.

Tucker, M., and Fishbein, W. (2009). The impact of sleep duration and subject intelligence on declarative and motor memory performance: how much is enough? *J Sleep Res, 18*(3), 304-312.

Tucker, M., Hirota, Y., Wamsley, E. J., Lau, H., Chaklader, A., and Fishbein, W. (2006). A daytime nap containing solely non-REM sleep enhances declarative but not procedural memory. *Neurobiology of Learning and Memory, 86*(2), 241-247.

Tulving, E. (1972). Episodic and semantic memory. In E. Tulving & W. Donaldson (Eds.), *Organization of memory* (pp. 381-403). New York: Academic Press.

Tulving, E. (1983). *Elements of episodic memory*. New York: Oxford University Press.

Tulving, E. (1985). Memory and consciousness. *Canadian Psychology, 26*, 1-12.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., and Joliot, M. (2002). Automated anatomical labeling of activations in SPM using a

macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage, 15*(1), 273-289.

Vartanian, O., and Goel, V. (2004). Neuroanatomical correlates of aesthetic preference for paintings. *Neuroreport, 15*(5), 893-897.

Vecsey, C. G., Baillie, G. S., Jaganath, D., Havekes, R., Daniels, A., Wimmer, M., Huang, T., Brown, K. M., Li, X. Y., Descalzi, G., Kim, S. S., Chen, T., Shang, Y. Z., Zhuo, M., Houslay, M. D., and Abel, T. (2009). Sleep deprivation impairs cAMP signalling in the hippocampus. *Nature, 461*(7267), 1122-1125.

Vertes, R. P. (2004). Memory consolidation in sleep; dream or reality. *Neuron, 44*(1), 135-148.

Vertes, R. P., and Eastman, K. E. (2000). REM sleep is not committed to memory. *Behavioral and Brain Sciences, 23*(06), 1057-1063.

Vertes, R. P., and Siegel, J. M. (2005). Time for the sleep community to take a critical look at the purported role of sleep in memory processing. *Sleep, 28*(10), 1228-1229; discussion 1230-1223.

Voermans, N. C., Petersson, K. M., Daudey, L., Weber, B., Van Spaendonck, K. P., Kremer, H. P., and Fernandez, G. (2004). Interaction between the human hippocampus and the caudate nucleus during route recognition. *Neuron, 43*(3), 427-435.

Vyazovskiy, V., Borbely, A. A., and Tobler, I. (2000). Unilateral vibrissae stimulation during waking induces interhemispheric EEG asymmetry during subsequent sleep in the rat. *J Sleep Res, 9*(4), 367-371.

Wagner, A. D., Shannon, B. J., Kahn, I., and Buckner, R. L. (2005). Parietal lobe contributions to episodic memory retrieval. *Trends Cogn Sci, 9*(9), 445-453.

Wagner, U. (2004). *Schlaf-assoziierte Konsolidierungs- und Restrukturierungsprozesse in der emotionalen und kognitiven Gedächtnisbildung.* University of Luebeck.

Wagner, U., Gais, S., Haider, H., Verleger, R., and Born, J. (2004). Sleep inspires insight. *Nature, 427*(6972), 352-355.

Wagner, U., Kashyap, N., Diekelmann, S., and Born, J. (2007). The impact of post-learning sleep vs. wakefulness on recognition memory for faces with different facial expressions. *Neurobiol Learn Mem, 87*(4), 679-687.

Walker, M. P. (2008a). Cognitive consequences of sleep and sleep loss. *Sleep Med, 9 Suppl 1*, S29-34.

Walker, M. P. (2008b). Sleep-dependent memory processing. *Harv Rev Psychiatry, 16*(5), 287-298.

Walker, M. P., Brakefield, T., Hobson, J. A., and Stickgold, R. (2003a). Dissociable stages of human memory consolidation and reconsolidation. *Nature, 425*(6958), 616-620.

Walker, M. P., Brakefield, T., Morgan, A., Hobson, J. A., and Stickgold, R. (2002). Practice with sleep makes perfect: sleep-dependent motor skill learning. *Neuron, 35*(1), 205-211.

Walker, M. P., Brakefield, T., Seidman, J., Morgan, A., Hobson, J. A., and Stickgold, R. (2003b). Sleep and the time course of motor skill learning. *Learn Mem, 10*(4), 275-284.

Walker, M. P., and Stickgold, R. (2004). Sleep-dependent learning and memory consolidation. *Neuron, 44*(1), 121-133.

Wamsley, E. J., Tucker, M., Payne, J. D., Benavides, J. A., and Stickgold, R. (2010). Dreaming of a Learning Task Is Associated with Enhanced Sleep-Dependent Memory Consolidation. *Current Biology*, 1-6.

Wamsley, E. J., Tucker, M., Payne, J. D., and Stickgold, R. (2010). A brief nap is beneficial for human route-learning: The role of navigation experience and EEG spectral power. *Learn Mem, 17*(7), 332-336.

Wilhelm, I., Diekelmann, S., Molzow, I., Ayoub, A., Molle, M., and Born, J. (2011a). Sleep selectively enhances memory expected to be of future relevance. *J Neurosci, 31*(5), 1563-1569.

Wilhelm, I., Wagner, U., and Born, J. (2011b). Opposite Effects of Cortisol on Consolidation of Temporal Sequence Memory during Waking and Sleep. *J Cogn Neurosci,* 10.1162/jocn_a_00093.

Wilson, R. A., and Keil, F. C. (1999). *The MIT Encyclopedia of the Cognitive Sciences*. Cambridge, MA: Massachusetts Institute of Technology.

Winocur, G., Moscovitch, M., and Bontempi, B. (2010). Memory formation and long-term retention in humans and animals: convergence towards a transformation account of hippocampal-neocortical interactions. *Neuropsychologia, 48*(8), 2339-2356.

Witt, K., Margraf, N., Bieber, C., Born, J., and Deuschl, G. (2010). Sleep consolidates the effector-independent representation of a motor skill. *Neuroscience, 171*(1), 227-234.

Wixted, J. T. (2004). The psychology and neuroscience of forgetting. *Annu Rev Psychol, 55*, 235-269.

Yaroush, R., Sullivan, M. J., and Ekstrand, B. R. (1971). Effect of sleep on memory. II. Differential effect of the first and second half of the night. *J Exp Psychol, 88*(3), 361-366.

Yonelinas, A. P. (1994). Receiver-operating characteristics in recognition memory: Evidence for a dual-process model. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 20*(6), 1341-1354.

Yonelinas, A. P. (2002). The nature of recollection and familiarity: a review of 30 years of research. *Journal of Memory and Language, 46*, 441-517.

Yoo, S. S., Hu, P. T., Gujar, N., Jolesz, F. A., and Walker, M. P. (2007). A deficit in the ability to form new human memories without sleep. *Nat Neurosci, 10*(3), 385-392.

Yotsumoto, Y., Sasaki, Y., Chan, P., Vasios, C. E., Bonmassar, G., Ito, N., Nanez, J. E., Sr., Shimojo, S., and Watanabe, T. (2009). Location-specific cortical activation changes during sleep after training for perceptual learning. *Curr Biol, 19*(15), 1278-1282.

10 Appendix

10.1 Terms and Explanations

Term	Explanation
BOLD	The blood oxygen level dependent contrast, abbreviated as BOLD contrast, is measured and used to demonstrate variations in brain perfusion (in case of brain imaging) related to neuronal activity.
Cued recall	Cued recall is a type of retrieval of previously learned information in which the subject is asked to recall the information with given cue support: a cue, such as one word of a two-word pair, aids memory recall. For example, words from two lists, A and B, are presented as A-B pair during the learning session. The word on the left side is from list A, the one on the right from list B. During retrieval, word A is presented to the subject. Word A serves as the cue for word B. In cued recall, subjects need to recall word B. See section 1.3
Free recall	Free recall is a type of retrieval of previously learned information in which the subject is asked to recall the information with minimal external cue support. Thus, subjects need to generate the answer themselves. It is a test of controlled memory search. In the literature of cognitive psychology free recall is often termed *serial* recall if items have to be recalled in the same order as they are presented. In this case, the order serves as cue support. In this thesis I do *not* distinguish between the order of the recalled items. The term free recall used here applies to both the recall in the order of item presentation and the order chosen by subject. See section 1.3
FWE	The family wise error rate represents the probability of type I errors (false positive results) in multiple statistical testing. One single brain volume contains over 100 000 voxels and when looking for activation, statistical tests treat each voxel individually, requiring tens of thousands of statistical tests (multiple comparisons) such that the probability of at least one false positive is very high. Family wise error corrections limit the amount of false positives across the whole brain.
PET	Positron emission tomography (PET) is a nuclear imaging technique showing the distribution of compounds labeled with positron-emitting isotopes in the body. First, a radioactive tracer is injected into the bloodstream (e.g. of a patient) and takes approximately 30 to 60 minutes to distribute throughout the body. In the PET, gamma rays (formed when a positron from the radioactive substance collides with an electron of a tissue) are detected. Gamma rays are converted to electrical signals and processed into images. Depending on the tracer, PET results in images of blood flow or other biochemical functions. Related to this thesis, PET can be used to measure regional cerebral blood flow (rCBF) during cognitive tasks, allowing researchers to make inferences about the function of specific brain regions. However, many other aims in neuroimaging exist for PET scans, such as the diagnosis and staging of dementia, cancer and other diseases (Nadel and Moscovitch, 1997; Stephan et al., 2010).
Place Cells	Place cells are specialized neurons in the hippocampus with a spatially confined firing field. O'Keefe and Dostrovsky (1971) first reported about hippocampal cells that fire when the animal is at a specific place in its environment. Place cells are part of a broader circuit of self-location, relative to the external environment. Together with other cells, such as grid cells (located in the entorhinal cortex, the main input to the hippocampus), place cells play an important role in spatial navigation.

Recognition	Recognition is a type of retrieval of previously learned information in which the subject is presented the correct answer among false choices. An example of a recognition test is the multiple choice test. The correct answer needs to be *recognized*. See section 1.3
Semantic	Semantic memoria includes facts, organized knowledge about words rules, concepts and relations, which can all be retrieved in the absence of a connection to a specific spatio-temporal context, (Hoscheidt et al., 2010) which concerns the “what”, “when” and “where” of the happenings (Aggleton and Pearce, 2001). In contrast to episodic memory, retrieval of semantic information is independent of the re-experience of the event in which the information was originally learned (Tulving, 1972).

10.2 German words learned in study 1

Fuchs	Tinte	Pflanze
Frosch	Zigarre	Kirche
Hammer	Brett	Larve
Stuhl	Fahne	Gehirn
Nagel	Reptil	
Fass	Pelz	
Stein	Bauch	
Arm	Schaum	
Gabel	Butter	
Papier	Pfirsich	
Kaffee	Pferd	
Pfeil	Sessel	
Raupe	Ofen	
Schlange	Klavier	
Flasche	Diamant	
Feuer	Gras	
Kugel	Wolle	
Vulkan	Gletscher	
Pfeife	Gold	
Stange	Salat	
Nonne	Küche	
Insekt	Getreide	
Palast	Pudding	
Junge	Schmied	
Plakat	Dampfer	
Metall	Vogel	
Sturm	Brief	
König	Fleisch	
Kleidung	Wiege	
Doktor	Stern	
Richter	Sänger	

10.3 German words learned in study 2

Burg	Schwan
Tourist	Becher
Uhr	Schrank
Feld	Moor
Freund	Korb
Straße	Garten
Zug	Hürde
Hütte	Keller
Zeitung	Taxi
Stirn	
Tür	
Hund	
Wein	
Dose	
Kern	
Wand	
Bahnhof	
Rathaus	
Leiter	
Klinik	
Zeugnis	
Maler	
Kranz	
Radio	
Koffer	
Schleier	
Hemd	
Gasthaus	
Fabrik	
Klub	
Lampe	

10.4 Questionnaire used for study 3

Alter _____

Geschlecht □ w □ m

Sind Sie Linkshänder? □ ja □ nein

Haben Sie in den letzten 6 Wochen Schichtarbeit geleistet? □ ja □ nein

Rauchen Sie? □ ja □ nein

Nehmen Sie zur Zeit Medikamente ein? □ ja □ nein

Trinken Sie regelmäßig Kaffee zum Frühstück? □ ja □ nein

Trinken Sie regelmäßig Kaffee nach der Mittagspause? □ ja □ nein

Wann gehen Sie normaler Weise zu Bett (im Durchschnitt)?_____ Uhr

Wann stehen Sie normaler Weise auf (im Durchschnitt)? _____ Uhr

10.5 Questionnaire used for study 4

Alter _____

Geschlecht □ w □ m

Bist du Linkshänder? □ ja □ nein

Hast du in den letzten 6 Wochen Schichtarbeit geleistet? □ ja □ nein

Rauchst du? □ ja □ nein

Nimmst du zur Zeit Medikamente ein? □ ja □ nein

wenn ja, welche _____

Trinkst du regelmäßig Kaffee zum Frühstück? □ ja □ nein

Trinkst du regelmäßig Kaffee nach der Mittagspause? □ ja □ nein

Hast du heute Kaffee/Tee/Alkohol getrunken? □ ja □ nein

Wenn ja, um wieviel Uhr? _____ Uhr

Wann gehst du normaler Weise zu Bett (im Durchschnitt)?_____ Uhr

Wann stehst du normaler Weise auf (im Durchschnitt)? _____ Uhr

Hälst du gelegentlich tagsüber ein Nickerchen? □ ja □ nein

Wenn ja, um wieviel Uhr generell _____ Uhr

Für wie lange? _____ min

10.6 Questionnaire used for study 4

Studienfach: _____ oder Beruf: _____

Alter: _____

Geschlecht □ w □ m

Bist du Linkshänder? □ ja □ nein

Hast du in den letzten 6 Wochen Schichtarbeit geleistet? □ ja □ nein

Rauchst du? □ ja □ nein

Spielst du Klavier? □ ja □ nein

wenn ja, seit wie vielen Jahren _____ und wie oft? _____

Ist deutsch deine Muttersprache? □ ja □ nein

Hast du Schlafstörungen? □ ja □ nein

Hast du schon mal an einer Studie teilgenommen, bei der du Silben (Buchstaben, die kein sinnvolles Wort ergaben) auswendig lernen musstest? □ ja □ nein

Nimmst du gerade an einer Studie unseres Schlaflabors (in der Martiusstr.) teil ? (z.B. Kernspinstudie von Melanie G. und Caro oder Morgenschlafstudie von Melanie P.) □ ja □ nein

Hattest du in den letzten 5 Tagen einen Schlafentzug (weniger als 6 Stunden geschlafen?) □ ja □ nein

Wenn ja, vor wie vielen Tagen? ____ Wie viele Std. Schlaf hattest du? _____

Nimmst du Medikamente ein (außer der Pille)? □ ja □ nein

wenn ja, welche _____

Trinkst du regelmäßig Kaffee zum Frühstück? □ ja □ nein

Trinkst du regelmäßig Kaffee nach der Mittagspause? □ ja □ nein

Hast du **heute** Kaffee/Tee/Alkohol getrunken? □ ja □ nein

Wenn ja, um wieviel Uhr? _____ Uhr

Wann gehst du normaler Weise zu Bett (im Durchschnitt)?_____ Uhr

Wann stehst du normaler Weise auf (im Durchschnitt)? _____ Uhr

Hältst du gelegentlich tagsüber ein Nickerchen? □ ja □ nein

Wenn ja, um wieviel Uhr generell _____ Uhr

Für wie lange? _____ min

Wie hast du von unserer Studie gehört?

Aushang, wenn ja, wo? (z.B. Bibliothek Geschwister–Scholl–Platz)

_____

Sonstiges: _____

10.7 Accuracy and Speed of Motor Sequences in study 5

Accuracy and speed of a main and new sequences for all conditions and groups.

Table 12: Accuracy for the 12h group.
Rpresented are the conditions (Contition): S= Sleep; SD = Sleep Deprivation and W = Wake. Accuracy (= correctly typed sequences, see 6.2.4) was captured once for the new sequence (New) and twice for the main sequence (Main), that is during Learning and during Recall. Recall occurred after the retention time of 12h. The new sequence was only offered to subjects once, therefore "Session" does not apply. Represented are mean accuracy (Mean), standard deviation (S.D.) and standard error (S.E.). N = 12.

Condition	S	S	S	SD	SD	SD	W	W	W
Session	Learning	Recall		Learning	Recall		Learning	Recall	
Sequence	Main	Main	New	Main	Main	New	Main	Main	New
MEAN	20.36	23.31	17.17	20.58	22.22	15.70	19.78	21.75	17.03
S.D.	4.00	6.99	4.52	4.58	6.84	6.31	6.70	7.57	6.09
S.E.	1.15	2.02	1.30	1.32	1.98	1.82	1.93	2.19	1.76

Table 13: Speed for the 12h group.
Rpresented are the conditions (Contition): S= Sleep; SD = Sleep Deprivation and W = Wake. Speed (= total number of sequences typed, see 6.2.4) was captured once for the new sequence (New) and twice for the main sequence (Main), that is during Learning and during Recall. Recall occurred after the retention time of 12h. The new sequence was only offered to subjects once, therefore "Session" does not apply. Represented are mean speed (Mean), standard deviation (S.D.) and standard error (S.E.). N = 12.

Condition	S	S	S	SD	SD	SD	W	W	W
Session	Learning	Recall		Learning	Recall		Learning	Recall	
Sequence	Main	Main	New	Main	Main	New	Main	Main	New
MEAN	21.97	24.56	18.86	22.25	23.53	17.25	21.39	23.53	18.56
S.D.	4.34	6.69	4.55	4.88	6.42	5.85	6.78	7.63	6.29
S.E.	1.25	1.93	1.31	1.41	1.85	1.69	1.96	2.20	1.82

Table 14: Accuracy for the 72h group.
Rpresented are the conditions (Contition): S= Sleep; SD = Sleep Deprivation and W = Wake. Accuracy (= correctly typed sequences, see 6.2.4) was captured once for the new sequence (New) and twice for the main sequence (Main), that is during Learning and during Recall. Recall occurred after the retention time of 72h. The new sequence was only offered to subjects once, therefore "Session" does not apply. Represented are mean accuracy (Mean), standard deviation (S.D.) and standard error (S.E.). N = 12.

Condition	S	S	S	SD	SD	SD	W	W	W
Session	Learning	Recall		Learning	Recall		Learning	Recall	
Sequence	Main	Main	New	Main	Main	New	Main	Main	New
MEAN	21.44	24.33	18.42	20.00	25.33	16.78	19.20	21.92	18.36
S.D.	3.58	4.38	4.42	5.12	6.85	4.23	5.11	7.10	5.93
S.E.	1.03	1.26	1.28	1.48	1.98	1.22	1.48	2.05	1.71

Table 15: Speed for the 72h group.
Rpresented are the conditions (Contition): S= Sleep; SD = Sleep Deprivation and W = Wake. Speed (= total number of sequences typed, see 6.2.4) was captured once for the new sequence (New) and twice for the main sequence (Main), that is during Learning and during Recall. Recall occurred after the retention time of 72h. The new sequence was only offered to subjects once, therefore "Session" does not apply. Represented are mean speed (Mean), standard deviation (S.D.) and standard error (S.E.). N = 12.

Condition	S	S	S	SD	SD	SD	W	W	W
Session	Learning	Recall		Learning	Recall		Learning	Recall	
Sequence	Main	Main	New	Main	Main	New	Main	Main	New
MEAN	23.47	26.33	19.97	22.92	26.81	18.70	21.75	23.67	20.09
S.D.	3.93	4.89	4.38	4.49	6.45	4.23	4.84	6.99	5.96
S.E.	1.13	1.41	1.26	1.30	1.86	1.22	1.40	2.02	1.72

Table 16: Accuracy for the 144h group.
Rpresented are the conditions (Contition): S= Sleep; SD = Sleep Deprivation and W = Wake. Accuracy (= correctly typed sequences, see 6.2.4) was captured once for the new sequence (New) and twice for the main sequence (Main), that is during Learning and during Recall. Recall occurred after the retention time of 144h. The new sequence was only offered to subjects once, therefore "Session" does not apply. Represented are mean accuracy (Mean), standard deviation (S.D.) and standard error (S.E.). N = 11.

Condition	S	S	S	SD	SD	SD	W	W	W
Session	Learning	Recall		Learning	Recall		Learning	Recall	
Sequence	Main	Main	New	Main	Main	New	Main	Main	New
MEAN	18.03	21.88	14.06	18.21	21.70	15.73	17.33	21.45	15.70
S.D.	4.66	5.98	4.22	4.98	6.70	4.64	5.20	6.78	6.07
S.E.	1.41	1.80	1.27	1.50	2.02	1.40	1.57	2.04	1.83

Table 17: Speed for the 144h group.
Rpresented are the conditions (Contition): S= Sleep; SD = Sleep Deprivation and W = Wake. Speed (= total number of sequences typed, see 6.2.4) was captured once for the new sequence (New) and twice for the main sequence (Main), that is during Learning and during Recall. Recall occurred after the retention time of 144h. The new sequence was only offered to subjects once, therefore "Session" does not apply. Represented are mean speed (Mean), standard deviation (S.D.) and standard error (S.E.). N = 11.

Condition	S	S	S	SD	SD	SD	W	W	W
Session	Learning	Recall		Learning	Recall		Learning	Recall	
Sequence	Main	Main	New	Main	Main	New	Main	Main	New
MEAN	20.06	23.06	15.55	20.12	23.36	17.39	19.42	22.61	17.39
S.D.	4.67	6.03	4.56	5.06	6.78	4.97	5.29	6.79	5.78
S.E.	1.41	1.82	1.38	1.53	2.04	1.50	1.59	2.05	1.74

10.8 Sleep Log

Wann bist du **gestern** zu Bett gegangen? _____ Uhr

Wann bist du **gestern** eingeschlafen? _____ Uhr

Wann bist du **heute** aufgewacht? _____ Uhr

Wann bist du **heute** aufgestanden? _____ Uhr

[This is an exemplary sleep log, part of all sleep studies of this thesis. Subjects filled out the questions as often as necessary for the studies.]

10.10 Stanford Sleepiness Scale as given to German subjects

Grad der Schläfrigkeit	**Skala**
Fühle mich aktiv, vital, voll da, hellwach	1
Habe einen klaren Kopf, bin aber nicht in Top-Form; kann mich konzentrieren	2
Wach, aber entspannt; reagiere, bin aber nicht so ganz da	3
Etwas benommen, kämpfe mit dem Schlaf; würde mich gerne hinlegen	6
Kämpfe nicht mehr gegen den Schlaf, schlafe gleich ein; traumartige Gedanken	7
Schlafe	X

11 Acknowledgements

I had the great opportunity to attend the interdisciplinary program of the Graduate School of Systemic Neurosciences, GSN-LMU. I would like to thank my three supervisors of the Thesis Advisory Committee, Prof. Steffen Gais, Prof. Stefan Glasauer and Dr. Virginia Flanagin, for their time and guidance throughout the program. I want to express my gratitude to Steffen for being my first supervisor and giving me the opportunity to join his Emmy Noether Group in sleep research. Thank you for introducing me to the techniques of fMRI, EEG and sleep research. I appreciate your scientific ideas, guidance on all projects and help with the interpretation of results. I also very much appreciate the problem-solving attitude of my second supervisor, Stefan, who especially helped me with scientific courses, talks, posters and finding office space. Virginia, thank you for all the weekends and late nights spent working with me on fMRI data collection. At this point I would like to thank Prof. Brückmann for providing the MR tomograph at the Department of Neuroradiology at the Clinic of the University Munich in Grosshadern. Sincere thanks to Prof. Müller and Renate Matsche for integrating me into the Department of Psychology of the LMU.

I want to express my gratitude to the managers of the GSN, especially Lena Bittl and Dr. Alexandra Stein for structuring the program, their guidance and supporting me with all PhD issues. Special thanks to Maj-Catherine Botheroyd for organizing events for us students.

I want to say a big thank you to my sister for all her help with graphics. Thank you mom for always being there for me. I would like to thank Ulli and Annedore for their thorough proof-reading. Jakob, thank you for helping me solve Matlab problems. Big thanks to Rainer, Melanie, Melina, Iskra, Eva and Markus for your friendship.

I would like to thank my entire research group for their scientific discussions. A special thanks goes to all research assistants and students of the Emmy Noether sleep research group. Special thanks to Monika, Jun, Anna, Caro and Franziska: Thank you for spending weekends at the clinic and nights awake during sleep deprivation sessions!

Curriculum Vitae
Melanie Grätsch

Contact Details

Email	melanie@graetsch.org

Current Position

Since 06.2013	Clinical Research Organization (CRO), Germany Dpt. of Clinical Operations - Therapeutic Fields: Neurology and Hematology - Clinical Trial Phases: II and III

Work History

08. – 12.2005	**Internship** Pharmaceutical Company, Germany Dpt. of Pharma Operations Biotechnology - Research and development in molecular biology and biochemistry - Techniques: Expression cloning, PCR, gel electrophoresis, sequencing, SDS-PAGE, ELISA, chromatography (HPLC; IMAC, TAC) - Immobilization of a monoclonal antibody using controlled pore glass
06. – 09.2003	**PR-Assistant** Public Relations Agency, Germany - Drafting and translation (English-German) of press releases - Print and online media support for the customers: Texas Instruments Inc., Baycom Multimedia and Broadcast Solutions GmbH, GTCO CalComp, Christie Digital Systems Inc. - Liaising with international customers to meet project specific goals

Further Education

11.2012 – 04.2013	**ATV GmbH, Munich, Germany** Certified qualification in Life Science Management

Education

Since 10.2009	**Graduate School of Systemic Neurosciences (GSN^{LMU})**, Germany Ph.D. fellow
Since 11.2008	Ludwig-Maximilians-University Munich, Germany Dept. of General and Experimental Psychology **Topics of Ph.D. research: Learning, memory and sleep** **Methods: Functional magnetic resonance imaging and electroencephalography**
10.2006 – 10.2008	University of Bielefeld, Germany **Degree: Master of Science,** grade: 1.0 Programme: Systems Biology of Brain and Behaviour
09.2007 – 02.2008	Université Pierre et Marie Curie, Paris, France Erasmus programme: Master of Neurosciences
10.2003 – 09.2006	Westfaelische Wilhelms-University Muenster, Germany **Degree: Bachelor of Science**, grade: 2.0 Programme: Biosciences (OWL Scholarship, Studienfonds OWL e.V.)

Posters

Graetsch, M.; Flanagin, V.L.; Roselli, C.; Glasauer, S.; Gais, S., "A Hippocampal Contribution to the Recall of Autobiographical, but not Spatial Memory". Multimodal and Sensorimotor Bionics, TUM-IAS, Munich, July 2011

Graetsch, M.; Flanagin, V.L.; Roselli, C.; Glasauer, S.; Gais, S., "Mutual and Distinct Brain Areas Activated by different forms of Declarative Memory". Berlin School of Brain and Mind, Berlin, May 2010

Graetsch, M.; Pawlizki, A.; Scherer, J.; Gais, S., "The influence of sleep on recall and recognition of non-semantic material". APM Congress, Leipzig, June 2009

Languages

German	Native Language
English	Fluent
French	Very good
Korean	Basic Knowledge

Computer Skills

Clinical Trial Management Systems (CTMS) Siebel Clinical, electronic trial master file (eTMF), Lawson, Crystal Reports, Microsoft Office, GanttProject, EndNote, SigmaPlot, PASW/SPSS, MATLAB, SPM, Brain Vision Analyzer

Community Service

- Mentor at the Brother-Sister-Program, dpt. of international relations, University of Bielefeld, Germany
- Lecturer in General Chemistry at the Westfaelische Wilhelms-University Muenster, Germany

10-Sep-2014

Affidavit / Eidesstattliche Erklärung

I hereby confirm that the dissertation with the title: *Neuroimaging and Behavioral Investigations of Memory Consolidation during Sleep on Time Scales from Hours to Months* is the result of my own work and that I have only used sources or materials listed and specified in the dissertation.

Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation mit dem Titel: *Neuroimaging and Behavioral Investigations of Memory Consolidation during Sleep on Time Scales from Hours to Months* selbstständig angefertigt habe, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

_____ _____

Place, date / Ort, Datum Signature / Unterschrift

List of Author Contributions

Abbreviation	Name	Institution
Gais, S.	Prof. Dr. Steffen Gais	1. Ludwig-Maximilians-University Munich, Graduate School of Systemic Neurosciences (GSN^{LMU}) 2. Eberhard Karls University Tuebingen, Institute for Medical Psychology and Behavioural Neurobiology
Glasauer, S.	Prof. Dr. Stefan Glasauer	Ludwig-Maximilians-University Munich 1. Graduate School of Systemic Neurosciences (GSN^{LMU}) 2. University Clinic Grosshadern, Dept. of Neurology
Flanagin, V.L.	Dr. Virginia Flanagin	Ludwig-Maximilians-University Munich 1. Graduate School of Systemic Neurosciences (GSN^{LMU}) 2. University Clinic Grosshadern, Dept. of Neurology
Leyer, K.	Katja Leyer	n/a
Steiner, U.	Dr. Ulrich Steiner	n/a
Pawlizki, A.	Dr. Annedore Pawlizki	n/a
Scherer, J.	Jakob Scherer	n/a
Roselli, C.	Anna Carolina Roselli	n/a
Graetsch, M.	Melanie Graetsch	Ludwig-Maximilians-University Munich, Graduate School of Systemic Neurosciences (GSN^{LMU})

Study 1 –The Relation between Episodic Memory and Sleep on a Functional and Behavioral Level, after Short and Long Delays

Designed the study: Gais, S.; Flanagin, V.L.; Graetsch, M.; Glasauer, S.

Conducted the experiment: Graetsch, M.; Flanagin, V.L.; Roselli, C.

Programmed the study: Gais, S.; Graetsch, M.; Flanagin, V.L.

Analyzed the data: Graetsch, M.; Flanagin, V.L.; Roselli, C.

Discussed the data: Gais, S.; Flanagin, V.L.; Graetsch, M.; Glasauer, S.

Wrote the chapter: Graetsch, M.

Designed the figures: Graetsch, M.; Leyer, K.

Commented the chapter: Gais, S; Steiner, U; Pawlizki, A.

Revised the chapter: Graetsch, M.

Study 2 – The Contribution of the Hippocampus to the Recall of Autobiographical, but not Spatial Memory

Designed the study: Gais, S.; Flanagin, V.L.; Graetsch, M.; Glasauer, S.

Conducted the experiment: Graetsch, M.; Flanagin, V.L.; Roselli, C.

Programmed the study: Gais, S.; Graetsch, M.; Flanagin, V.L.

Analyzed the data: Roselli, C.; Flanagin, V.L.; Graetsch, M.

Discussed the data: Gais, S.; Flanagin, V.L.; Graetsch, M.; Glasauer, S.

Wrote the chapter: Graetsch, M.

Designed the figures: Graetsch, M.; Flanagin, V.L.; Leyer, K.

Commented the chapter: Gais, S.; Steiner, U.; Pawlizki, A.

Revised the chapter: Graetsch, M.

Study 3 – Is the Type of Retrieval crucial for an Effect of Sleep on Declarative Memory?

Designed the study: Gais, S; Graetsch, M.

Conducted the experiment: Graetsch, M.

Programmed the study: Gais, S; Graetsch, M.

Analyzed the data: Graetsch, M.

Discussed the data: Gais, S; Flanagin, V.L.; Graetsch, M.; Glasauer, S.; Pawlizki, A.; Scherer, J.

Wrote the chapter: Graetsch, M.

Designed the figures: Graetsch, M.; Leyer, K.

Commented the chapter: Gais, S; Steiner, U; Pawlizki, A.

Revised the chapter: Graetsch, M.

Study 4 – Declarative Memory Retrieval after a Nap versus Wakefulness: Testing Cued Recall and Recognition of unrelated Word Pairs

Designed the study: Gais, S; Graetsch, M.

Conducted the experiment: Graetsch, M.

Programmed the study: Gais, S; Graetsch, M.

Analyzed the data: Graetsch, M.

Discussed the data: Gais, S.; Flanagin, V.L.; Graetsch, M.; Glasauer, S.; Pawlizki, A.; Scherer, J.

Wrote the chapter: Graetsch, M.

Designed the figures: Graetsch, M.; Leyer, K.

Commented the chapter: Gais, S.; Steiner, U.; Pawlizki, A.

Revised the chapter: Graetsch, M.

Study 5 – Declarative and Procedural Memory Retrieval after 12, 72 and 144 hours of nocturnal Sleep, Sleep Deprivation or Diurnal Wakefulness

Designed the study: Gais, S.; Graetsch, M.

Conducted the experiment: Graetsch, M.

Programmed the study: Gais, S.; Graetsch, M.

Analyzed the data: Graetsch, M.

Discussed the data: Gais, S.; Flanagin, V.L.; Graetsch, M.; Glasauer, S.; Pawlizki, A.; Scherer, J

Wrote the chapter: Graetsch, M.

Designed the figures: Graetsch, M.; Leyer, K.

Commented the chapter: Gais, S.; Steiner, U; Pawlizki, A.

Revised the chapter: Graetsch, M.

_____	_____	_____
Place	Date	Signature 1st supervisor Prof. Dr. Steffen Gais
_____	_____	_____
Place	Date	Signature Melanie Graetsch