Neurochemical mechanisms of sleep-dependent memory consolidation

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### II. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid</td>
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<td>DCS</td>
<td>d-cycloserine</td>
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<td>EEG</td>
<td>Electroencephalogram</td>
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<td>EMG</td>
<td>Electromyogram</td>
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<td>EOG</td>
<td>Electrooculogram</td>
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<td>FTT</td>
<td>Finger sequence tapping task</td>
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<td>GABA</td>
<td>γ-aminobutyric acid</td>
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<td>HEOG</td>
<td>Horizontal electrooculogram</td>
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<td>ISI</td>
<td>Inter stimulus interval</td>
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<td>i.v.</td>
<td>Intravenous</td>
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<td>LTP</td>
<td>Long term potentiation</td>
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<td>ML task</td>
<td>Motivated learning task</td>
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<td>NAcc</td>
<td>Nucleus accumbens</td>
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<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<td>NonREM</td>
<td>non rapid eye movement sleep</td>
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<td>PAL</td>
<td>Paired associate learning task (word pairs)</td>
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<td>PANAS</td>
<td>Positive and negative affective scale</td>
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<td>PKMζ</td>
<td>Protein kinase M zeta</td>
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<td>PVT</td>
<td>Psychomotor vigilance task</td>
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<tr>
<td>p.o.</td>
<td>Per os (orally)</td>
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<td>REM sleep</td>
<td>Rapid eye movement sleep</td>
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<tr>
<td>RMS</td>
<td>Root mean square</td>
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<td>SEM</td>
<td>Standard error of the mean</td>
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<td>SWS</td>
<td>Slow wave sleep</td>
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<td>TST</td>
<td>Total sleep time</td>
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<tr>
<td>VEOG</td>
<td>Vertical electrooculogram</td>
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<td>VTA</td>
<td>Ventral tegmental area</td>
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<td>WFT</td>
<td>Word fluency task</td>
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1 Introduction

1.1 General introduction

An organism’s ability to adapt to its sensory encounters, reactions and their outcome by forming lasting memories has evolutionary advantages. This has led to the development and preservation of many forms of plastic mechanisms in neurons throughout the animal kingdom. First demonstrated in *Aplysia californica* by Eric Kandel, Hebbian learning, i.e., the adaption of synaptic strength depending on firing contingencies, has been shown to be the basis of many of these mechanisms. However, the brain does not indiscriminately maintain all experiences but forms lasting representations mainly for the information that it considers to be of future use due to its salience or due to repeated encounters. The discrimination between relevant and irrelevant information may be made during learning but mechanisms of memory maintenance also contribute to this selection process. In humans, selection mechanisms driven purely by the knowledge that specific information will be required at a later point in time exist, but the amount of reward promised for retention is also effective. Advances in the field of sleep and memory during the last decade have shown that sleep is critically involved in the fate of memory traces acquired during prior wakefulness. Neurons that have encoded a specific memory are active again together during subsequent sleep and this reactivation is related to strengthening of memory traces. The delicate interplay of brain wide neuronal oscillations and the neuromodulatory milieu during sleep offer an ideal environment for transforming memories from their initially labile state into stable representations. The present work focuses on pharmacologically manipulating different neurotransmitter systems to identify their role in sleep-dependent memory consolidation. Glutamatergic neuroplasticity is manipulated to infer if Hebbian plasticity is also involved in sleep-dependent memory consolidation. Dopaminergic neuromodulation is perturbed to elucidate the neurochemical mechanism of reward driven sleep-dependent memory consolidation. γ-aminobutyric acid (GABA) levels are manipulated to induce slow wave sleep and thereby enhance sleep-dependent memory consolidation. Parts of the present work have already been published in the journals *Sleep* and *Neuropsychopharmacology* (Feld, Lange, Gais, &
1.2 Memory

1.2.1 Memory systems

While the plastic processes involved may be similar, different forms of memory involve different brain regions depending on the type of memory that is stored. Brenda Milner’s pioneering work with the patient Henry Gustav Molaison (H.M.) indicated that remote and recent memory have different anatomical correlates and that motor skills can be trained without persistence of explicit knowledge of the episode in which this training occurred (Scoville & Milner, 1957). Today a division between the non-declarative and declarative memory systems is classically made (Squire, 1992; Squire & Zola, 1996; see Figure 1.1).

Declarative memory that can be further divided into semantic memory (memory for facts) and episodic memory (memory for events) is defined anatomically by its dependence on the hippocampus. Episodic memories can be considered precursors of semantic memories, as the repeated experience of the same information (e.g., sweet foods are often served at the end of a dinner) within different events can lead to the abstraction of facts (e.g., desert is the last course), which does not always allow the reconstruction of the first event where this fact was encountered. Sleep may be ideally suited to extract this gist from the constant stream of events (Inostroza & Born, 2013; Lewis & Durrant, 2011) as is detailed in the sections below. While we can usually retrieve most of our experiences from the day before, information that is not required is usually forgotten rather fast (Hardt, Nader, & Nadel, 2013).

Non-declarative memory spans different types of memories that are typically acquired implicitly. For example, perceptual tasks such as the visual texture discrimination task that relies mainly on plasticity in the visual cortex or conditioning that relies on amygdala activation have been shown to benefit from sleep (Gais, Plihal, Wagner, & Born, 2000; Menz et al., 2013). However, the non-declarative tasks that are used in the present work are limited to procedural skills and the following discussion will therefore focus on these types of non-declarative memory. Motor and perceptual skills are considered part of the procedural memory system and typically rely on many repetitions within a short time frame to be learned. This form of learning leads
to rather robust traces and can be performed with or without awareness of the material to be learned. Most times the task binds explicit attention at first and when performance increases implicit processes, e.g., automation, take over. Procedural motor skills were not thought to rely on hippocampal areas at initial learning, however, it has recently been shown that at least initially hippocampal activation can be seen in the fMRI for the serial reaction time task during explicit and implicit learning (Schendan, et al., 2003). While during wakefulness striatum and hippocampus may interfere with each other concerning task performance, during subsequent sleep their signals may be integrated to improve performance (Albouy et al., 2008; Albouy et al., 2013).

<table>
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<td>Declarative memory</td>
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<td>Episodic memory</td>
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<td>Non-declarative memory</td>
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<td>Skill learning</td>
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*Figure 1.1. Multiple systems of long-term memory (Squire & Zola 1996)*

1.2.2 *Stages of memory formation*

The process of memory formation can be subdivided into three basic stages: (1) encoding is the stage of information uptake and storage, (2) consolidation or retention is the phase of memory upkeep and (3) retrieval is the stage of reproduction (Squire & Wixted, 2011). In the declarative domain encoding initially relies on the hippocampus, which acts as a hub that binds the activations in other brain regions forming the new memory into a representation (Battaglia, Benchenane, Sirota, Pennartz, & Wiener, 2011). Retrieval of declarative memory initially relies on hippocampal networks that bind the representation, but research in patients with lesions of the medial temporal lobe, such as H.M., or in intentionally lesioned animals, has demonstrated that retrieval can gradually become independent of the hippocampus (Frankland & Bontempi, 2005). While some reports of conditioning during sleep exist (Arzi et al., 2012; Ikeda & Morotomi, 1996), it is assumed that learning and retrieval are most effective during wakefulness (Diekelmann & Born, 2010).

While research on the retention period initially focused on rates of forgetting and the question whether it is produced by interference or decay (McGeoch, 1932; Thorndike, 1913), it is clear that some memories are not forgotten at all. Interference
Memory

theories are a version of the plasticity stability dilemma, i.e., that the brain relies on stable representations that must be protected from being continuously overwritten by new traces, but at the same time needs to be plastic, so that new representations can be written into the same storage system (Carpenter & Grossberg, 1988). One solution of this problem is assuming a two stage model of memory formation, where isolated memory traces are rapidly formed initially and are only later gradually integrated into long-term memory stores (Marr, 1971; McNaughton, Barnes, Rao, Baldwin, & Rasmussen, 1986). This two stage model forms the basis of standard consolidation theory and recent developments thereof (Winocur, Moscovitch, & Bontempi, 2010). The model assumes that initial encoding established in a fast learning system is transient but fast. This system is accompanied by a slow learning system, into which the trace can be transferred for stable safekeeping. As of late this transfer process is explicitly assumed to also lead to a transformation of the trace and is therefore now called trace transformation theory. Encoding is thought to be established in the fast learning system by binding of information that is already present in the long-term stores. Repeated reactivation of the newly encoded information together with already established long term memories then leads to the integration of the new information. This process can lead to the abstraction of invariant features of individual episodes, as overlapping features are reactivated more frequently (Lewis & Durrant, 2011). Marginal reactivation of irrelevant features may lead to their erasure. In the declarative domain the role of the fast learning system is attributed to the hippocampus and long term memory relies on the networks of the neocortex. Repeated reactivation of the information stored in the hippocampus is thought to be the basis of memory transfer to the neocortex, so that retrieval, over the course of days to years, can become independent of the hippocampus (Frankland & Bontempi, 2005; see Figure 1.2; McClelland, McNaughton, & O'Reilly, 1995; Zola-Morgan & Squire, 1990).

![Figure 1.2](image)

Figure 1.2. A model for the transfer of memory from the hippocampus, which initially has a hub-like function binding the new trace and disengages as soon as the cortical trace can represent the memory without aid (Frankland & Bontempi, 2005).
System consolidation that involves the transfer of traces from one system to another is one of two forms of consolidation that are generally differentiated and is distinguished from synaptic consolidation (Dudai, 2004). Synaptic consolidation involves the strengthening of those synapses involved in encoding of the trace per se and can be perturbed, e.g., by administering protein synthesis blockers shortly after learning (Bourtchouladze et al., 1998; Xia, Feng, & Guo, 1998). However, synaptic (re-)consolidation also occurs after reactivation (Milekic & Alberini, 2002). System consolidation is assumed to be most effective during sleep, when the brain is offline, i.e., deprived from the constant influx of sensory information (Diekelmann & Born, 2010), evidence for this notion will be discussed in the section on Memory and sleep.

1.2.3 Long term potentiation and other forms of synaptic plasticity

In 1949 Donald O. Hebb postulated that learning in neuronal networks is established by increasing the connection between two neurons that fire together. Synaptic long term potentiation (LTP) describes a process of strengthening the connection between two neurons by increasing their signal transduction efficacy and requires correlated firing. Signal transduction efficacy in this model is expressed by the amplitude and duration of post synaptic currents generated by action potentials arriving at the synapse. The most thoroughly investigated form of LTP is related to glutamatergic signalling. At the glutamatergic synapse presynaptic and postsynaptic modifications have been shown to be responsible for the expression of LTP (Bliss & Collingridge, 2013). Presynaptic mechanisms are thought to involve the amount of glutamate released into the synaptic cleft and the speed of clearance therefrom. Postsynaptic mechanism include modifications of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid (AMPA) receptor properties, e.g., changes in opening time or opening probability due to glutamate binding and, the amount of AMPA receptors in the active zone. Changes in synapse morphology probably involve increase in size of both pre- and postsynaptic structures.

A popular mechanism of N-methyl-D-aspartate (NMDA) receptor dependent LTP in the hippocampus is described in this section and serves to explain the empirical findings (see Figure 1.3). The basis of this model is that NMDA receptors act as coincidence detectors that detect correlated activity and react by strengthening the synaptic connection (Malenka & Nicoll, 1999). For the opening of their ion channels NMDA receptors require binding of glutamate and a co-agonist, i.e., glycine or d-
serine (Kleckner & Dingledine, 1988; Mothet et al., 2000). At resting membrane potentials NMDA receptor Ca\(^{2+}\) permeability is blocked by Mg\(^{2+}\). Time and/or location summation of post synaptic potentials that rely on AMPA receptor activation can push the membrane potential across the threshold, which releases the Mg\(^{2+}\)-block. Opening of the NMDA receptor ion channels allows the influx of Ca\(^{2+}\), which combines with calmodulin and activates downstream targets such as calmodulin dependent kinase II. This signalling cascade can lead to, e.g., phosphorylation of AMPA receptors and trafficking of AMPA receptors to the active zone and, thus, increases signal transduction.

A discussion of all types of synaptic plasticity goes beyond the scope of the present work, but, importantly, plasticity is not a one way street and mechanisms of NMDA receptor dependent long term depression have been identified (Malenka & Bear, 2004). Also decay of LTP at potentiated synapses relies on NMDA receptors (Villarreal, Do, Haddad, & Derrick, 2002). There is evidence that the exact timing of firing of the pre- and postsynaptic neuron can influence what kind of plasticity is exhibited and this model has been termed spike timing dependent plasticity (Caporale & Dan, 2008). Finally, considerable evidence is amounting that NMDA receptor subunit composition can account for differences in the direction of plasticity (Paoletti, Bellone, & Zhou, 2013).

Figure 1.3. Schematic overview of processes for long term potentiation. (A) Repeated activation of the post synaptic AMPA receptor induced Na\(^{+}\)-currents leads to the depolarization of the post synaptic membrane; (B) the release of the magnesium block from the NMDA receptor. The NMDA receptor can now induce Ca\(^{2+}\)-influx, which starts signalling cascades that lead to plastic processes.
1.2.4 Neuromodulation of synaptic plasticity

Neuromodulators are neurotransmitters that are not involved in direct synaptic transmission but rather modulate ongoing synaptic communication of the brain. Molecules that are considered neuromodulators for some cells can also be involved in direct communication at others, e.g., acetylcholine is responsible for signal transduction at the neuromuscular junction but can influence learning and attention by modulating neuronal function in the brain (e.g., Sarter, Bruno, & Givens, 2003).

In mammals the adaptive effect of rewards is thought to be induced by dopamine signalling modulating ongoing plastic processes in the brain (Schultz, 2000, 2007, 2013; Wise, 2004; Wise & Rompre, 1989). Plasticity in the hippocampus is gated by dopaminergic activity (e.g., Edelmann & Lessmann, 2013). Dopaminergic afferents from the ventral tegmental area (VTA) to the hippocampus that are activated by striatal inputs are thought to convey information about stimulus value and novelty (Lisman & Grace, 2005). Consequently, rewards can improve both the retention of declarative and non-declarative memory (Abe et al., 2011; Adcock, Thangavel, Whitfield-Gabrieli, Knutson, & Gabrieli, 2006), which is probably achieved by modulation of hippocampal plasticity and/or modulation of plasticity in extra-hippocampal brain areas.

1.3 Sleep

1.3.1 Sleep stages

Sleep is a phylogenetically well preserved behaviour that is present also in organisms that have nervous systems with low complexity, such as *Drosophila melanogaster* (Huber, Hill, et al., 2004) or *Caenorhabditis elegans* (Raizen et al., 2008) and Cirelli and Tononi (2008) argue that it may even be essential in any organism that learns according to Hebbian rules. To identify sleep in non-mammalian species the following attributes have been used to define sleep: (1) immobility, (2) species specific posture, (3) increased arousal threshold and (4) homeostatic regulation. The mammalian brain produces electrical signals (Electroencephalogram - EEG) that together with physiological data (from the periphery: Electromyogram – EMG and sometimes Electrooculogram – EOG) clearly differentiate sleep from wakefulness. Aserinsky and Kleitman (1953) were the first to identify periods of rapid eye movement (REM) sleep in humans, which eventually led to the first consensus-based recommendation of methods and criteria for scoring sleep (Rechtschaffen & Kales, 1968). And while
there has been a recent effort by the American Academy of Sleep Medicine to introduce a new standard for clinical polysomnography (Silber et al., 2007), the majority of basic scientific labs still score according to the 1968 guidelines. The following text briefly summarizes these guidelines so that differences in the procedure used in the present work can be appreciated.

The minimum recommendation for polysomnography is two EEG channels placed at C3 and C4 (according to the 10-20 system) and referenced to the respective contralateral mastoids, EOG recorded from two electrodes placed left above the left eye and right below the right eye and EMG recorded from two electrodes placed on the chin. Sleep scoring is performed offline on 30 second epochs of the polysomnographic data after filtering. The sleep stages are split into wakefulness (wake), defined by a high muscle tone and > 50% alpha rhythm (8 - 13 Hz) in the EEG, REM sleep, defined by a mixed frequency EEG, eye movements and absence or near absence of muscle tone, and Non-REM (NonREM) sleep. NonREM sleep is further subdivided into sleep stage 1 (NonREM 1), which is defined by < 50% alpha rhythm in the EEG, sleep stage 2 (NonREM 2), defined by the occurrence of sleep spindles (waxing and waning 12-14 Hz oscillation with a duration > 0.5 s) and/or K-complexes (sharp negative high-voltage deflexion of the EEG followed by a slower positive wave with a duration > 0.5 s), sleep stage 3 (NonREM 3), defined by > 20% delta waves (< 2 Hz, peak-to-peak amplitude > 75 µV) in the EEG, and, sleep stage 4, defined by > 50% delta waves. NREM 3 and 4 are often summarized by the term slow wave sleep (SWS).

More fine grained analyses of polysomnographic data are performed by calculating spectral data for the individual sleep stages and comparing specific frequency bands, i.e., the slow oscillation band (0.5-1 Hz), the delta band (1-4 Hz), the slow spindle band (9-12 Hz) and the fast spindle band (12-15 Hz). Additionally, slow oscillation (very slow oscillations peaking at 0.75 Hz that consist of an up-state with wake-like neuronal firing and a down-state with wide-spread neuronal silence) and sleep spindle events can be detected to investigate effects on their morphology or to perform analyses on the time locked EEG (see Molle & Born, 2011 for an overview of work related to memory consolidation).
1.3.2 Neurotransmitters and sleep

Many neurotransmitters that are indicated in memory formation and maintenance are also reciprocally related to sleep, i.e., they can influence sleep and are influenced by sleep (or other circadian factors) in turn. Sleep architecture and occurrence is tightly regulated by neuromodulators and neurotransmitters in the central nervous system, e.g., acetylcholine, GABA, histamine, orexin (Pace-Schott & Hobson, 2002). Specifically, SWS is accompanied by a marked decrease in cortisol, acetylcholine and noradrenaline levels (Aston-Jones & Bloom, 1981; Born, Lange, Hansen, Molle, & Fehm, 1997; Marrosu et al., 1995; Weitzman et al., 1971). Conversely, gonadotropin, growth hormone and prolactin reach their maximum concentration during SWS (Gore, 1998; Spiegel et al., 1994; Spratt et al., 1988; Van Cauter et al., 1992). Glutamate levels have been shown to increase over periods of wakefulness and REM sleep and reduce over NonREM sleep (Dash, Douglas, Vyazovskiy, Cirelli, & Tononi, 2009), similarly, dopamine levels seem to be reduced during sleep (Feenstra, Botterblom, & Mastenbroek, 2000; Sowers & Vlachakis, 1984), which speaks for homeostatic regulation in these systems. Finally, GABA levels in the cortex are increased during Non-REM sleep and reduced during wakefulness and REM sleep (Vanini, Lydic, & Baghdoyan, 2012), which probably pertains to GABA’s role in the regulation of sleep. Importantly, as many neurotransmitter pathways exert influence on the generation of sleep per se the effects of their manipulation on sleep can contribute a potential confound to studies looking at memory consolidation during sleep.

1.4 Memory and sleep

1.4.1 The history of sleep and memory research

While there is still a considerable debate about the core function of sleep, the recent advances in sleep and memory research have painted an ever clearer picture of sleep’s beneficial influence on memory. The most prominent classical semi-systematic observation of reduced forgetting due to sleep in the retention interval was reported by Jenkins and Dallenbach (1924). Their data replicated reports by (Ebbinghaus, 1885), who had attributed reduced forgetting during sleep to errors in his data. This experiment contributed to the establishment of the interference theory of sleep and memory, as the authors reasoned that forgetting was lower during sleep because interference through incoming sensory information is reduced compared to
wakefulness. During the 20th century the beneficial effect of sleep on memory was revisited several times (Barrett & Ekstrand, 1972; Benson & Feinberg, 1975; Ekstrand, 1967; Ekstrand, Barrett, West, & Maier, 1977). However, the recent upsurge of experiments on sleep and memory points towards a theory involving an active role of sleep for memory maintenance (Rasch & Born, 2013). The most prominent proponent the active system consolidation hypothesis is detailed in the next section.

1.4.2 The active systems consolidation hypothesis

While obscure only a decade ago, the mechanisms of memory maintenance have received growing attention over the last years. This research has led to the conclusion that, whereas memory is most effectively encoded during wakefulness, sleep promotes the consolidation of memory. In the declarative domain, memory consolidation during sleep is thought to be an active process, which involves reactivating neuronal ensembles that encoded these memories during wakefulness (Diekelmann & Born, 2010; Oudiette & Paller, 2013; Rasch & Born, 2013).

Work in rodents shows that neuron assemblies that displayed correlated activity during wakefulness are more likely to fire together during subsequent sleep (Wilson & McNaughton, 1994). This replay occurs in the same sequence as during wakefulness and is coordinated between the hippocampus and neocortex (Ji & Wilson, 2007; Skaggs & McNaughton, 1996).

A causal role of replay during SWS for the consolidation of hippocampus-dependent declarative memory (Rasch, Buchel, Gais, & Born, 2007; Rudoy, Voss, Westerberg, & Paller, 2009) and motor skill memory (Antony, Gobel, O’Hare, Reber, & Paller, 2012; Schonauer, Geisler, & Gais, 2014) was demonstrated in humans. In these studies participants learned while being exposed to olfactory or auditory stimuli that became associated with the learning material. During sleep after learning, they were re-exposed to these stimuli or sham stimulation. At retrieval after sleep the participants who were exposed to the stimuli performed significantly better than the controls. Also, stimulation during REM sleep and wakefulness was not successful in boosting memory.

The enhancing effect on memory consolidation appears to be mediated in particular by the neocortical <1 Hz slow oscillation that hallmarks the EEG during SWS, and synchronizes the neuronal reactivation of newly acquired memory representations
that takes place during SWS in distributed networks, to the excitable depolarizing up-state of these slow oscillations (Molle & Born, 2011, see Figure 1.4). This allows the redistribution of the reactivated memory representations and their stabilization for the longer term (Diekelmann & Born, 2010). The memory consolidating effect of the slow oscillations appears to additionally result from the fact that in parallel with reactivations, they also synchronize thalamo-cortical spindles (12-15 Hz) to the depolarizing slow oscillation up-state (Bergmann, Molle, Diedrichs, Born, & Siebner, 2012; Mölle, Bergmann, Marshall, & Born, 2011). Post-learning spindle activity has been consistently found to be associated with the retention of declarative and procedural memories (Fogel & Smith, 2011; Schabus et al., 2004; Tamaki, Matsuoka, Nittono, & Hori, 2009), especially when occurring during the depolarizing phase of the slow oscillation (Ruch et al., 2012). Enwrapping reactivated memory information, spindles might provoke processes, like enhancing cellular calcium influx, in neocortical networks that prime plastic processes underlying the longer-term storage of the reactivated information in these networks (Ribeiro et al., 2007).

**Figure 1.4.** Model of the interplay between different oscillations during slow wave sleep. Reactivation of memory traces in the hippocampus, in form of sharp-wave ripples, occur most prominently during the slow oscillation up-state. The coupling of fast spindles to the up-state leads to the occurrence of spindle-ripple events. This allows the reactivated memories to reach the cortex, possibly represented by gamma-oscillations, during windows of high plasticity (Feld & Born, 2012).

### 1.4.3 Selective benefit of sleep for memory

The role of sleep for memory is not limited to a mere stabilization of traces, but also leads to qualitative changes of the trace. For example, Wagner and colleagues (2004) showed that memory transformation during sleep can lead to insight. This was demonstrated by letting participants solve mathematical problems that were constructed to have a long and a short way to their solution. Participants solved some of
these problems before sleeping or staying awake. The sleep group showed significantly greater rates of detecting the short-cut. Another demonstration of this qualitative change is the induction of false memories by sleep. In these studies participants learn a list of words that are congruent with a lure that is presented within the retrieval list. Sleep seems to increase the participants’ susceptibility to falsely remember these words (Diekelmann, Born, & Wagner, 2010; Payne et al., 2009).

Another way in which sleep’s benefit for memory is specific is that it only facilitates the retention of memories that will be retrieved at a later time point (Wilhelm, Diekelmann, et al., 2011). This was shown by letting participants learn word pairs and instructing half of the participants that the words will be retrieved at a later time point. Sleep only benefited retention, if participants knew they would be tested again, whereas uninformed participants performed as badly as the wake control group. Sleep’s effect on memory can also be manipulated by granting rewards for successful retrieval (Fischer & Born, 2009). In this study participants significantly increased their retention of a finger tapping task, if they were told they would receive a reward for successful retrieval the next day. Oudiette, Antony, Creery, and Paller (2013) showed that this enhancing reward effect can be levelled out by externally cueing low rewarded memory traces, which may suggest that reactivation probability is influenced by rewards. Interestingly, during sleep, cueing half of the low rewarded items in this study improved retention of the whole set of low reward items, whereas cueing whilst awake specifically enhanced the cued items.

1.4.4 Pharmacological influences on sleep-dependent memory consolidation

While the specific roles of different neurotransmitters during sleep for memory is not fully understood, a number of studies that have pharmacologically manipulated neurotransmitter systems demonstrate their influence on sleep-dependent memory consolidation. Usually, in these studies, participants learn a task in the evening before a retention interval containing sleep and the pharmacological agent is administered thereafter. This leads to a manipulation of sleep-dependent mechanisms by the agent and after it is removed from the system, retrieval is tested to reveal the agents influence on consolidation.

As mentioned above, SWS is typically accompanied by low levels of neuromodulators such as acetylcholine (Marrosu et al., 1995) and the direction of information flow between hippocampus and neocortex is thought to rely on cholinergic tone, as
low levels of acetylcholine release feedback synapses in the hippocampus (Buzsaki, 1986; Hasselmo, 1999). Consequently, increasing cholinergic activity with cholinesterase blocker physostigmine during SWS disrupts declarative memory consolidation (Gais & Born, 2004b). The opposite procedure, blocking muscarinic and nicotinergic receptors, improves the consolidation of declarative memory during a wake interval (Rasch, Born, & Gais, 2006), but interferes with motor memory consolidation during REM sleep (Rasch, Gais, & Born, 2009).

Similar findings have been reported for low levels of cortisol that are commonly found during SWS, inasmuch as, administration of hydrocortisone or dexamethasone impaired sleep-dependent declarative memory consolidation (Plihal & Born, 1999; Plihal, Pietrowsky, & Born, 1999; Wilhelm, Wagner, & Born, 2011). Interestingly and demonstrating the delicate balance achieved in the sleeping brain, further decreasing the already low levels of cortisol during SWS also impairs declarative memory consolidation (Wagner, Degirmenci, Drosopoulos, Perras, & Born, 2005). These counterintuitive effects may be due to different sensitivity of mineralocorticoid- and glucocorticoid receptors to cortisol and their opposing contribution to memory processing (Groch, Wilhelm, Lange, & Born, 2013; Rimele, Besedovsky, Lange, & Born, 2013).

As mentioned above, noradrenaline levels are typically low during SWS, however, locus coeruleus bursts can be observed during sleep spindles and slow oscillations (Aston-Jones & Bloom, 1981), which seem to be related to pre-sleep episodes of learning (Eschenko & Sara, 2008). Accordingly, the inhibition of noradrenergic signalling by administering the noradrenaline autoreceptor agonist clonidine impaired memory for odours, while the noradrenaline reuptake inhibitor reboxetine increased performance (Gais, Rasch, Dahmen, Sara, & Born, 2011). Administration of clonidine also blocked differences between emotional and neutral memories that are usually observed across sleep-dependent memory consolidation (Groch et al., 2011) and reboxetine increased performance on a finger sequence tapping task together with sleep spindle density (Rasch, Pommer, Diekelmann, & Born, 2009).

Some work has also been done concerning the impact of GABAergic signalling on sleep-dependent declarative memory consolidation. However, researchers applying the GABA A positive modulator zolpidem have reported mixed effects (Hall-Porter, Schweitzer, Eisenstein, Ahmed, & Walsh, 2014; Kaestner, Wixted, & Mednick, 2013; Mednick et al., 2013; Melendez et al., 2005). Administration of zolpidem during an interval including a nap improved retention of word pairs (Mednick et al., 2013) and
influences emotional memory (Kaestner et al., 2013). However, other studies using a whole night of sleep found no effect on word list memory (Melendez et al., 2005) or even detrimental effects on declarative memory for word pairs and motor skill memory for a finger tapping task (Hall-Porter et al., 2014).

Also glutamatergic signalling has been shown to be involved in sleep-dependent consolidation of sensory memory (Gais, Rasch, Wagner, & Born, 2008). In this study participants learned a procedural visual texture discrimination task that mainly relies on glutamatergic plasticity in the visual cortex. During a whole night of retention sleep caroverine, an AMPA receptor blocker, and ketamine, a NMDA receptor blocker, were infused. Participants receiving either of the blockers performed worse on subsequent retrieval of the task.

1.5 Objectives and hypotheses

For communication brain cells rely on neurotransmitters and this communication can elicit complex interactions. Sleep is a highly regulated behaviour that depends on and elicits neurochemical changes from a system scale down to individual neurons. Sleep has been shown to benefit the maintenance of memories acquired during prior sleep, but it is currently unclear which neurotransmitters contribute to this effect. The present work aimed to shed light onto some of these processes by influencing the major neurotransmitters involved in memory formation during waking.

The first study aimed at influencing LTP by disturbing or enhancing glutamatergic neurotransmission to infer if it is important for declarative memory consolidation. While, one report of disturbed memory due to AMPA and NMDA receptor blockade during sleep exists (Gais et al., 2008), this pertains to sensory learning, which is mainly established in cortical structures. Blockade of AMPA receptors was chosen to interfere with reactivation of glutamatergic neuron ensembles in the hippocampus during post learning sleep. Modulating NMDA receptors was expected to influence consolidation by influencing to what extent glutamatergic LTP could be expressed by the reactivated neuronal circuitry. A declarative word pair associates task was chosen as the main dependent variable, as reactivation has been demonstrated most convincingly in humans in the hippocampal formation (Rasch et al, 2007). AMPA or NMDA receptor blockade by antagonists should therefore reduce, whereas facilitating NMDA receptor activity by administering NMDA receptor co-agonist d-cycloserine (DCS) should increase the amount of word pairs retained compared to placebo.

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The second study was conducted to examine if reactivation of memory during sleep includes dopaminergic reward circuitry. To this end, a motivated learning task was chosen to represent the dependent variable, which has been shown to rely on hippocampal striatal interactions during encoding. The dopamine agonist pramipexole was chosen to interfere with physiological dopaminergic signalling and it was expected that reward contingencies in this task would be disturbed, if dopamine’s role in determining the strength of a memory trace extends beyond the encoding situation.

The last study elucidated the role of GABAergic circuitry for the expression of physiologic slow wave sleep and associated processes of memory consolidation. While some work has been done on GABAergic neurotransmission (Kaestner et al., 2013; Mednick et al., 2013), these studies did not manipulate GABAergic tone, instead they affected GABA binding efficiency. Also there exist contrary findings in studies using the same substance (Hall-Porter et al., 2014; Melendez et al., 2005). Therefore the GABA reuptake inhibitor tiagabine was used to enhance SWS. The increase of SWS was expected to improve the amount of words retained in a declarative word pair associates task.
2 Studies

2.1 General methods

The following section contains a short introduction to the pharmacological design and general methods applied for testing memory and for registering sleep stages.

2.1.1 Memory tasks

The declarative verbal paired associates task required learning a list of 40 pairs of semantically related words (e.g., clock–church). Different wordlists were used on the participant’s two experimental nights. During the learning phase, the word pairs were presented sequentially on a computer screen, each for 4 sec, separated by inter-stimulus intervals of 1 sec. After presentation of the entire list, performance was tested using a cued recall procedure, i.e., the first word (cue) of each pair was presented and the subject had to name the associated second word (response). The correct response word was then displayed for 2 sec, regardless of whether the response was correct or not, to allow re-encoding of the correct word pair. The cued-recall procedure was repeated until the subject reached a criterion of 60% correct responses. Retrieval in the evening after sleep was tested using the same cued recall procedure as during the learning phase, except that no feedback of the correct response word was given. Absolute differences between word pairs recalled at retrieval testing and on the criterion trial during learning, served as a measure of overnight retention. Several studies showed that consolidation of word pairs profits particularly from SWS (e.g., Ekstrand et al., 1977; Plihal & Born, 1997).

The finger sequence tapping task was adopted from earlier studies, indicating very robust sleep-dependent improvements in this task (Walker et al., 2003). It requires the subject to press repeatedly one of two 5-element sequences (e.g., 4-1-3-2-4 or 4-2-3-1-4) with the fingers of the non-dominant hand on a keyboard as fast and as accurately as possible for 30-sec epochs interrupted by 30-sec breaks. The numeric sequence was displayed on the screen at all times to keep working memory demands at a minimum. A key press resulted in a white asterisk appearing underneath the current element of the sequence. Each 30-sec trial was scored for speed (number of correctly completed sequences) and errors. After each 30-sec trial, feedback was given about the number of correctly completed sequences and error rate.
At learning, subjects trained on twelve 30-s trials. The average score for the last three of these trials was used to indicate learning performance. At retrieval in the evening after sleep, subjects were tested on another three trials. Overnight changes in performance were calculated as absolute differences in speed and error rate between the three trials at retrieval and the last three trials at learning. Effects unspecific to the actually learned sequence, i.e., general increases in reaction time, were measured during the retrieval phase after sleep by assessing performance on three blocks of a new sequence after recall of the trained sequence.

Additionally, in the DCS study one hundred emotional and neutral pictures (taken from the International Affective Picture System, Lang et al, 2008) were used to measure emotional memory consolidation. In the learning phase, fifty pictures of high arousal and negative valence were chosen for the emotional category and fifty pictures of low arousal and medium valence were chosen for the neutral category. Twenty-five pictures of each category were presented to the participants on a computer screen for 4 sec each with an inter stimulus interval (ISI) of 1 sec. During retrieval testing at the end of the session, participants were asked to recall the pictures they had seen as accurately as possible and to record this in a written description of each picture. These descriptions were then compared to the pictures and correct answers were used as score for emotional memory performance.

The Motivated Learning (ML) task was adapted from the reward learning paradigm reported by Adcock et al. (2006) and required the participants to memorize 160 pictures of landscapes and living rooms (Figure 2.3 B). Presentation of eighty of these pictures was preceded (delay 2000-2500 msec) by a 1 Euro symbol the other eighty were preceded by a 2 Cents symbol, and participants were informed they would receive the respective reward for every hit during subsequent recognition. They were also informed that a correct rejection would earn them 51 cents and that for a miss or a false alarm they would lose 51 cents. This was done to exclude potential strategy effects, e.g., only choosing items that would, with high certainty, yield high rewards. Forty pictures each of the two reward conditions were presented for 750 msec and 1500 msec, respectively, in order to control for effects of encoding depth. Encoding depth was manipulated as the reward manipulations may also have influenced encoding depth and we were interested if the effect of pramipexole would be independent of this. Each picture was followed by three items of a distraction task where participants had to press one of two buttons according to the orientation of an
arrow presented on the screen, and 1 sec later the next trial started. Participants were allowed to train the task for three items including the recognition procedure before learning the pictures and the first two and last two pictures that were added in addition to the 160 pictures were excluded from later recognition testing to buffer recency and primacy effects. They were also informed that recognition would be tested twice, immediately after learning and in the evening of the next day. Immediate recognition started 15 min after learning had finished and, beforehand, participants were reminded of the reward contingencies (also by training on three pictures). They were then shown eighty of the original pictures together with eighty new pictures in a pseudorandom order and asked to indicate for each picture if they remembered or knew the picture (correct answers were summed and used to calculate individual hit rates) or if it was new by pressing a key on the keyboard (1, 2 or 3, respectively). They also pressed a key (1 or 2, respectively) according to whether they believed to receive a high or a low reward for the answer (thus incorrect remember and know judgements allowed us to calculate individual false alarm rates for high and low reward categories). All participants received mock feedback (“You performed slightly above average and will receive xx €” with amounts varying between 47.5 and 52.5 €) of how much they had earned after each recognition test. This was done to keep participants motivated, while controlling effects of high or low performance. Delayed recognition that was performed the next evening was identical, but the other eighty learned pictures were used and eighty completely new pictures were shown in comparison. D-prime, i.e., the z-value of the hit rate minus the z-value of the false alarm rate, was calculated as dependent variable which is independent of response strategies. We also calculated the accuracy of participants reward knowledge, i.e., the proportion of correctly categorized high and low reward hits. For constructing task stimuli, thirty-two similar groups of twenty pictures each were generated with regard to mean valence and arousal ratings as assessed in a pilot study (n = 5). The presentation of the groups was then balanced across the old/new, immediate/delayed recognition, short/long presentation and high/low reward conditions for the different participants.

2.1.2 Sleep scoring

The EEG was recorded continuously from electrodes (Ag-AgCl) placed according to the 10–20 System, referenced to two linked electrodes attached to the mastoids.
EEG signals were filtered between 0.16 and 35 Hz and sampled at a rate of 250 Hz using a BrainAmp DC (BrainProducts GmbH, Munich, Germany). Additionally, horizontal and vertical eye movements (HEOG, VEOG) and the EMG (via electrodes attached to the chin) were recorded for standard polysomnography. Sleep architecture was determined according to standard polysomnographic criteria using EEG recordings from C3 and C4 (Rechtschaffen & Kales, 1968). Scoring was carried out independently by two experienced technicians who were blind to the assigned treatment. Differences in scoring between the scorers were resolved by consulting a third experienced technician. For each night, total sleep time (TST), and time spent in the different sleep stages (wake; sleep stages 1, 2, 3, 4; SWS, i.e., sum of sleep stage 3 and 4; REM sleep) was calculated in minutes.
Study 1 – The role of glutamatergic neuroplasticity for sleep-dependent memory consolidation

2.2 Study 1 – The role of glutamatergic neuroplasticity for sleep-dependent memory consolidation

2.2.1 Introduction

LTP is considered a candidate for the plastic mechanism underlying sleep-dependent consolidation, which has been most prominently studied at the glutamatergic synapse (Malenka & Bear, 2004). As stated above, in this model, the postsynaptic membrane is depolarized above threshold by excitatory inputs that add up across time and space via AMPA receptors, thereby releasing the magnesium block of the NMDA receptor thus allowing for calcium influx and, downstream, for strengthening of the synapse, e.g., by including further AMPA-receptors into the postsynaptic membrane (Malenka & Nicoll, 1999). Blocking of both NMDA- and AMPA-receptors disturbs encoding of information, but only AMPA-blockade impairs retrieval (Bast, da Silva, & Morris, 2005; Day, Langston, & Morris, 2003). Conversely, enhancing NMDA-receptor activation by administration of DCS, i.e., a co-agonist at the glycine binding site of the receptor, benefited declarative memory encoding (Onur et al., 2010). Yet, the role of glutamatergic neurotransmission for sleep-dependent offline consolidation of memories has been scarcely examined. In the developing cortex of cats, sleep-dependent ocular dominance plasticity was inhibited after blocking NMDA-receptors (Aton et al., 2009). In adult humans, sleep-dependent consolidation of visual texture discrimination skill is deteriorated by the non-competitive NMDA-receptor blocker ketamine or the competitive AMPA-receptor blocker caroverine (Gais et al., 2008). However, these findings pertain to non-declarative types of memory not essentially relying on hippocampal networks.

Here, we tested contributions of glutamatergic neurotransmission to sleep-dependent consolidation of hippocampus-dependent declarative memory. As sleep-dependent consolidation of these memories is caused by the reactivation of firing patterns during SWS in neuron assemblies likely comprising glutamatergic activation, we expected that consolidation would be sensitive to blocking or enhancing glutamatergic neurotransmission during retention sleep. First we investigated the effects of blocking AMPA-receptors (by caroverine) or NMDA-receptors (by ketamine) during

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Study 1 – The role of glutamatergic neuroplasticity for sleep-dependent memory consolidation

retention sleep. Then we tested the effects of enhancing NMDA-receptor function by post-learning administration of DCS.

2.2.2 Methods

2.2.2.1 Participants

Altogether, 58 participants completed the study (caroverine: n = 15, ketamine: n = 13, DCS n = 30; see Supplementary Methods for details of methods). Participants were healthy, non-smoking, native German speaking men (18-30 years). The experiments were approved by the ethics committee of the University of Luebeck. Written informed consent was obtained from all participants prior to participation. One participant revoked his consent after data acquisition in the DCS experiment and his data were deleted.

2.2.2.2 Design and procedures

Each of the experiments followed a randomized, double-blind, placebo-controlled, within-subject, crossover design. In the DCS study, two different groups were recruited to compare effects of DCS (versus placebo) during retention intervals of sleep (n = 16) and wakefulness (n = 14), respectively. Participants took part in two experimental sessions scheduled at least 14 days apart. Both sessions were identical but for the administration of placebo or substance (caroverine: Calmaverine®, intravenously, 16 mg/h, corresponding to a total dose of 40 mg/kg, Taphlan, Switzerland, plasma halftime: 25 min, ketamine: Ketanest-S®, intravenously, 0.1 mg/kg/h, corresponding to a total dose of 0.25 mg/kg, Pfizer, USA, plasma halftime: 10-15 min, DCS: Cycloserine Capsules®, 175 mg, The Chao Center for Industrial Pharmacy & Contract Manufacturing, USA, plasma halftime: 10 h, plasma maximum: 1–2 h; Figure 1 summarizes study designs). The administration of placebo and substance was performed in balanced order, i.e., half of the sample received first placebo and then the active agent, for the other half the order of substance administration was reversed.

On experimental nights, the participants arrived at the sleep lab at 8:00 pm and received a venous catheter for blood sampling. Following preparations for EEG and polysomnographic recordings, participants in all experiments learned declarative word pair associates. In the DCS experiments, participants additionally learned emo-
tional and neutral pictures and a procedural task, i.e., finger sequence tapping. Learning always took place between 9:00 pm and 10:30 pm and participants were asked to refrain from active rehearsal during the retention interval. Afterwards, in the caroverine and ketamine experiments participants received a second catheter for intravenous substance infusion. Infusions of ketamine and caroverine and respective placebo infusions (saline solution) started immediately after first signs of sleep stage 2 and lasted for 2.5 hours. Participants were woken up 3 hours after sleep onset (i.e., the transition from sleep stage 1 to sleep stage 2) and retrieval was tested 30 min later. A retention interval of three hours during the first half of the night was chosen, to restrict the effects of the substances to early nocturnal SWS rich sleep, which has been shown to be the phase for reactivation of declarative memory (Rasch et al., 2007; Rudoy et al., 2009).

DCS and placebo were administered orally immediately before lights out (11:00 pm), and participants were woken after 7.5 h. This longer sleep period was chosen to ameliorate potential sleep deprivation effects of reducing sleep to 3 hours as, due to the substantially longer half-life of DCS, retrieval was shifted to the evening, i.e., a time when plasma levels of DCS were negligible (Zhu, Nix, Adam, Childs, & Peloquin, 2001). After the sleep period, participants then left the lab for approximately 12 h (during this time participants engaged in their usual activities) and returned for retrieval assessment. The wake control group of the DCS study was subjected to an identical protocol but the beginning of the session was shifted by 10 h (arrival 10:00 am, learning: 11:00 am) to ameliorate effects of prolonged wakefulness; participants remained in the lab for the whole retention interval to prevent unintentional sleep.

2.2.2.3 EEG Analysis

Average power spectra were calculated at Cz for all sleep epochs of SWS and sleep stage 2 individually for the first and the second half of the night. Power spectra were calculated by Fast Fourier Transformations with a Hanning window applied to subsequent blocks of 2048 data points (~10.24 sec, 3 blocks per 30-sec epoch). Spectra were filtered by a 5-point moving average to produce a smoothing of the FFT outcome. In the averaged spectra, mean power was determined for 0.5-1 Hz slow oscillation, 1-4 Hz delta, 9-12 Hz slow spindle and the 12-15 Hz fast spindle frequency bands.
Control measures – vigilance, sleepiness, and mood ratings and test of encoding

At the retrieval phase, vigilance, alertness, sleepiness and mood was assessed using self-report measures including the “Eigenschaftswörterliste” (Janke & Debus, 1978), the Stanford sleepiness scale (SSS; Hoddes, Zarcone, Smythe, Phillips, & Dement, 1973) and the Positive and Negative Affective Schedule (PANAS; Watson et al, 1988). In the DCS experiments vigilance was additionally assessed by mean reaction times in a 5-min version of the psychomotor vigilance task (PVT; Dinges et al., 1997) that required pressing a button as fast as possible whenever a bright millisecond clock presented on a dark computer screen started counting upward. After the button press, this clock displayed the reaction time. General capabilities of long-term memory retrieval were also tested in these experiments using a word generation task. Participants had to generate as many words as possible starting with a certain letter (P or M) or belonging to a defined category (hobby or profession) during a time of 2 minutes each.

Only in the wake control group of the DCS experiments, encoding (of a list of 16 three digit numbers) was measured. This measure was applied to test if DCS has an effect on encoding at high plasma concentrations during the wake retention interval. At the end of a session all participants were asked if they believed to have received an active agent or placebo.

Analyses of adrenocorticotropin (ACTH) and cortisol

Because blockers of glutamatergic transmission like ketamine can stimulate pituitary adrenal activity (Herman et al, 2004), we sampled blood once before and after learning as well as after retrieval. Additionally, blood was sampled during the retention interval, i.e., hourly during the first four hours after substance intake and, in the DCS study, every two hours during the second four hours. Sampling during the retention interval was performed via a long plastic tube from an adjacent room, leaving the participant’s sleep undisturbed. Blood samples were immediately centrifuged and then stored at -80°C until assay. Serum cortisol concentrations were assessed using the Immulite (Siemens Medical Solutions Diagnostics, Los Angeles, CA; serum sensitivity, 0.2 µg/dl, interassay coefficient of variation < 10 %). ACTH was assessed in
plasma (Immulite, Siemens Medical Solutions Diagnostics, Los Angeles, CA; sensitivity, 9 pg/ml, interassay coefficient of variation < 9.6 %).

2.2.3 Results

2.2.3.1 Memory Tasks

Neither caroverine nor ketamine significantly changed retention of word pairs in comparison with respective placebo treatments (all p > 0.53, see Table 2.1 and Figure 2.1 for a summary of results). Learning performance also did not differ between the active agents and respective placebo conditions (all t ≤ 1.61, p ≥ 0.13).

DCS administration before the sleep-retention interval distinctly improved recall of word pairs at retrieval testing after the retention interval. This effect was confirmed by significance for the ANOVA treatment x time point interaction (F(1,12) = 9.33, p ≤ 0.01, Table 2.1 and Figure 2.1). By contrast, DCS administered before a wake retention interval did not improve word pair retention (p ≥ 0.99). During learning there were no evident differences between placebo and DCS conditions concerning amounts of learned word pairs and trials to criterion (all t ≤ 1.19, p ≥ 0.19). An ANOVA including both the sleep and wake groups of the DCS study (represented by an additional ‘sleep/wake’ factor) revealed a trend for the treatment x time point x sleep/wake interaction (F(1,25) = 3.15, p = 0.09).

The emotional memory task did not reveal any differences as measured by the amount of freely recalled emotional and neutral pictures between DCS and placebo conditions both in the sleep group and in the wake group of this study (p ≥ 0.29, for respective treatment main and interaction effects, Table 2.2). Independent of the treatment condition, generally more emotional than neutral pictures were remembered (sleep: F(1,13) = 21.06 and p ≤ 0.01, wake: F(1,13) = 26.74, p ≤ 0.01).

Procedural finger sequence tapping was not differentially affected by DCS or placebo. The overnight gains in tapping speed and accuracy were comparable in both conditions (all p ≥ 0.27, Table 2.2), and this was also true for the wake control group (all p ≥ 0.16). There was also no difference evident between DCS and placebo conditions at training or concerning performance on the untrained control sequence during the retrieval phase (all p ≥ 0.26). The ANOVA including both the sleep and the wake condition of the DCS study revealed that the sleep group improved their performance more during the retention interval (F(1,24) = 8.64, p ≤ 0.01 for time point x sleep/wake).
Figure 2.1. (A) Study design: In the caroverine and ketamine studies participants learned at 9:00 pm and went to bed at 11:00 pm. The retention interval was 3.5 hours and half an hour after waking the participant retrieval was tested at 2:30 am. In the DCS study sleep condition participants also learned at 9:00 pm and went to bed from 11:00 pm to 7:30 am. The retention interval was 22 hours and retrieval was tested at 9:00 pm. In the wake condition learning was shifted 10 hours to 11:00 am and participants remained awake the whole retention interval until retrieval was tested at 11:00 am the next day. Approximate times of learning and retrieval are indicated, during learning criterion trials were the last cued recall during learning the word pairs and the last three blocks of learning the finger sequence. p.o. – oral administration, i.v. – intravenous administration, PAL - word pair associates task, FTT –finger sequence tapping task, Pics – emotional and neutral pictures. (B) Overnight retention of word pairs and finger sequence tapping skills in the substance (black bars) and placebo condition (empty bars). Retention of word pairs is indicated by the mean (±SEM) percentage of word pairs recalled at retrieval testing after the retention interval relative to recall performance on the criterion trial at learning before sleep (Please note that retention in the DCS experiments is generally lower than in the caroverine and ketamine experiments due to the longer retention interval). Overnight gains in finger sequence tapping are indicated by the mean (±SEM) percentage of correctly tapped sequences per 30-sec trial at retrieval testing relative to the average performance on the last three trials during training before the retention interval. ** p ≤ 0.01, for pairwise comparisons between the effects of the treatments (caroverine: n = 15, ketamine: n = 12, DCS: n = 13 for sleep condition, n = 14 for wake condition).
Table 2.1. Word Pair Memory Task: Mean (± SEM) values are given for the active agent and placebo conditions. Total amount of recalled words is given for criterion trials at learning and at retrieval, additionally, percent values of retrieved words are provided relative to learning performance (set to 100%). ** p ≤ 0.01 and ns = not significant.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Placebo</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caroverine + sleep</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blocks to criterion</td>
<td>1.53 ±0.17</td>
<td>1.60 ±0.16</td>
</tr>
<tr>
<td>Learning</td>
<td>29.60 ±0.83</td>
<td>28.13 ±0.84</td>
</tr>
<tr>
<td>Retrieval</td>
<td>32.53 ±0.98</td>
<td>31.67 ±0.98</td>
</tr>
<tr>
<td>Absolute difference</td>
<td>2.93 ±0.64</td>
<td>3.53 ±1.0</td>
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<tr>
<td>% of learning</td>
<td>110.14 ±2.27</td>
<td>113.43 ±4.00</td>
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<tr>
<td><strong>Ketamine + sleep</strong></td>
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<tr>
<td>Blocks to criterion</td>
<td>1.50 ±0.15</td>
<td>1.58 ±0.15</td>
</tr>
<tr>
<td>Learning</td>
<td>29.19 ±0.98</td>
<td>27.93 ±1.13</td>
</tr>
<tr>
<td>Retrieval</td>
<td>31.35 ±0.91</td>
<td>30.23 ±0.69</td>
</tr>
<tr>
<td>Absolute difference</td>
<td>2.16 ±0.94</td>
<td>2.29 ±0.93</td>
</tr>
<tr>
<td>% of learning</td>
<td>108.12 ±3.58</td>
<td>109.65 ±3.90</td>
</tr>
<tr>
<td><strong>DCS + sleep</strong></td>
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<td></td>
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<tr>
<td>Blocks to criterion</td>
<td>1.85 ±0.15</td>
<td>2.00 ±0.25</td>
</tr>
<tr>
<td>Learning</td>
<td>27.85 ±0.83</td>
<td>28.31 ±1.11</td>
</tr>
<tr>
<td>Retrieval</td>
<td>27.00 ±0.87</td>
<td>25.08 ±1.16</td>
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<tr>
<td>Absolute difference</td>
<td>-0.85 ±0.55</td>
<td>-3.23 ±0.59 **</td>
</tr>
<tr>
<td>% of learning</td>
<td>97.10 ±1.99</td>
<td>88.56 ±2.27 **</td>
</tr>
<tr>
<td><strong>DCS + wake</strong></td>
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<td>Blocks to criterion</td>
<td>1.50 ±0.14</td>
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</tr>
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<td>Learning</td>
<td>28.64 ±0.90</td>
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<tr>
<td>Retrieval</td>
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<td>27.93 ±1.32</td>
</tr>
<tr>
<td>Absolute difference</td>
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<td>-1.29 ±1.07</td>
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<tr>
<td>% of learning</td>
<td>95.78 ±3.34</td>
<td>95.68 ±3.69</td>
</tr>
</tbody>
</table>
2.2.3.2 Sleep

Infusion of ketamine compared with placebo, reduced the time spent in stage 2, SWS, and REM sleep and increased time in wakefulness (Wake: $t_{(12)} = 2.55$, $p \leq 0.05$, stage 2: $t_{(12)} = -2.75$, $p \leq 0.05$, SWS: $t_{(12)} = -2.14$, $p \leq 0.05$, stage 4: $t_{(12)} = -2.15$, $p \leq 0.05$, REM: $t_{(12)} = -2.50$, $p \leq 0.05$, Table 2.3). Under caroverine there was a trend towards less time spent in stage 4 sleep ($t_{(13)} = 1.79$, $p = 0.10$). Oral administration of DCS before sleep increased time in wakefulness ($t_{(12)} = 2.66$, $p \leq 0.05$) and stage 1 sleep ($t_{(12)} = 2.27$, $p \leq 0.05$), and reduced REM sleep ($t_{(12)} = -3.51$, $p \leq 0.01$; Table 3). There was no evident correlation between DCS induced changes in sleep architecture and improvements in the retention of word pairs (all $r \leq 0.34$ and $p \geq 0.26$, changes were calculated individually with reference to the placebo condition).

More fine-grained analyses of EEG power spectra at Cz revealed a power reduction around the spindle maximum during stage 2 sleep following DCS (Figure 2.2). ANOVA on the fast spindle band (12-15 Hz) confirmed significance for the sleep stage x treatment interaction ($F_{(1,11)} = 9.47$, $p \leq 0.01$; $t_{(11)} = 2.71$, $p \leq 0.05$ for post hoc comparison between the treatments for stage 2 spindle power). The reducing effect of DCS on stage 2 sleep spindle power appeared to be less pronounced during the first than the second half of sleep ($t_{(11)} = -2.95$, $p \leq 0.01$, for pairwise comparison between the effects of treatment, $F_{(1,11)} = 7.87$, $p \leq 0.05$, for treatment x night half). However, this was due to the fact that, during the first half, DCS simultaneously enhanced beta power with this effect extending into the upper (> 14-Hz) range of fast spindle frequencies (Figure 2). Analyses on the other bands did not show any significant effects of treatment ($p \geq 0.16$). There was no correlation between DCS induced changes in the spindle band and differences in the retention of word pairs (all $r \leq 0.26$ and $p \geq 0.39$).

2.2.3.3 Control measures

In the caroverine study we found no differences between the treatments in subjective measures of vigilance, alertness, sleepiness or mood during the retrieval phase ($p \geq 0.19$). These measures also did not differ between treatments in the ketamine study ($p \geq 0.58$); however, three participants reported slight nausea after awakening on the ketamine nights. Cortisol and ACTH levels were not differentially affected by caroverine or placebo ($p \geq 0.31$). Under ketamine, cortisol was increased at the end of the
infusion (between 01:00 am and 02:00 pm; ketamine: 4.54 ± 3.67 µg/dl; placebo 2.80 ± 1.96 µg/dl; p ≤ 0.05) and ACTH concentrations showed a corresponding trend (p = 0.10).

Table 2.2. Emotional and Procedural Memory: Mean (± SEM) values are given for the DCS and placebo condition. Top: number of correctly remembered emotional, neutral and of total pictures in the emotional memory task. Bottom: average number of correctly tapped sequences for the finger sequence tapping during the last three 30-sec trials at learning, the three trials at retrieval and for the untrained sequence at retrieval. Additionally, percent values of correctly tapped sequences at retrieval are provided relative to learning performance (set to 100%). ** p ≤ 0.01, * p ≤ 0.05 and ns = not significant.

<table>
<thead>
<tr>
<th>Emotional and neutral pictures</th>
<th>Sleep</th>
<th>DCS</th>
<th>Placebo</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotional</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Emotional</td>
<td>7.54 ±0.83</td>
<td>8.23 ±0.74</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Neutral</td>
<td>4.77 ±0.86</td>
<td>4.85 ±0.85</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>12.31 ±1.29</td>
<td>13.08 ±1.36</td>
<td>ns</td>
</tr>
<tr>
<td>Wake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emotional</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Emotional</td>
<td>6.14 ±0.72</td>
<td>6.79 ±0.88</td>
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<tr>
<td></td>
<td>Neutral</td>
<td>4.00 ±0.60</td>
<td>4.57 ±0.60</td>
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<tr>
<td></td>
<td>Total</td>
<td>10.14 ±1.08</td>
<td>11.36 ±1.34</td>
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<table>
<thead>
<tr>
<th>Finger sequence tapping</th>
<th>Sleep</th>
<th>DCS</th>
<th>Placebo</th>
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<tr>
<td>Learning</td>
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<td></td>
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<tr>
<td></td>
<td>Learning</td>
<td>17.11 ±1.18</td>
<td>16.36 ±1.21</td>
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<tr>
<td>Retrieval</td>
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<tr>
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<td>Retrieval</td>
<td>21.03 ±1.26</td>
<td>19.75 ±1.26</td>
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<tr>
<td>Absolute difference</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Absolute difference</td>
<td>3.91 ±0.72</td>
<td>3.39 ±0.89</td>
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</tr>
<tr>
<td>% of learning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% of learning</td>
<td>124.87 ±5.69</td>
<td>122.66 ±5.75</td>
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<tr>
<td>Untrained sequence</td>
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<tr>
<td></td>
<td>Untrained sequence</td>
<td>13.50 ±1.55</td>
<td>13.47 ±0.83</td>
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<td>Wake</td>
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<td></td>
</tr>
<tr>
<td>Learning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Learning</td>
<td>18.83 ±1.00</td>
<td>18.78 ±1.00</td>
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<td>Retrieval</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Retrieval</td>
<td>20.60 ±1.21</td>
<td>20.10 ±1.29</td>
<td>ns</td>
</tr>
<tr>
<td>Absolute difference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absolute difference</td>
<td>1.76 ±0.54</td>
<td>1.31 ±0.93</td>
<td>ns</td>
</tr>
<tr>
<td>% of learning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% of learning</td>
<td>109.56 ±3.18</td>
<td>107.86 ±5.04</td>
<td>ns</td>
</tr>
<tr>
<td>Untrained sequence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Untrained sequence</td>
<td>12.86 ±0.72</td>
<td>12.66 ±0.72</td>
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</tr>
</tbody>
</table>
Subjective measures of vigilance, sleepiness and mood, as well as behavioural measures of vigilance and general retrieval capabilities at retrieval all remained unaffected after administration of DCS ($p \geq 0.11$). An additional control test performed in the wake control group of the DCS study, which tested if DCS influences encoding during wakefulness, revealed that 4 hours after DCS intake intrusions (from previous

![Figure 2.2. Mean EEG power (±SEM) during sleep stage 2 (A) and slow wave sleep (B) between 0.1 – 20 Hz in the first half of night (left panels) and second half of night (middle panels). Respective bottom panels indicate p-values for pairwise comparisons between the treatment conditions (placebo – thick red line, DCS – thin black line). Right panels show mean (±SEM) power in the fast spindle (12-15 Hz) band during the first and the second night half (Please note that only differences were considered meaningful that remained for the mean power of the entire frequency band of interest). ** $p \leq .01$, for pairwise comparisons between the effects of the treatments (n=12).]
Table 2.3. Sleep parameters: *Mean (± SEM) values of minutes spent in the different sleep stages are given for the active agent and placebo conditions. REM – rapid eye movement sleep; SWS – slow wave sleep, TST – total sleep time; ** p ≤ .01, * p ≤ .05, t p ≤ .10 and ns = not significant.*

<table>
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<td>Wakefulness</td>
<td>2.80 ±1.21</td>
<td>2.41 ±0.60</td>
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<tr>
<td>Stage 1</td>
<td>22.29 ±4.59</td>
<td>29.5 ±4.78</td>
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</tr>
<tr>
<td>Stage 2</td>
<td>96.18 ±5.63</td>
<td>87.57 ±7.65</td>
<td>ns</td>
</tr>
<tr>
<td>Stage 3</td>
<td>44.61 ±4.39</td>
<td>42.14 ±3.35</td>
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</tr>
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<td>Stage 4</td>
<td>5.46 ±1.64</td>
<td>8.07 ±2.11</td>
<td>t</td>
</tr>
<tr>
<td>REM</td>
<td>11.67 ±2.51</td>
<td>9.96 ±3.32</td>
<td>ns</td>
</tr>
<tr>
<td>SWS</td>
<td>50.07 ±5.18</td>
<td>50.21 ±4.49</td>
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<tr>
<td>TST</td>
<td>184.28 ±2.05</td>
<td>182.07 ±3.36</td>
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<td>Wakefulness</td>
<td>47.96 ±13.87</td>
<td>14.69 ±4.29</td>
<td>*</td>
</tr>
<tr>
<td>Stage 1</td>
<td>26.38 ±4.49</td>
<td>20.00 ±2.76</td>
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</tr>
<tr>
<td>Stage 2</td>
<td>77.69 ±9.32</td>
<td>104.12 ±5.05</td>
<td>*</td>
</tr>
<tr>
<td>Stage 3</td>
<td>19.50 ±4.61</td>
<td>23.92 ±3.25</td>
<td>ns</td>
</tr>
<tr>
<td>Stage 4</td>
<td>2.58 ±1.18</td>
<td>6.08 ±1.96</td>
<td>*</td>
</tr>
<tr>
<td>REM</td>
<td>12.62 ±3.61</td>
<td>22.96 ±4.17</td>
<td>*</td>
</tr>
<tr>
<td>SWS</td>
<td>22.08 ±5.53</td>
<td>30.00 ±4.35</td>
<td>*</td>
</tr>
<tr>
<td>TST</td>
<td>186.73 ±1.73</td>
<td>191.77 ±5.5</td>
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<td>19.54 ±3.43</td>
<td>9.84 ±2.62</td>
<td>*</td>
</tr>
<tr>
<td>Stage 1</td>
<td>31.27 ±2.89</td>
<td>25.30 ±2.03</td>
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<tr>
<td>Stage 2</td>
<td>213.77 ±8.88</td>
<td>215.69 ±10.52</td>
<td>ns</td>
</tr>
<tr>
<td>Stage 3</td>
<td>60.46 ±6.37</td>
<td>56.69 ±6.59</td>
<td>ns</td>
</tr>
<tr>
<td>Stage 4</td>
<td>32.96 ±7.61</td>
<td>33.19 ±7.77</td>
<td>ns</td>
</tr>
<tr>
<td>REM</td>
<td>83.73 ±5.39</td>
<td>101.85 ±6.95</td>
<td>**</td>
</tr>
<tr>
<td>SWS</td>
<td>93.42 ±10.07</td>
<td>89.88 ±10.47</td>
<td>ns</td>
</tr>
<tr>
<td>TST</td>
<td>445.38 ±3.18</td>
<td>447.08 ±3.76</td>
<td>ns</td>
</tr>
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</table>
testing immediately after and 2 hours after substance intake) were reduced (DCS: 0.21 ± 0.11; placebo: 0.93 ± 0.27, \( t_{(13)} = -2.92, p \leq 0.01, F_{(1,13)} = 8.29, p \leq 0.01 \) for treatment x time point). Levels of cortisol and ACTH did not differ between treatment conditions (\( p \geq 0.26 \)). A positive relation between differences in cortisol level and differences in word pair retention in the sleep condition was found (2:00 am: \( r = 0.62 \) and \( p \leq 0.05 \)), however, it did not survive multiple comparison correction.

Participants could differentiate ketamine and placebo (\( X^2_{(1)} = 22.29 \ p \leq 0.01 \)). However, caroverine and placebo as well as DCS and placebo could not be discriminated (\( p \geq 0.25 \)).

2.2.4 Discussion

Evidence from animal and humans studies indicates that the consolidation of hippocampus-dependent declarative memory relies on the reactivation of newly encoded neuronal representations during post-learning SWS (Diekelmann & Born, 2010; Rasch et al., 2007; Ribeiro et al., 2007; Wilson & McNaughton, 1994). In hippocampal neuron assemblies the same sequential firing patterns are observed during SWS as during encoding during preceding wake (O’Neill, Pleydell-Bouverie, Dupret, & Csicsvari, 2010). These reactivations that typically coincide with sharp wave-ripples in the hippocampal EEG are thought to involve excitatory glutamatergic synapses (Behrens, van den Boom, de Hoz, Friedman, & Heinemann, 2005; Dupret, O’Neill, Pleydell-Bouverie, & Csicsvari, 2010; King, Henze, Leinekugel, & Buzsaki, 1999). Repeated reactivations are hence expected to induce plastic synaptic changes within these hippocampal assemblies and in extra-hippocampal output structures that generally contribute to the strengthening of respective memory representations. A candidate mechanism for mediating memory consolidation in this context is glutamatergic LTP, where the AMPA-receptor is responsible for fast signal transfer and the NMDA-receptor acts as coincidence detector for the induction of LTP (Malenka & Nicoll, 1999). Consequently, here we found that facilitating the response of the NMDA receptor to glutamate with the co-agonist d-cycloserine (DCS) improved consolidation of hippocampus-dependent word pair memories during sleep. Yet, contrary to our expectation, the straightforward approach of blocking AMPA- or NMDA-receptors did not impair declarative memory consolidation. This pattern suggests that effective hippocampal memory replay during sleep does not simply rely on the reactivation of synaptic AMPA and NMDA receptors that contributed to encoding, thereby suggest-
ing that processes other than classical glutamatergic LTP essentially contribute to sleep-dependent declarative memory consolidation.

The improvement in word pair recall after DCS, administered before the retention period of sleep, can easily be explained by the co-agonistic effect of DCS enhancing activity of the NMDA-receptor and, consequently, enhancing LTP after assembly re-activation in hippocampal networks. Whether the DCS effect reflects plastic changes at glutamatergic synapses within and/or outside the hippocampus, cannot be inferred from the present data. Observations that retrieval of word pair memories within two days after retention sleep was associated with an increase in activity primarily within the hippocampus (Gais et al., 2007) suggest that the immediate effects of DCS on the first night after learning strengthen the memory trace within the hippocampus itself. Also arguing for this position, DCS reduced power of the fast sleep spindles that have been considered a mechanism supporting the transfer of reactivated memory information to extra-hippocampal sites (Inostroza & Born, 2013; Molle & Born, 2011; Siapas & Wilson, 1998). However, it is also possible that the positive effect of DCS on the representation outweighs the negative impact of reduced sleep spindles.

Notably, DCS did not influence retention of word pairs when given before a wake interval. Assuming spontaneous reactivations occurred also during wake retention intervals, this finding supports the concept that reactivations during sleep serve different functions from reactivations during wakefulness (Diekelmann, Buchel, Born, & Rasch, 2011). Indeed during waking, reactivations are thought to exert twofold functions, i.e., to transiently labilize the representation and to support re-encoding of the stimulus (Hardt et al., 2013; Nader & Hardt, 2009) and the same reactivation of memories by odour cues during sleep that facilitated memory, when applied during wake, led to decreased declarative memory retention (Diekelmann et al., 2011). Missing interference together with the instruction not to rehearse the learned tasks may have led to DCS not changing memory in any direction during the wake retention interval, as could have been expected. As to encoding of hippocampal memories, improving effects of DCS have been revealed in most (e.g., Kuriyama, Honma, Soshi, Fujii, & Kim, 2011; Lee, Milton, & Everitt, 2006; Onur et al., 2010), but not in all studies (e.g., Kuriyama, Honma, Koyama, & Kim, 2011), and an improving effect of DCS on encoding also fits our findings of reduced intrusions at immediate recall of numbers after DCS administration in the wake condition – although, the effect was not marked. Thus, speculating that DCS has a similar benefiting effect on re-
encoding, the missing effect of DCS on retention of hippocampal memories across wake intervals might point towards a parallel enhancing effect of DCS on the reactivation induced labilization of memories (Ben Mamou, Gamache, & Nader, 2006; Lee et al., 2006).

DCS did not enhance overnight gains in procedural finger sequence tapping skills and also failed to affect memory after sleep for emotional and neutral pictures, which might reflect that sleep-associated consolidation of these memories is not primarily a consequence of hippocampal reactivation (Debas et al., 2010; Diekelmann & Born, 2010; Karni et al., 1998; Wagner, Gais, & Born, 2001) but see also (Albouy et al., 2008). The findings concur with previous studies that likewise failed to observe DCS induced changes in overnight gains in cognitive skill, although benefits in retention occurred for working memory training and emotional memories when participants were awake (Kalisch et al., 2009; Kuriyama, Honma, Shimazaki, et al., 2011). Interestingly, in a previous study of ours both blocking of NMDA receptors and of AMPA receptors impaired sleep-dependent gains in a procedural visual texture discrimination task (Gais et al., 2008). Indeed, against this backdrop, the present pattern of a selective enhancing influence of DCS on sleep-associated declarative memory consolidation in the absence of changes in overnight benefits for procedural skills or recognition memories, supports the view that DCS specifically acts on hippocampal memory reactivations during sleep as sleep-associated gains in those memories are less dependent on such reactivations.

Paradoxically, whereas the NMDA-receptor co-agonist DCS significantly enhanced hippocampus-dependent memory consolidation of word-pair associations during sleep, the consolidation process remained entirely unaffected after disrupting glutamatergic neurotransmission by administration of either the NMDA-receptor antagonist ketamine or the AMPA-receptor antagonist caroverine. It is unlikely that the missing effect after ketamine or caroverine is due to a too low dosing of the substances because in a previous study of ours (Gais et al., 2008) infusion of the substances at very similar concentrations (in case of caroverine even slightly lower concentrations were used in that study) effectively blocked sleep-dependent consolidation of visual texture discrimination skills. Still, it could be argued that the duration of administration matters, as in that study substances were infused for a longer (6 hours) interval whereas here we restricted administration to a 2.5-hours interval in which neuronal reactivations of memories are thought to preferentially occur in hip-
pocampal networks. Although a longer infusion duration cannot be entirely excluded as a prerequisite for the substances to become effective at hippocampal sites, increased levels of cortisol and reports of side effects like nausea observed after ketamine confirmed central nervous efficacy of the substance even with the shorter infusion interval. Studies in guinea pigs and using PET imaging in humans indicate that both ketamine and caroverine quickly reach central nervous sites of action within minutes after intravenous administration (Z. Chen, Duan, Lee, Ruan, & Ulfendahl, 2003; Hartvig et al., 1995). Also power analyses did not provide any hint that putative blocking effects of ketamine and caroverine on consolidation of hippocampal memories during sleep were just not strong enough to be revealed with the limited sample size of our study. While the effect size for DCS improving word-pair memory retention during sleep was large (d = 0.85; Cohen 1992) power analysis (using G*Power; Faul, Erdfelder, Lang, & Buchner, 2007) with (1 - $\beta$) set to 0.80 led to unreasonably large sample sizes of n = 423 and n = 6808 to reach significance ($\alpha = 0.05$) for the differences reported in the caroverine and ketamine conditions. Another factor to be considered in this context is that the ketamine and caroverine studies differed from the DCS study with respect to the amount of sleep employed, which in the DCS study covered the whole night due to its longer plasma half-life and the resulting longer retention interval, however, its plasma maximum falls into the first half of the night. Nevertheless, numerous studies have consistently shown that restricting manipulations to a 3-hour period of early nocturnal SWS-rich sleep can effectively change consolidation of declarative memory (e.g., Barrett & Ekstrand, 1972; Marshall, Molle, Hallschmid, & Born, 2004; Plihal & Born, 1997), excluding the amount of sleep per se as factor preventing effects of ketamine or caroverine. Still, although previous work has demonstrated that sleep-dependent consolidation of our word-pair task involved the hippocampus (Gais et al., 2007), not investigating other hippocampus-dependent forms of memory, e.g., object location, sequence or episodic memory, or other doses of the substances limits the explanatory power of our findings.

While any explanation of these findings remains tentative the data imply that efficacy of hippocampal memory reactivation during sleep might not rely exclusively on activation mediated by AMPA- and NMDA-receptors, respectively. Reactivations during waking associated with memory retrieval do not appear to rely on activation of NMDA receptors anyway, although, they involve AMPA-receptor activation (Bast et al., 2005; Day et al., 2003). Moreover, ketamine induced blockade of NMDA recep-
tors can be ameliorated by activating metabotropic glutamate receptor 5 (mGluR5) (H. H. Chen, Liao, & Chan, 2011). Thus, in synapses potentiated during encoding, glutamatergic signalling might shift to mGluR5 making them insensitive to ketamine blockade, although, such a shift itself would not explain why synaptic efficacy can still be enhanced via DCS.

A more plausible explanation might derive from work indicating that activation of hippocampal NMDA-receptors, and possibly also AMPA-receptors, are not only responsible for LTP induction, but are also involved in subsequent depotentiation, i.e., LTD, thereby mediating forgetting (Rosenzweig, Barnes, & McNaughton, 2002; Villarreal et al., 2002). In the hippocampus LTP is preferentially mediated by NMDA-receptors containing the NR2A subunit whereas LTD is mediated by NR2B containing receptors (Liu et al., 2004). Furthermore, there is evidence that DCS preferentially acts via NR2A containing receptors to enhance LTP (Billard & Rouaud, 2007; Kochlamazashvili et al., 2012; Sheinin, Shavit, & Benveniste, 2001), whereas ketamine provides an unspecific blockade of both these NMDA-receptor subtypes. These findings suggest the following scenario for the present experiments: Encoding of word pairs leads to the potentiation of specific hippocampal assemblies, which is accompanied by a selective up-regulation of NMDA-receptors containing the NR2A subunit (Baez et al., 2013). Glutamatergic reactivation of these assemblies during sleep preferentially enhances NR2A mediated LTP, whereas NR2B mediated LTD prevails in networks not specifically potentiated during waking. Ketamine (and caroverine) leave the reactivation-dependent memory enhancement during sleep unaffected as the proportional activation of LTP and LTD inducing NMDA-receptors remains unchanged. By contrast, DCS by preferentially activating NR2A containing receptors strengthens LTP and thus enhances the consolidating effect of sleep on hippocampal memories. This view is very much in line with a recently proposed account on the role of sleep in active decay processes and forgetting (Hardt et al., 2013). Following this view, a factor mediating the effect of sleep induced LTP and LTD on retention may be represented by the protein kinase C isoform M-zeta (PKMζ), which has been shown to sustain hippocampal memories by regulating AMPA receptor trafficking to the active zone of the synapse and might simultaneously regulate forgetting of such memories as LTD has been shown to degrade PKMζ (Hardt, Migues, Hastings, Wong, & Nader, 2010; Hrabetova & Sacktor, 2001; Migues et al., 2010). Together with the present research this account offers a mechanism for an active
process of synaptic consolidation working in balance with processes of synaptic downscaling to sustain hippocampus function and long term memory (Born & Feld, 2012; Diekelmann & Born, 2010; Tononi & Cirelli, 2006).

This scenario relating glutamatergic signalling and reactivation in hippocampal neuron assemblies to balanced processes of memory consolidation and forgetting is clearly in need of further experimentation, but would also plausibly reconcile findings of SWS being simultaneously involved in freeing of capacity for new learning (Antonenko, Diekelmann, Olsen, Born, & Molle, 2013; Van Der Werf et al., 2009) and memory consolidation (Diekelmann & Born, 2010; Marshall, Helgadottir, Molle, & Born, 2006).
2.3 Study 2 – The role of dopaminergic neuromodulation for sleep-dependent memory consolidation

2.3.1 Introduction

Memory formation is adaptive, and behaviour that is associated with high reward increases in frequency while other behaviour dwindles. This process has been linked to dopaminergic neuromodulation of learning processes (Shohamy & Adcock, 2010; Wise & Rompre, 1989). However, it remains unclear to what extent post-encoding consolidation processes contribute to this effect, in addition to the immediate influence of reward at encoding. A large body of evidence has accumulated supporting sleep’s beneficial role for memory consolidation. Sleep-dependent declarative memory consolidation is thought to rely mainly on the reactivation of traces that were encoded during prior wakefulness (Diekelmann & Born, 2010; Rasch & Born, 2013), and memories associated with high rewards benefit more from this process (Fischer & Born, 2009; Wilhelm, Diekelmann, et al., 2011). However, it remains unclear if sleep leads to the preferential consolidation of highly rewarded memories because reward present at learning tags these memories so that they are reactivated more frequently during subsequent sleep, or rather the sleep-associated consolidation process itself involves reactivation of the dopaminergic reward circuitry associated with a specific memory. Here we probed the latter assumption by testing the effects of a dopaminergic agonist (pramipexole) on the sleep-associated consolidation of memories, which were associated with high or low rewards.

Correlated activity of neurons active that encoded information during wake predicts their firing together during subsequent sleep in rodents (Wilson & McNaughton, 1994). This replay of neural representations during sleep occurs in the same sequence as during wakefulness and is coordinated between the hippocampus and the neocortex (Ji & Wilson, 2007; Skaggs & McNaughton, 1996). In humans a causal role for these reactivations has been shown for declarative and skill memory (Antony et al., 2012; Rasch et al., 2007; Rudoy et al., 2009). During SWS, hippocampal reactivations lead striatal reactivations of place-reward information in rats, consistent with the view that striatal dopaminergic activation contributes to the consolidation of re-

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ward-related memory traces during sleep (Lansink et al., 2008; Lansink, Goltstein, Lankelma, McNaughton, & Pennartz, 2009).

Dopamine is a major neuromodulator and has been put forward as the main neurotransmitter mediating the preferential encoding of highly rewarding stimuli (Schultz, 2007; Wise, 2004; Wise & Rompre, 1989), by influencing plasticity in the hippocampus (e.g., Edelmann & Lessmann, 2013; Manahan-Vaughan & Kulla, 2003; Zhang, Lau, & Bi, 2009) and extra-hippocampal reward related structures like the VTA and the nucleus accumbens (NAcc) (e.g., Goto & Grace, 2005; Schotanus & Chergui, 2008; Thomas, Malenka, & Bonci, 2000). These processes do not exclusively depend on immediate reward but are likewise triggered by the anticipation of reward in the future (Shohamy & Adcock, 2010). The preferential retention of high-reward items in anticipated reward paradigms such as the Motivated Learning (ML) task has been shown to rely on activity in the NAcc and VTA, and the connectivity of these regions to the hippocampus during encoding (Adcock et al., 2006). Two major groups of dopamine receptor subtypes, the D1-like (D1/5) and D2-like receptors (D2/3/4), have been identified (Missale, Nash, Robinson, Jaber, & Caron, 1998). In the hippocampus, postsynaptic D1 and D2 receptors are most highly expressed, which corresponds to their agonists’ ability to influence plasticity (Edelmann & Lessmann, 2013; Manahan-Vaughan & Kulla, 2003) and plasticity at hippocampal and prefrontal inputs to the NAcc is likewise modulated by D1 and D2 receptors (Goto & Grace, 2005), while D3 receptors have been shown to act as autoreceptors blunting reward signals (Sokoloff et al., 2006).

Pramipexole is an agonist of the D2-like dopamine receptors, i.e., the D2 and D3 dopamine receptors (Antonini & Calandrella, 2011). It is widely used in the treatment of Parkinson’s disease (Jankovic & Poewe, 2012) and of restless legs syndrome (Buchfuhrer, 2012), which are both related to pathological changes of midbrain dopaminergic neurons projecting to the basal ganglia (Connor et al., 2009; Dauer & Przedborski, 2003). Pramipexole has been shown to have reinforcing properties in conditioned place preference and self-administration paradigms in rodents (Engeln et al., 2012; Riddle, Rokosik, & Napier, 2012). Here, we administered the drug to increase dopaminergic activity during the sleep-associated consolidation of memories (pictures) associated with high or low reward. We expected that beyond generally enhancing consolidation of memories during sleep, the D2-like receptor agonist would nullify preferential consolidation of memories associated with high reward, in-
asmuch as, reward circuitry would be equally active during reactivation of low reward memories.

2.3.2 Methods

2.3.2.1 Participants

Sixteen young men aged 24.5 years (range 19-30 years) participated in the study. Participants were non-smoking, native German speaking. They underwent a routine health examination prior to participation to exclude any mental or physical disease, also excluding a history of psychiatric disorders by a structured interview. Participants did not take any medication at the time of the experiments, and reported having a normal sleep–wake cycle for at least 6 weeks before the experiments. They were instructed to get up at 07:00 am on experimental days, and during these days not to take any naps and not to ingest alcohol or, after 01:00 pm, caffeine-containing drinks. Before the experiment proper, participants took part in an adaption night under conditions of the experiment (i.e., including the placement of electrodes for polysomnographic recordings and insertion of an intravenous catheter). The experiments were approved by the local ethics committee. Written informed consent was obtained from all participants prior to participation.

2.3.2.2 Design and procedure

The study followed a balanced, double-blind, placebo-controlled, within-subject, crossover design. Participants took part in two experimental sessions scheduled at least 14 days apart. Both sessions were identical but for the oral administration of placebo or pramipexole (Pramipexol Winthrop 0.35 mg – corresponding to 0.5 mg pramipexole dihydrochloride monohydrate, Fa. Winthrop Arzneimittel GmbH, Germany, plasma halftime 8 h, plasma maximum: 2 h). To prevent periphery side effects of pramipexole participants additionally received domperidone, a dopamine antagonist that does not cross the blood-brain barrier, in both sessions (Motilium 20 mg, Nycomed GmbH, Germany, plasma halftime 8 h, plasma maximum: 1 h), a procedure proved effective in several forgoing studies (e.g., Riba, Kramer, Heldmann, Richter, & Münte, 2008; Ye, Hammer, Camara, & Münte, 2011).

Figure 2.3 A summarizes the experimental procedure. On experimental nights, participants arrived at the laboratory at 07:30 pm. Following insertion of an intrave-
nous catheter and preparations for EEG and polysomnography, the participants learned the ML task between 08:30 and 09:30 pm. Afterwards, they learned control tasks (declarative word pair associates and procedural sequence finger tapping) with a 10-min break between each of the tasks. This order was chosen so that participants would be most attentive during encoding of the reward task. Fifteen minutes before lights were turned off (at 11:15 pm) to enable sleep, the participants were orally administered a capsule containing pramipexole or placebo, as well as, the domperidone tablet. They were woken at 07:15 am and left the lab. During the following day participants engaged in their usual activities. They were instructed to refrain from any stressful mental or physical activities, and to keep a record of their activities during this day. In the evening they returned to the lab at 08:00 pm and retrieval of the memory tasks was tested – in reverse order of learning. (This was done as retrieval procedures for the word pairs and the sequence finger tapping were short (i.e., < 8 min) compared to the longer picture recognition test taking about 30 min). At learning and retrieval, as a control, tests of vigilance, mood and subjective sleepiness were also performed. Blood was sampled before and after learning, after retrieval and at 1.5 h intervals during the night. For this purpose the intravenous catheter was connected to a long thin tube to enable blood collection from an adjacent room without disturbing the participant’s sleep.

2.3.2.3 Control measures – general retrieval performance, vigilance, sleepiness and mood

At retrieval, to exclude effects of the drug on general retrieval performance, participants were tested on a WFT (table 2 for means and SEMs of the control measures). They were asked to generate as many words as possible within a two minute interval after being cued with either a letter (p or m) or a category (professions or hobbies).

The following control measures were assessed once before and once after each learning and retrieval phase. Mean reaction times were assessed as a measure of vigilance in a 5-min version of the PVT (Dinges et al., 1997) that required pressing a button as fast as possible whenever a bright millisecond clock presented on a dark computer screen started counting upward. After the button press this clock displayed the reaction time. The median reaction speed (i.e., 1/[reaction time in msec]) was calculated for each participant. Mood was measured using the 10 positive and 10 negative items of the PANAS (Watson, Clark, & Tellegen, 1988), where participants
respond to items (e.g., “Do you momentarily feel scared?”) on a 5 point Likert scale ranging from 1 = "not at all" to 5 = “very much”. Subjective sleepiness was assessed with the 1-item SSS (Hoddes et al., 1973) ranging from 1 = “Feeling active, vital, alert, or wide awake” to 8 = “Asleep”. At the end of the experiment participants were asked if they believed to have received an active agent or placebo.

2.3.2.4 Control measures – blood samples

Samples for measuring hormone concentrations were kept frozen at -80°C until assay. Cortisol, growth hormone, and prolactin levels were determined in serum using commercial assays (Immulate, Siemens Medical Solutions Diagnostics, Los Angeles, USA). Intra- and interassay coefficients of variation were < 10 %.

2.3.2.5 Data reduction and statistical analysis

Data from two participants were completely discarded because of poor sleep during the placebo night. Data from one participant were not included in the analysis of the ML task, as he remembered an unusual amount more low reward items than high reward items in the placebo condition (i.e., the difference between high and low reward was more than 2 standard deviations from the group mean, probably reflecting a misunderstanding of the rather complex task instruction or an unusual encoding strategy). For two participants, hormonal data sets were incomplete because of problems with blood sampling during sleep. Statistical analyses generally relied on analyses of variance (ANOVA; SPSS version 21.0.0 for Windows) including a repeated measures factor ‘treatment’ (substance vs placebo) and, where appropriate, the factor ‘phase’ (learning vs retrieval). As analyses revealed a strong suppressive influence of pramipexole on both SWS and REM sleep, main analyses of memory performance included the individual difference in wake time between treatment conditions as covariate to account for this sleep disruption. Wake time (i.e., the amount of time spent awake between sleep onset and lights on) was used for these analyses because it did not differ significantly between treatment conditions. For analysis of pictures additional ‘reward’ and ‘duration’ factors were introduced, representing recognition of high vs low reward pictures and long vs short stimulus presentation, respectively. The analyses of the pictures did not include a factor ‘phase’ as immediate and delayed recognition were performed on different sets of stimuli. Significant
ANOVA interactions were specified by post-hoc t-tests. Degrees of freedom were corrected according to Greenhouse-Geisser where appropriate.

2.3.3 Results

2.3.3.1 Sleep parameters

Total sleep time was 441.71 min and 435 min for placebo and pramipexole, respectively, and mean (SEM) minutes spent in the different sleep stages are provided in Figure 2.3 C. Time spent in sleep stages 3 and 4, SWS and REM sleep was significantly reduced by pramipexole ($t_{(13)} = 6.91, p \leq 0.001$, $t_{(13)} = 2.38, p \leq 0.05$, $t_{(13)} = 6.11, p \leq 0.001$, $t_{(13)} = 11.04, p \leq 0.001$, respectively), whereas, sleep stages 1 and 2 were increased ($t_{(13)} = -6.76, p \leq 0.001$, $t_{(13)} = -3.29, p \leq 0.01$).

2.3.3.2 Memory tasks

Pramipexole significantly increased the retrieval of low reward pictures after sleep ($F_{(1,11)} = 5.91, p \leq 0.05$, see Figure 2.3 D for means and standard error of mean (SEM)). The analysis of retrieval performance after sleep revealed that longer duration pictures and high reward pictures were retained better ($F_{(1,11)} = 18.99, p \leq 0.01$, $F_{(1,11)} = 5.41, p \leq 0.05$). There was also an interaction between treatment and reward ($F_{(1,11)} = 5.20, p \leq 0.05$). The lower order ANOVAs revealed a superiority of high reward over low reward for the placebo condition ($F_{(1,11)} = 8.19, p \leq 0.01$) but not for the pramipexole condition ($p = 0.80$).

Note that in these analyses we used differences in wakefulness during the sleep interval as covariate to account for the sleep disruption observed after pramipexole. However, analyses without the covariate showed a similar picture, with statistical trends for increased retention of low reward pictures ($t_{(12)} = -2.12, p = 0.056$) in the pramipexole condition as compared to placebo, as well as for the reward main effect and the treatment x reward interaction ($F_{(1,12)} = 4.19, p = 0.063$, $F_{(1,12)} = 3.63, p = 0.081$, respectively). Also, the difference between high and low reward conditions was only prominent for the placebo condition ($t_{(12)} = 3.00, p \leq 0.01$) but not for the pramipexole condition ($p = 0.60$). During immediate recognition before sleep, there was a main effect of duration ($F_{(1,12)} = 11.65, p \leq 0.01$, see Table 2.4 for means and SEMs) but, interestingly, no main or interaction effects for reward ($p > 0.14$). An analysis including immediate and delayed recognition in the placebo condition revealed...
main effects of phase, reward and duration (\(F_{1,12} = 39.32, p < 0.001\), \(F_{1,12} = 9.04, p < 0.01\), \(F_{1,12} = 5.51, p < 0.05\)) but no interaction effects (\(p > 0.58\)).

**Figure 2.3.** (A) Study design: Participants took part in two identical experimental sessions, but for the administration of placebo or pramipexole. Following preparation for blood sampling, the learning phase started at 8:30 pm. Thereafter, and 15 min before the participant went to bed (at 11:15 pm) the capsules were orally administered. Participants were awakened at 7:15 am in the next morning. The retention interval was approximately 24 hours and retrieval was tested at 8:00 pm. Blood was drawn before and after learning, after retrieval and in 1.5-h intervals during the night. ML – Motivated learning task (reward learning), PAL – paired associate learning (word-pairs), FTT – finger tapping task (sequence finger tapping). (B) The ML task was adapted from Adcock et al (2006). At learning participants were presented 160 pictures for 750 (short presentation) or 1500 ms (long presentation). Each picture was preceded by a slide indicating a high (1 €) or a low (2 Cents) reward for correctly identifying the picture at later recognition. After each picture participants performed on three items of a distractor task, which afforded pressing the arrow key corresponding to the orientation of an arrow presented on the screen. At immediate and delayed recognition testing participants were shown different groups of 80 new and 80 old pictures and had to identify them correctly, which earned them their reward (see Methods for details). (C) Mean (±SEM) time (in minutes) spent in NonREM sleep stages S1, S2, S3, and S4, in slow wave sleep (SWS, i.e., the sum of S3 and S4), and in REM (rapid eye movement) sleep is provided for the pramipexole (empty bars) and placebo condition (black bars). (D) Performance on the ML task for the delayed recognition test during the retrieval phase after sleep. Mean (±SEM) performance is indicated as d-prime, i.e., the z-value of the hit rate minus the z-value of the false alarm rate. \(n = 14\) (\(n = 13\) for ML task), ***: \(p < 0.001\), **: \(p < 0.01\), *: \(p < 0.05\) and ns: \(p > 0.10\)
Table 2.4. Memory tasks - Mean (±SEM) values are given for the pramipexole and placebo conditions. Motivated Learning Task (reward learning): d-prime is provided for performance during the learning phase. Paired Associates Learning Task (word-pairs): Total amount of recalled words is given for criterion trials at learning and at retrieval. Additionally, percent values of retrieved words are provided relative to learning performance at the criterion trial (set to 100%). Finger Tapping Task: Average number of correctly tapped sequences per 30-sec trial and error rates (in percent) for finger sequence tapping during the last three 30-sec trials at learning, the three trials at retrieval, and for the untrained control sequence at retrieval. Additionally, percent values of correctly tapped sequences at retrieval are provided relative to learning performance (set to 100%). ns: p > 0.10

<table>
<thead>
<tr>
<th>Motivated Learning Task Immediate recognition</th>
<th>Placebo</th>
<th>Pramipexole</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>High reward</td>
<td>2.52</td>
<td>(0.24)</td>
<td>2.42</td>
</tr>
<tr>
<td>Low reward</td>
<td>2.10</td>
<td>(0.23)</td>
<td>2.33</td>
</tr>
<tr>
<td>Long duration</td>
<td>2.41</td>
<td>(0.21)</td>
<td>2.47</td>
</tr>
<tr>
<td>Short duration</td>
<td>2.21</td>
<td>(0.20)</td>
<td>2.28</td>
</tr>
</tbody>
</table>

| Paired Associates Learning Task               |         |             |   |
| Blocks to criterion                           | 1.64    | (0.31)      | 1.86 |  (0.33) | ns |
| Learning                                      | 28.86   | (1.16)      | 29.07 | (1.10) | ns |
| Retrieval                                     | 28.21   | (0.93)      | 28.43 | (0.61) | ns |
| Absolute difference                           | -0.64   | (0.93)      | -0.64 | (0.61) | ns |
| % of learning                                 | 98.10   | (3.58)      | 97.78 | (2.21) | ns |

| Finger Tapping Task - Correct sequences        |         |             |   |
| Learning                                      | 17.52   | (1.20)      | 18.30 | (1.38) | ns |
| Retrieval                                     | 20.67   | (1.30)      | 21.40 | (1.66) | ns |
| Absolute difference                           | 3.14    | (0.96)      | 3.10 |  (0.58) | ns |
| % of learning                                 | 120.39  | (6.49)      | 117.03 | (3.51) | ns |

| Finger Tapping Task - Error rates              |         |             |   |
| Learning                                      | 9.34    | (2.40)      | 7.78 |  (1.54) | ns |
| Retrieval                                     | 6.50    | (1.08)      | 6.92 |  (1.64) | ns |
| Absolute difference                           | 2.84    | (2.53)      | 0.86 |  (1.35) | ns |

| Finger Tapping Task - Control sequence         |         |             |   |
| Correct sequences                             | 15.10   | (1.25)      | 15.45 | (1.51) | ns |
| Error rate in percent                         | 9.16    | (2.07)      | 8.71 |  (1.60) | ns |
Response bias calculated as the negative mean of the z-value of the hit rate and the z-value of the false alarm rate were comparable between the treatment conditions at delayed recognition (p > 0.19, see Table 2.5 for a summary of means and SEMs of hits, false alarms and response bias). Analysis of response bias during immediate recognition, however, revealed that participants were more conservative for high reward pictures (F(1,12) = 4.88, p ≤ 0.05), there was also an interaction between treatment and reward (F(1,12) = 6.55, p ≤ 0.05), which was reflected by a more conservative strategy for high reward pictures in the placebo condition (t(12) = 2.68, p ≤ 0.05). This argues toward concentrating the analyses on the d-prime measures reported above, as they are independent of response bias. At delayed recognition and immediate recognition, hit rates were higher for longer duration pictures (F(1,12) = 14.11, p ≤ 0.01 and (F(1,12) = 9.82, p ≤ 0.01, respectively). There were statistical trends for false alarm rates being reduced for high reward pictures during delayed (F(1,12) = 3.00, p ≤ 0.10) and immediate recognition (F(1,12) = 4.48, p ≤ 0.10). No main or interaction effects for participants’ accurate categorization of hits to reward category were found at immediate (pramipexole: high 0.48 ± 0.06 low 0.49 ±0.07, placebo: high 0.57 ± 0.05 low 0.47 ±0.06, p > 0.46) or delayed recognition (pramipexole: high 0.50 ± 0.09 low 0.46 ±0.07, placebo: high 0.52 ± 0.07 low 0.49 ±0.08, p > 0.46) and accuracy did not differ from chance (p > 0.20, tested against 0.5 chance level).

The declarative and procedural memory tasks did not yield differences between placebo and pramipexole (see Table 1 for means and SEMs). Neither the difference between word-pairs recalled at learning and retrieval (p > 0.99), nor performance at learning, blocks needed to reach criterion, or performance at retrieval in the word paired associates task (p > 0.34) differed between placebo and pramipexole conditions. An analysis comparing learning and retrieval for individual treatment conditions revealed no significant differences (p > 0.31). Likewise, in the finger sequence tapping task, the differences between correctly tapped sequences as well as error rate at learning and retrieval (p > 0.46) were not significantly affected by treatment, and performance at learning before treatment, at retrieval after treatment and on the control sequence at retrieval was comparable between treatments (p > 0.28). However, at retrieval participants tapped more correct sequences than during learning (F(1,13) = 22.03, p ≤ 0.001) and this was also true in an individual analysis for both of the treatment conditions (pramipexole: t(13) = 5.30, p ≤ 0.001 and placebo: t(13) = 3.28, p ≤ 0.01). No such effect was evident for error rates (p > 0.26).
Table 2.5. Motivated Learning Task - additional response information: *Mean (±SEM)* values are given for the pramipexole and placebo conditions. *: p ≤ 0.05 and ns: p > 0.10.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Pramipexole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Immediate recognition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High reward</td>
<td>0.72 (0.05)</td>
<td>0.74 (0.05)</td>
</tr>
<tr>
<td>Low reward</td>
<td>0.73 (0.05)</td>
<td>0.76 (0.04)</td>
</tr>
<tr>
<td>Long duration</td>
<td>0.75 (0.05)</td>
<td>0.78 (0.04)</td>
</tr>
<tr>
<td>Short duration</td>
<td>0.69 (0.05)</td>
<td>0.73 (0.05)</td>
</tr>
<tr>
<td><strong>Delayed recognition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High reward</td>
<td>0.61 (0.07)</td>
<td>0.62 (0.06)</td>
</tr>
<tr>
<td>Low reward</td>
<td>0.57 (0.07)</td>
<td>0.64 (0.06)</td>
</tr>
<tr>
<td>Long duration</td>
<td>0.61 (0.07)</td>
<td>0.67 (0.06)</td>
</tr>
<tr>
<td>Short duration</td>
<td>0.57 (0.07)</td>
<td>0.58 (0.06)</td>
</tr>
<tr>
<td><strong>False alarms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Immediate recognition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High reward</td>
<td>0.04 (0.01)</td>
<td>0.06 (0.01)</td>
</tr>
<tr>
<td>Low reward</td>
<td>0.11 (0.03)</td>
<td>0.10 (0.03)</td>
</tr>
<tr>
<td><strong>Delayed recognition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High reward</td>
<td>0.06 (0.02)</td>
<td>0.08 (0.02)</td>
</tr>
<tr>
<td>Low reward</td>
<td>0.13 (0.02)</td>
<td>0.11 (0.03)</td>
</tr>
<tr>
<td><strong>Response Bias</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Immediate recognition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High reward</td>
<td>0.59 (0.08)</td>
<td>0.44 (0.10)</td>
</tr>
<tr>
<td>Low reward</td>
<td>0.34 (0.12)</td>
<td>0.35 (0.11)</td>
</tr>
<tr>
<td><strong>Delayed recognition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High reward</td>
<td>0.67 (0.15)</td>
<td>0.63 (0.13)</td>
</tr>
<tr>
<td>Low reward</td>
<td>0.51 (0.16)</td>
<td>0.49 (0.13)</td>
</tr>
</tbody>
</table>
2.3.3.3 General retrieval performance, vigilance, mood and subjective sleepiness

There were no significant differences in general retrieval performance (as measured by the word fluency task), in reaction times on the PVT, and mood (as assessed by the PANAS) between pramipexole and placebo conditions at learning or retrieval (p > 0.25, Table 2.6 for means and SEMs). At retrieval, there was a trend toward increased subjective sleepiness in the pramipexole condition (before retrieval: \( t_{13} = -1.75, p \leq 0.10 \), after retrieval: \( t_{13} = -2.11, p \leq 0.06 \)). Participants could not differentiate if they had received placebo or an active substance (\( X^2(1) = 0.14, p = 0.70 \)).

2.3.3.4 Blood hormone concentrations

For cortisol and growth hormone levels, there was a trend for main effect of treatment (\( F_{1,11} = 4.68 \) and \( p = 0.054 \), \( F_{1,11} = 3.89 \) and \( p = 0.074 \), respectively). This was due to increased cortisol (pramipexole: 7.09 µg/dL ±0.90, placebo: 3.67 µg/dL ±0.97 at 03:30 am) and growth hormone (pramipexole: 3.01 ng/mL ±1.04, placebo: 0.36 ng/mL ±0.11 at 05:00 am) concentrations at night following pramipexole intake (\( t(11) = 3.30, p \leq 0.01, t(11) = 2.44, p \leq 0.05 \)). Serum prolactin levels were not significantly different between pramipexole and placebo conditions (\( p = 0.45 \)).

2.3.4 Discussion

In the present study, we aimed to clarify whether the preferential consolidation of memories associated with reward during sleep involves the reactivation of dopaminergic reward circuitry during sleep. For this purpose, we enhanced dopaminergic activity during a period of retention sleep by administration of the D2-like receptor agonist pramipexole, which, if reactivation of dopaminergic circuitry is of relevance, should enhance memory consolidation during sleep, in particular for memories associated with low rather than high reward. Our data of the placebo condition replicate findings by Adcock et al. (2006) in showing a robust reward effect on memory 24 hours after learning. Importantly, as we expected, rather than enhancing memories that were associated with a high reward, pramipexole wiped out differences in retention performance between low and high reward memories. Unexpectedly, overall memory consolidation in the reward task, and also in the procedural and declarative control tasks, was not increased by pramipexole, which may be due to the fact that
the D2-like receptor agonist distinctly impaired SWS and REM sleep (Dzirasa et al., 2006). This direct effect of pramipexole on sleep limits the explanatory power of the present study.

Table 2.6. Control measures: *Mean (±SEM) values are given for the pramipexole and placebo conditions. SSS – Stanford sleepiness scale (subjective sleepiness), PANAS – Positive and negative affective scale (mood), PVT – Psychomotor vigilance task (reaction speed = 1/(reaction time in msec)) and WFT – Word fluency test (Regensburg Wortfluessigkeitstest) measuring general retrieval capabilities. t (trend): 0.05 ≤ \( p \) ≤ 0.10 and ns: \( p > 0.10 \).*  

<table>
<thead>
<tr>
<th>Sleepiness (SSS)</th>
<th>Placebo</th>
<th>Pramipexole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before learning</td>
<td>2.71 (0.24)</td>
<td>2.71 (0.24)</td>
</tr>
<tr>
<td>After learning</td>
<td>3.57 (0.43)</td>
<td>4.00 (0.26)</td>
</tr>
<tr>
<td>Before retrieval</td>
<td>2.43 (0.20)</td>
<td>2.71 (0.28)</td>
</tr>
<tr>
<td>After retrieval</td>
<td>2.64 (0.23)</td>
<td>3.00 (0.26)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Positive affect (PANAS)</th>
<th>Placebo</th>
<th>Pramipexole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before learning</td>
<td>26.71 (1.80)</td>
<td>25.21 (1.30)</td>
</tr>
<tr>
<td>After learning</td>
<td>21.79 (1.68)</td>
<td>19.93 (1.53)</td>
</tr>
<tr>
<td>Before retrieval</td>
<td>25.43 (1.77)</td>
<td>25.36 (1.61)</td>
</tr>
<tr>
<td>After retrieval</td>
<td>24.21 (1.83)</td>
<td>24.64 (1.66)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative affect (PANAS)</th>
<th>Placebo</th>
<th>Pramipexole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before learning</td>
<td>11.14 (0.39)</td>
<td>10.71 (0.29)</td>
</tr>
<tr>
<td>After learning</td>
<td>11.36 (0.55)</td>
<td>11.21 (0.43)</td>
</tr>
<tr>
<td>Before retrieval</td>
<td>10.64 (0.17)</td>
<td>11.36 (0.62)</td>
</tr>
<tr>
<td>After retrieval</td>
<td>10.50 (0.17)</td>
<td>11.00 (0.55)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Psychomotor Vigilance Task (PVT)</th>
<th>Placebo</th>
<th>Pramipexole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before learning</td>
<td>3.40 (0.07)</td>
<td>3.35 (0.09)</td>
</tr>
<tr>
<td>After learning</td>
<td>3.22 (0.10)</td>
<td>3.20 (0.10)</td>
</tr>
<tr>
<td>Before retrieval</td>
<td>3.48 (0.09)</td>
<td>3.49 (0.10)</td>
</tr>
<tr>
<td>After retrieval</td>
<td>3.36 (0.10)</td>
<td>3.35 (0.11)</td>
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</table>

<table>
<thead>
<tr>
<th>Word Fluency Test (WFT)</th>
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<th>Pramipexole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
<td>19.36 (1.18)</td>
<td>18.71 (0.87)</td>
</tr>
<tr>
<td>Letter</td>
<td>16.50 (1.37)</td>
<td>15.64 (1.59)</td>
</tr>
</tbody>
</table>
The finding in the placebo condition, that reward only differentially affected recognition performance of pictures at delayed recognition after sleep, but not at immediate recognition testing right after learning before sleep, lends to the idea that sleep substantially contributes to forming memories specifically associated to reward, beyond supporting the preferential maintenance of memories associated with high reward, which corresponds to findings that monetary reward effects are stronger after retention intervals of several days (Murayama & Kuhbandner, 2011). However, the lack of clear differential effects of low versus high reward at immediate recognition could also be due to ceiling effects as here all recognition scores were rather high, additionally, the treatment conditions differed regarding bias at immediate recognition. In an analysis of the placebo condition the respective phase x reward interaction term failed to reach significance, however, this analysis is limited by the fact that different recognition stimuli were tested at immediate and delayed recognition. All in all, the issue of sleep being critical for the formation of representations distinctly differing in strength depending on the associated reward remains to be further explored.

Whatever the cause for the absence of differences in immediate recognition of memories associated with low and high reward, at the delayed recognition after sleep, high reward memories were clearly better recognized than low reward memories in the placebo condition, and this difference was wiped out by pramipexole. In rats during sleep reactivation of cell assemblies that were active together during prior wake has been shown in the hippocampus (Ji & Wilson, 2007; Skaggs & McNaughton, 1996) and ventral striatum (Lansink et al., 2008; Pennartz et al., 2004). Therefore, the preferential consolidation of high reward memories might be mediated by reactivation within the hippocampus that initiates the reactivation of the striatal reward centres (Lansink et al., 2009) leading to a feedback of reward signals from the striatum to the hippocampus during sleep, via a feedback-loop that may also include the VTA (Lisman & Grace, 2005). Another possibility is that reward-associated memories that are deemed important for future behaviour are already tagged before sleep by prefrontal processes for preferential reactivation during sleep (Wilhelm et al., 2011). Indeed, it has been shown that the reactivation frequency of cells within the hippocampus during sleep can be preferentially enhanced by exogenous cues (Bendor & Wilson, 2012), and that such reactivations induced by exogenous cues in particular benefit low value representations (Oudiette et al., 2013). However, differential effects of reward on consolidation during sleep being solely conveyed by a tag-
ging that takes place prior to sleep, would not explain that enhancing D2-like receptor activation during sleep nullifies any difference in recognition between memories associated with low and high reward.

It is probable that the reward promised for later retrieval increased encoding strength and we, therefore, additionally manipulated this factor by presenting pictures for a short or a long duration. Consequently, the longer duration led to a robust increase in recognized pictures. However, our finding that the reward related effect of pramipexole did not depend on or interact with the duration of stimulus presentation, precludes that effects of D2-like receptor activation were conveyed via encoding strength per se, as a mechanism that might directly regulate reactivation frequency in hippocampal circuitry (Drosopoulos, Schulze, Fischer, & Born, 2007).

While reinforcing effects of pramipexole have been consistently demonstrated in rats (Engeln et al., 2012; Riddle et al., 2012) in human fMRI studies, reward-related effects of pramipexole expressed themselves in reduced activation of reward networks probably reflecting the inhibition of endogenous dopamine release via presynaptic autoreceptors (McCabe, Harwood, Brouw, Harmer, & Cowen, 2013; Riba et al., 2008). Moreover, performance on the same task as was used here, was shown to rely on NAcc and VTA activity during the encoding session and the connectivity of these brain areas to the hippocampus as measured by blood oxygen dependent activity (Adcock et al., 2006); and reactivation of brain areas involved in prior encoding have been proposed to be causal to sleep-dependent memory consolidation (Rasch et al., 2007; Rudoy et al., 2009). Combining these pieces of evidence, we suppose that in the placebo condition of our experiment, when pictures were reactivated, inputs from the reward circuits modulated memory according to the reward contingencies learned during encoding. Under pramipexole, however, with inhibition of the reward centres via pre-synaptic D2-like receptor activity and globally enhanced activation of post-synaptic D2-like receptors in the NAcc and the hippocampus, reactivation efficacy is balanced out for memories with high and low rewards (see Figure 2 for an overview of the proposed mechanisms). In line with this assertion, pramipexole during encoding blocks the discrimination between high reward stimuli and low reward stimuli in a reward learning task (Pizzagalli et al., 2008; Santesso et al., 2009). This interpretation also fits well with reports of increases in compulsive behaviour in patients with restless-leg syndrome and Parkinson’s disease treated with pramipexole (Aiken, 2007; Pourcher, Remillard, & Cohen, 2010; Weintraub et al., 2010). It is quite
possible that these patients feel the urge to perform certain maladaptive behaviour because pramipexole leads to a blunting of reward contingent consolidation of adaptive behaviour during sleep.

Unexpectedly, pramipexole did not increase the overall amount of pictures that were retained or improve performance on any of the other memory tasks, which may be due to the disrupting effects of the drug on sleep suppressing both SWS and REM sleep. Alternatively, this may also indicate that the effect of pramipexole is conveyed mostly by inhibiting the reward centres via autoreceptors, thus, leaving unrewarded memories unchanged.

The causal role of SWS for hippocampus-dependent memory has been repeatedly shown (e.g., Marshall et al., 2006; Marshall, Kirov, Brade, Molle, & Born, 2011). To the best of our knowledge, the present study is the first to examine effects of pramipexole on sleep in healthy volunteers. However, the present findings fit well to observations in restless-leg patients exhibiting massive changes in sleep architecture after acute administration of the D2-like receptor agonist, which likewise comprised marked reductions in SWS and REM sleep in favour of sleep stages 1 and 2 (Saletu, Anderer, Saletu-Zyhlarz, Hauer, & Saletu, 2002).

Ultimately, our data in combination with foregoing animal studies suggest that sleep-dependent consolidation adapts behaviour to future rewards through the hippocampus-driven feedback of reward contingencies from the reward system to the hippocampus thereby selectively strengthening those memories during reactivation that promise high rewards. This strengthening might be achieved by the modulatory effect of dopamine on plasticity in the hippocampus (e.g., Edelmann & Lessmann, 2013; Manahan-Vaughan & Kulla, 2003; Zhang et al., 2009), but could also occur in extra-hippocampal structures (Goto & Grace, 2005; Schotanus & Chergui, 2008; Thomas et al., 2000). The action of pramipexole obliterating this adaptation process by wiping out reward contingencies during consolidation sleep opens the possibility of manipulating maladaptive but highly rewarding behaviour after its encoding, e.g., to buffer effects of relapse in drug addicts.
2.4 Study 3 – The role of GABA for the induction of slow wave sleep and sleep-dependent memory consolidation

2.4.1 Introduction

The enhancing effect on memory consolidation appears to be mediated in particular by the neocortical <1 Hz slow oscillation that hallmarks the EEG during SWS, and synchronizes the neuronal reactivation of newly acquired memory representations that takes place during SWS in distributed networks, to the excitable depolarizing up-state of these slow oscillations (Molle & Born, 2011). This allows the redistribution of the reactivated memory representations and their stabilization for the longer term (Diekelmann & Born, 2010). Recent evidence suggests that procedural memory can also benefit from reactivation during NonREM sleep and that this effect is related to sleep spindles (Antony et al., 2012).

GABAergic mechanism in the preoptic region of the hypothalamus contribute to the generation of NonREM sleep and SWS (Benedetto, Chase, & Torterolo, 2012). Time spent in sleep is proportional to the activity of GABA producing neurons in the ventrolateral preoptic region of the hypothalamus (Sherin, Shiromani, McCarley, & Saper, 1996). GABA A agonists generally enhance SWS and also slow wave activity (0.5-4.0 Hz, including the < 1 Hz slow oscillations) during NonREM sleep, although this enhancement can be accompanied by a reduction in spindle activity (Lancel, 1999). Notably, these effects are opposite to those of benzodiazepines (and zolpidem) that are considered positive modulators of the GABA A receptor and increase spindle activity but reduce SWS or slow wave activity (Lancel, 1999). While these discrepant effects are difficult to reconcile they speak in favour of the use of agents non-specifically increasing extracellular GABA for investigating the role of GABAergic tone in the regulation of sleep and memory, rather than specific GABA receptor agonists. Against this backdrop, we tested here the effect of the GABA reuptake inhibitor tiagabine on sleep and associated memory consolidation in healthy young volunteers. tiagabine acts by selectively blocking the GABA-transporter GAT 1 (Borden et al., 1994; Fink-Jensen et al., 1992), and has been shown to improve sleep efficacy in healthy elderly, inasmuch as it strongly increased SWS without affecting other sleep stages or subjective sleep parameters (Mathias, Wetter, Steiger, &

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Lancel, 2001). With higher doses it also decreases time in rapid eye movement (REM) sleep (Walsh et al., 2005). We expected that the SWS promoting effects of tiagabine would be associated with an enhanced overnight consolidation of memory, and in particular of declarative materials which proved to be highly sensitive to SWS in previous studies (Marshall et al., 2006; Plihal & Born, 1997). We expected no benefits from tiagabine for overnight consolidation of procedural skills which in previous studies proved more sensitive to spindles rather than slow wave activity (Nishida & Walker, 2007; Rasch, Pommer, et al., 2009; Tamaki et al., 2009). As a control, we also examined effects on the retention of emotional materials which is known to profit from REM sleep (Baran, Pace-Schott, Ericson, & Spencer, 2012; Nishida, Pearsall, Buckner, & Walker, 2009; Wagner et al., 2001). While our study replicated a profound increase in SWS the data, unexpectedly, do not show an equivalent increase in sleep’s beneficial effect on memory consolidation, but an impaired gain in motor memory performance, possibly related to a concurrent decrease in slow oscillation phase-locked spindle activity after tiagabine.

2.4.2 Methods

2.4.2.1 Participants

Fourteen healthy young men aged 21.9 years (range 18 - 28 years) completed the study. Participants were non-smoking, native German speaking. Only males were included to reduce variance as cycling estradiol and progesterone levels in women can influence plasticity and GABA A receptors (Baudry, Bi, & Aguirre, 2012). They underwent a routine health examination prior to participation to exclude any mental or physical disease, did not take any medication at the time of the experiments, and reported a normal sleep–wake cycle. One additionally recruited subject did not complete the study due to adverse side effects. The participants were instructed to get up at 07:00 am on experimental days, and during these days not to take any naps and not to ingest alcohol or (after 01:00 pm) caffeine-containing drinks. Before the experiment proper, participants took part in an adaptation night under conditions of the experiment (i.e., including the placement of electrodes for polysomnographic recordings). The experiments were approved by the ethics committee of the University of Luebeck. Written informed consent was obtained from all participants prior to participation.
2.4.2.2 Design and procedure

The study followed a randomized, double-blind, placebo-controlled within-subject crossover design. Participants took part in two experimental sessions scheduled at least 14 days apart. Both sessions were identical but for the oral administration of placebo or tiagabine (Gabitril® 10 mg, Teva GmbH, German, plasma halftime 7 – 9 h, plasma maximum: 1 – 2.5 h after intake).

On experimental nights, participants arrived at the laboratory at 07:30 pm. Following preparations for EEG and polysomnographic recordings, the participants learned (between 09:00 and 10:30 pm always in the same order) neutral and emotional pictures, a declarative word pair associates task, and a procedural sequence finger tapping task, with a 10-min break between each of the tasks. Although this protocol introduces potential order effects, this approach was chosen to increase standardization. Also, consolidation of declarative and procedural tasks can influence each other if performed back to back. However, this retroactive interference does not eliminate sleep’s beneficial effect on declarative or procedural memory (Brown & Robertson, 2007). To further minimize such effects, we introduced longer breaks between the tasks. After the learning phase and 30 min before lights were turned off (at 11:00 pm) to enable sleep, the participants were orally administered a capsule containing either Tiagabine or Placebo. They were woken at 07:00 am and left the lab. During the following day participants engaged in their usual activities. They were instructed to refrain from any stressful mental or physical activities, and to keep a record of their activities during this day. In the evening they returned at 07:30 pm and retrieval of the memory tasks was tested – in reverse order of learning. At learning and recall tests of vigilance, mood and subjective sleepiness were performed to control these effects.

2.4.2.3 EEG Analysis

Average power spectra were calculated in Fz and Cz for all NonREM sleep epochs of the whole night. Power spectra were calculated by Fast Fourier Transformations with a Hanning window on subsequent blocks of 2048 data points (~10.24 sec, 3 blocks per 30-sec epoch). Spectra were filtered by a 5-point moving average. In the averaged spectra, mean power was determined for the frequency bands of interest, i.e., the 0.5-1 Hz slow oscillation band, the 1-4 Hz delta, 4-8 Hz theta, 9-12 Hz slow spindle and 12-15 Hz fast spindle frequency bands.
2.4.2.4  Spindles

Semiautomatic spindle detection was performed on an in-house program running in Matlab 2011a, which detects spindles by applying a standard algorithm (Mölle, Marshall, Gais, & Born, 2002), and calculates absolute spindle count and spindle density. In brief, first the peak frequency of fast spindle activity was assessed for each subject individually in the power spectra of sleep stage 2 (during Placebo nights) as the frequency of the power maximum between 12-15 Hz. The signal was then band pass filtered ±1.5 Hz around this peak frequency. A spindle was detected if the root mean square (RMS) of the filtered EEG signal was above the absolute spindle threshold for 0.5-3 sec. The absolute threshold for spindle detection was set for each participant individually at 1.5 standard deviations of the filtered RMS signal determined for sleep stage 2 of the Placebo night, and was on average: 5.65 ± 0.44 µV.

2.4.2.5  Slow oscillations

Detection of slow oscillations in NonREM sleep was based essentially on a standard algorithm described elsewhere in detail (Mölle et al., 2002), and was performed for Fz and Cz. In a first step, the EEG was low-pass filtered at 30 Hz and down-sampled to 100 Hz. Then a low-pass filter of 3.5 Hz was applied and time points of positive to negative zero crossings were computed in the resulting signal, for the identification of large slow oscillations. Then the lowest and highest value between these time points were detected (i.e., one negative and one positive peak) for all intervals of positive to negative zero crossings with a length of 0.9 to 2 sec. The means of these values were calculated across the participant's two experimental nights, and those intervals were marked as slow oscillation epochs whose negative peak amplitude was lower than 1.25 times the mean negative peak and whose amplitude difference (positive peak minus negative peak) was larger than 1.25 times the mean amplitude difference. Averages of original EEG potentials were calculated for a ±1.3-sec window around the peak of the negative half wave of all detected slow oscillations. To analyse spindle activity occurring phase-locked to slow oscillations, the average RMS activity in the slow (9-12 Hz) and fast (12-15 Hz) spindle bands was also calculated for the ±1.3-sec windows around the negative slow oscillation peak.
2.4.2.6 Reaction times, mood and sleepiness

Mean reaction times were assessed as a measure of vigilance in a 5 minute version of the PVT (Dinges et al., 1997) that required pressing a button as fast as possible whenever a bright millisecond clock presented on a dark computer screen started counting upward. After the button press this clock displayed the reaction time. Mood was measured using the 10 positive and 10 negative items of the PANAS (Watson et al., 1988), participants responded to items (e.g., “Do you momentarily feel scared?”) on a 5 point Likert scale ranging from 1 = “not at all” to 5 = “very much”. Subjective sleepiness was assessed with the one item SSS (Hoddes et al., 1973) ranging from 1 = “Feeling active, vital, alert, or wide awake” to 8 = “Asleep”. At the end of the experiment participants were asked if they believed to have received an active agent or Placebo.

2.4.2.7 Data reduction and statistical analysis

Data from one participant were discarded because of poor sleep during the Placebo night. For the finger tapping task, data from one further subject were discarded because of low performance (<2 standard deviations from the mean) during the Placebo session (Including these data increased the reported effect). Sleep data from one subject could not be evaluated due to data loss in the Tiagabine night (recorder malfunction). In one subject spindles and slow oscillations could not be reliably evaluated due to EEG artefacts. Statistical analyses generally relied on analyses of variance (ANOVA; SPSS version 20.0.0 for Windows) including a repeated measures factor ‘Treatment’ (Tiagabine vs Placebo) and, for analysis of pictures an additional ‘Emotionality’ factor, representing recall of neutral vs emotional pictures. Analyses of EEG measures included additional factors for ‘Topography’ (representing the recording sites) and ‘Sleep stage’ (stage 2 sleep, SWS). Significant ANOVA interactions were specified by post-hoc t-tests. Degrees of freedom were corrected according to Greenhouse-Geisser where appropriate. The level of significance was set to P ≤ 0.05.
Study 3 – The role of GABA for the induction of slow wave sleep and sleep-dependent memory consolidation

2.4.3 Results

2.4.3.1 Memory

For the word pair associates task, retention of word pairs as indicated by the difference in recall at the retrieval phase minus immediate recall performance after the learning phase did not differ between the tiagabine and placebo condition (mean difference of recalled word pairs (±SEM) tiagabine: -2.17 (1.18), Placebo: -1.58 (1.01), F < 0.14, p > .72, for respective effects of Treatment, Fig. 2.4 A). Also, numbers of recalled word pairs at retrieval testing did not differ between the treatment conditions (t(11) = -0.30, p = .77). There were also no hints of any difference between the conditions during the learning phase (number of trials to criterion tiagabine: 1.92 (0.26), placebo: 2.33 (0.43), t(11) = -1.33, p = .21, number of recalled words at criterion trials tiagabine: 27.50 (1.03), placebo: 27.50 (1.11), t(11) = - 0.00, p = 1).

Figure 2.4. Mean (±SEM) of overnight retention of memories (A) for word pair associates, (B) neutral and emotional pictures, and (C) for sequence finger tapping skills in the Tiagabine (empty bars) and Placebo condition (black bars). Retention of word pairs is indicated by the difference in the number of word pairs recalled at retrieval testing after sleep minus recall performance on the criterion trial at learning before sleep. Recall of pictures is indicated by the total number of pictures recalled during retrieval testing after sleep. Overnight gains in sequence finger tapping (C, left panel) are indicated by the difference in performance (number of correctly tapped sequences per 30-sec trial) at retrieval testing after sleep minus average performance on the last trials during training before sleep. Right panel indicates performance after sleep on a control sequence not trained before sleep. *p ≤ .05, for pairwise comparisons between the effects of the treatments (n=12).
Memory for emotional and neutral pictures was also not significantly affected by Tiagabine (number of recalled emotional pictures Tiagabine: 9.33 (1.15), Placebo: 9.25 (1.12) and neutral pictures Tiagabine: 5.17 (0.78), Placebo: 6.17 (0.74), F < 0.53, p > .48 for respective main and interaction effects of Treatment, Fig. 1B). Emotional pictures were remembered better than neutral pictures in both conditions (F(1,11) = 42.76, p ≤ .001).

For the sequence finger tapping task, the overnight gain expressed by the difference of correctly tapped sequences at recall minus performance at learning was significantly reduced by Tiagabine (Tiagabine: 2.50 (0.5) Placebo: 5.03 (1.17), F(1,11) = 5.58, p ≤ .05, Fig. 1C). At learning, the number of correctly tapped sequences did not differ significantly between the treatment conditions (tiagabine: 18.19 (1.56), placebo: 16.83 (1.13), t(11) = -1.54, p = .15). Also, tapping on the control sequence did not reveal any difference between the tiagabine and placebo conditions (t(11) = 0.47 and p = .65). Error rates were variable and there was a trend toward error rates reducing more across sleep in the placebo condition, i.e., participants made less errors in the Placebo condition (mean reduction in error rate tiagabine: -1.01% (1.25), placebo: -4.47% (3.25), t(11) = 1.91 , p = .08).

2.4.3.2 Sleep

Descriptive data for all sleep stages is provided in Table 2.7. During the tiagabine condition, participants spent distinctly more time in SWS (t(10) = -3.10, p ≤ .01) but less time in stage 1 sleep (t(10) = -3.46, p ≤ .01) than in the placebo condition. REM sleep was also reduced in the Tiagabine condition (t(10) = -2.54, p ≤ .05).

A more fine grained analysis of EEG power during NonREM sleep stages 2 and SWS indicated a significantly increased mean power density in the slow oscillation (0.5–1 Hz), delta (1 – 4 Hz), and theta (4 – 8 Hz) frequency bands during tiagabine in comparison with placebo (Fig. 2.5 A). All effects were apparent at Fz (slow oscillation: t(9) = 3.01, p ≤ .05, delta: t(9) = 11.64, p ≤ .01, theta: t(9) = 2.35 and p ≤ .05, Fig. 2B left) and Cz (slow oscillation: t(9) = 5.22, p ≤ .001, delta: t(9) = 4.58, p ≤ .001, theta: t(9) = 3.76 and p ≤ .01, Fig. 2.5 B right). There was no significant difference between the treatment conditions for fast (12-15 Hz) and slow (9-12 Hz) spindle power (t(9) < 1.69, p > .13).
Figure 2.5. Mean (±SEM) power spectra of EEG signal during NonREM sleep at Fz (left) and Cz (right) for the tiagabine (red thick line) and placebo condition (black thin line). Bottom panels indicate significance between the effects of tiagabine and placebo. (B) Average power for frequency bands of interest: 0.5-1 Hz slow oscillation, 1-4 Hz delta, 4-8 Hz theta, 9-12 Hz slow spindle, and 12-15 Hz fast spindle bands. *** p ≤ .001, ** p ≤ .01 and * p ≤ .05, for pairwise comparisons between the effects of the treatment (n=10).

Analysis of discrete fast spindles, with power maxima between 13-14 Hz in this sample, showed that overall fast spindle density (spindles per 30-sec epoch) during NonREM sleep was reduced in the tiagabine condition (t(9) = -3.24, p ≤ .01; Fig. 2.6). When differentiating sleep stage 2 and SWS, this effect was more consistent for stage 2 sleep (post-hoc pairwise comparisons for stage 2 sleep: t(9) = -2.71, p ≤ .05) than SWS (t(9) = -0.74, p = .47, F(1,9) = 12.20, p ≤ .01, for Treatment x Sleep stage interaction). A reducing effect of tiagabine was similarly apparent for absolute spindle counts (F(1,9) = 12.64, p ≤ .01, for Sleep stage x Treatment, t(9) = -2.00, p = 0.08, and t(9) = 1.45, p = .18, for pairwise comparisons between the treatments for stage 2 sleep and SWS, respectively).
In order to further characterize the effect of tiagabine on NonREM sleep, the morphology of slow oscillations as well as spindle activity occurring phase-locked during the slow oscillation cycle were analysed. Compared with placebo, the density of slow oscillations (slow oscillations per 30-sec epoch) detected during NonREM sleep under tiagabine was increased in Fz and Cz ($t(9) = 2.91$, $p \leq .05$ and $t(9) = 9.24$, $p \leq .001$, Fig. 2.7 B). The slow oscillation waveform detected at Cz did not differ significantly between placebo and tiagabine. At Fz, there were marginal differences occurring mainly during the increasing and decreasing flanks of the oscillation (Fig. 4A for waveforms and p-values). However, peak to peak amplitude, negative half wave amplitude and slope of the slow oscillation did not differ between the treatment conditions ($t(9) < 1.41$ $p > .19$). Under tiagabine, fast spindle activity (RMS) was significantly reduced during the slow oscillation up state, i.e., 200-600 msec following the negative half-wave peak of the slow oscillation, and this effect was more pronounced at Cz than Fz (Fig. 4B for data and p-values). Slow spindle activity was also reduced under tiagabine during the negative half wave at Fz (Fig. 4C).

2.4.3.3 Reaction times, mood and subjective sleepiness

There were no significant differences in reaction times, mood or subjective sleepiness between tiagabine and placebo at learning or retrieval (all $t(11) < 1.54$ $p > .15$, Table 2.7 for means and SEMs). There was a trend toward participants being able to tell, if they had received tiagabine or placebo, i.e., ~50 % of the sample correctly identified the active treatment and placebo in the respective conditions ($X^2(1) = 3.50$, $p = 0.06$).
Figure 2.7. (A) Averaged EEG signal within ±1.3 sec around the negative half-wave peak (0.0 sec) of identified slow oscillations. (B) Mean (±SEM) root mean square fast spindle (12-15 Hz) and (C) slow spindle band (9-12 Hz) activity averaged time-locked to negative half-wave peak of identified slow oscillations. Data are shown separately.
for recordings from Fz (left) and Cz (right), and separately for the Tiagabine (red thick lines, negative going error bars indicate SEM) and Placebo (black thin lines, positive going error bars indicate SEM) conditions. Respective bottom panels indicate significance between the effects of the Tiagabine and Placebo treatment for consecutive 5-ms bins.

2.4.4 Discussion

A consistent finding in sleep and memory research is that memory consolidation during sleep essentially relies on SWS, and in this regard especially on the synchronizing feature of the <1 Hz slow oscillations during this sleep stage (Diekelmann & Born, 2010; Marshall et al., 2006). The aim of this study was, through stimulating GABAergic neurotransmission, to enhance SWS in order to improve the consolidating effect on memory. The present data show that the administration of the GABA re-uptake inhibitor tiagabine (10 mg) has indeed the same effect on SWS in young adults as it had in previous studies in elderly (Mathias et al., 2001; Walsh et al., 2005), inasmuch as it promoted SWS in favour of REM sleep. Although the sample size of our study was relatively small, the more detailed analyses of the EEG signal during SWS that relied on the artefact free datasets indicated that tiagabine increases power in the lower frequency bands between 0.5-8 Hz and increased the density of slow oscillations. However, spindle activity was simultaneously reduced following tiagabine administration. Unexpectedly, analysis of the memory tasks show that the increase in SWS did not reflect in an enhancement of sleep’s beneficial effect on declarative memories, which in previous studies proved to be most consistently benefited by SWS (Gais & Born, 2004a). There also was no influence of tiagabine on the overnight retention of emotional memories. Procedural motor memory consolidation in terms of correctly tapped finger sequences was even significantly impaired by the GABA agonist which corresponds to findings in cats of impaired sleep-dependent ocular dominance plasticity after administration of the GABA A agonist zolpidem (Seibt et al., 2008).

The failure of tiagabine to improve overnight retention of declarative memory cannot be attributed to confounding effects of the substance on vigilance and sleepiness at the time of retrieval testing. Testing took place almost 24 hours after oral administration of tiagabine or Placebo, i.e., a time when most of the substance had cleared the system (plasma half time 7 – 9 h). Also, measures of vigilance (PVT), mood (PANAS) and self-reported tiredness as well as performance on a control finger tapping sequence were comparable in both treatment conditions at retrieval testing.
Table 2.7. Sleep parameters and control measures: Means (± SEM) values are given for the Tiagabine and Placebo condition (n = 12). ** p ≤ .01, * p ≤ .05, t p ≤ .10 and ns p > .15. aOvernight retention of word pairs and gains in finger sequence tapping skill are here provided additionally in per cent values, with performance at the end of learning before sleep set to 100%.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Placebo</th>
<th>Tiagabine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minutes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wakefulness</td>
<td>20.41 (4.98)</td>
<td>14.90 (2.53)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>37.50 (4.86)</td>
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<tr>
<td>Stage 2</td>
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<td>236.68 (16.89)</td>
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<tr>
<td>SWS</td>
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<td>98.95 (11.91)</td>
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<tr>
<td>REM</td>
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<td>55.86 (9.62)</td>
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<td>Movement time</td>
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<td>3.27 (0.54)</td>
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<td>Total sleep time</td>
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<td>429.00 (26.50)</td>
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<tr>
<td>Vigilance (PVT) at learning</td>
<td>294.25 (15.74)</td>
<td>284.58 (5.01)</td>
</tr>
<tr>
<td>Vigilance (PVT) at retrieval</td>
<td>271.25 (14.86)</td>
<td>282.33 (8.22)</td>
</tr>
<tr>
<td><strong>Percent of learning</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correct word pairs (PAL)</td>
<td>94.34 (4.28)</td>
<td>91.34 (4.46)</td>
</tr>
<tr>
<td>Correct sequences (FTT)</td>
<td>131.44 (7.18)</td>
<td>114.28 (2.68)</td>
</tr>
</tbody>
</table>
There were also no differences between conditions in learning performance before substance administration.

Slow wave activity, including the <1 Hz slow oscillations and the 1-4 Hz delta frequency band, is a primary marker of SWS and has been consistently shown to contribute to the enhancing effect of sleep on hippocampus-dependent declarative memories as well as on procedural skill memories not essentially relying on hippocampal function (e.g., Aeschbach, Cutler, & Ronda, 2008; Gais, Molle, Helms, & Born, 2002; Huber, Ghilardi, Massimini, & Tononi, 2004; Marshall et al., 2006). Against this background, the present negative finding that tiagabine-induced increases in SWS and slow wave activity fail to enhance these memories, indicates that phenotypic SWS per se is not a critical mechanism in the consolidation of these memories. Also, comparison of slow oscillations showed comparable amplitudes, slopes and morphology for these oscillations in the tiagabine and placebo condition, which questions the primary relevance of slow oscillations for memory consolidation.

Figure 2.8 Schematic overview over the phase relationships between the slow oscillation and slow and fast sleep spindles; x-axis in milliseconds is relative to the (surface) negative slow oscillation peak. Fast spindle activity (12-15 Hz) increases during the down-to-up transition, is most pronounced during the up state of the slow oscillation and coincides with memory reactivations in the hippocampus and neocortex (Ji & Wilson, 2007; Mölle et al., 2011). Slow spindle activity (9-12 Hz), on the other hand, has its maximum during the up-to-down state transition (Mölle et al., 2011). The surface EEG negative half wave of the slow oscillation (down state), which is related to reduced neuronal firing, corresponds to a positive field potential wave in deeper cortical layers, while the surface EEG positive half wave (up state), which is related to increased neuronal firing, corresponds to a depth negative wave (Contreras & Steriade, 1995; Mölle et al., 2002; Molle, Yeshenko, Marshall, Sara, & Born, 2006).
However, tiagabine distinctly reduced spindle activity. Importantly, this suppressive influence was most prominent when analysing spindle activity in synchrony with the slow oscillation cycle (Fig. 5 provides a schematic illustration of slow oscillation and spindle coupling) and its presumed relationship to hippocampal memory reactivations. Consistent with previous studies (e.g., Andrillon et al., 2011; Mölle et al., 2011; Mölle et al., 2002), classical fast spindle activity in the 12-15 Hz range which typically displays a more widespread centro-partietal cortical distribution, showed a distinct increase during the depolarizing up-phase of the slow oscillation, reflecting a driving influence slow oscillations exert on the thalamic generation of these spindles (Steriade, 2006). By contrast slow (9-12 Hz) frontal spindle activity, which is a separate kind of spindle activity whose function is less well understood, was synchronized to the up-to-down transition of the slow oscillation (Mölle et al., 2011). Tiagabine profoundly suppressed both types of synchronized spindle activity during the slow oscillation cycle. In particular the classical fast spindle activity has been consistently found to be associated with overnight retention of both declarative and procedural memories (Clemens, Fabo, & Halasz, 2005; Gais et al., 2002; Schabus et al., 2004; Tamaki et al., 2009). Recent studies suggest that the synchronization of fast spindle activity to the depolarizing slow oscillation up-state is critical to the consolidation effect (Cox, Hofman, & Talamini, 2012; Ruch et al., 2012). Specifically it has been proposed that the fast spindles represent a mechanism involved in the redistribution of memory representations that become reactivated during SWS, to neocortical and striatal sites of long-term storage (Bergmann et al., 2012; Clemens et al., 2011; Diekelmann & Born, 2010). Against this background, although density of slow oscillations was increased after tiagabine, the reduced efficacy of these slow oscillations to drive and synchronize fast spindle activity to the depolarizing upstate of these oscillations could well explain the failure of tiagabine to produce any improvement in declarative memory consolidation. An alternative explanation may be that SWS is already maximally beneficial in its unmedicated quantity. However this is not supported by data that show increasing slow oscillations and spindles above normal physiological levels by another method, i.e., through transcranial direct current stimulation, which did increase declarative memory consolidation in young healthy student participants (Marshall et al., 2006). Also, it could be speculated that tiagabine failed to enhance SWS-dependent memory consolidation because such enhancement was counteracted by immediate (retrograde) amnestic effects of the GABA agonist on hippocampal memory traces.
(Chang & Liang, 2012). Moreover, in studies of fear memory in mice, hippocampal microinjection of the GABA A receptor agonist muscimol impaired consolidation of fear context when given 4 or 6 hours following training, but not when given 1 hour after training (Misane, Kruis, Pieneman, Ogren, & Stiedl, 2013). Indeed, further research seems necessary to explore immediate GABAergic effects on consolidation in hippocampal networks.

Considering the particular robust association that has been revealed for overnight gains in procedural skills and spindle activity during retention sleep, the suppressing effect of tiagabine can also account for the significantly diminished sleep-associated increases in sequence finger tapping speed in this condition. Alternatively, the diminished gains in sequence finger tapping could be a consequence of tiagabine reducing REM sleep as REM sleep has been assumed to contribute to motor memory consolidation (Karni, Tanne, Rubenstein, Askenasy, & Sagi, 1994), and reducing cholinergic tone during REM sleep-rich sleep can impair motor memory consolidation (Rasch, Gais, et al., 2009). However, another study showed that benefits in performance on the same sequence motor task as used in the present study can occur in conditions of strongly suppressed REM sleep after administration of antidepressants (Rasch, Pommer, et al., 2009), (see Watts, Gritton, Sweigart, & Poe, 2012 for related results in rats). While REM sleep was suppressed, overnight gains in finger tapping were closely associated with fast spindle activity during retention sleep, which is well in line with motor memory consolidation relying on sleep spindles rather than on REM sleep for consolidation (Nishida & Walker, 2007; Tamaki et al., 2009).

Based on the present findings and the available literature, we can only speculate about the neurophysiological mechanisms mediating the effects of tiagabine, in particular those that enhance slow wave activity but simultaneously tend to reduce fast spindle activity. The generation of slow oscillation comprises a complex interplay of intrinsic voltage-dependent membrane currents, with miniature EPSPs and low threshold Ca2+ potential mediated bursts considered as initializing events within neocortical and thalamic networks, respectively (Crunelli & Hughes, 2010; Timofeev, Grenier, & Steriade, 2000). Its relatively stereotypical waveform remaining largely unaffected by Tiagabine argues against an immediate effect of the GABA agonist on the slow oscillation. Rather the general increase in slow oscillation density and power in lower EEG frequencies might origin from indirect GABAergic effects in brainstem and hypothalamic areas reducing cholinergic and histaminergic tone in the cortico-
thalamic system thereby reducing inhibition of the nucleus reticularis and depolarization of thalamocortical and cortical neurons (Steriade, 2003). Decreased brainstem cholinergic tone is a major factor shifting the thalamo-cortical system towards increased slow wave activity (Steriade, 2006). However, with regard to the suppression of spindle activity, a direct effect of tiagabine on the generating thalamic mechanisms cannot be excluded. Fast spindles (12 – 15 Hz) are locally generated in the thalamic reticular nucleus, which is composed entirely of GABAergic cells (Fuentealba & Steriade, 2005). Importantly, the thalamic GABAergic effects show a specific temporal dynamic, as tonic activation of GABA A receptors reduces the occurrence of spindles, whereas the action of agonistic modulators, which amplify the phasic response of the GABA A receptor, increases spindle occurrence (Lancel, 1999). Thus, the present data provide evidence that, although enhancing phenotypic SWS, Tiagabine severely disturbs fast spindle activity during NonREM sleep, possibly due to its action as a reuptake inhibitor, which increases GABAergic tone rather than increasing phasic GABAergic signalling (Lancel, Faulhaber, & Deisz, 1998).

In conclusion this study demonstrates that stimulating GABAergic activity by administration of the GABA reuptake inhibitor tiagabine strongly drives slow frequencies in the sleep EEG thus producing an increase in phenotypic SWS. However, this increase is not functionally effective as concurrently fast spindle activity, especially that occurring phase-locked to the slow oscillation up-states, is suppressed. Consequently, despite increasing phenotypic SWS, tiagabine fails to improve declarative memory consolidation during sleep and even impairs indicators of procedural memory consolidation.
3 Conclusions and general discussion

3.1 Summary of the main results

Sleep's role for memory has become clearer over the last decade, but the question remains which plastic processes are involved. To shed some light the present work manipulated the main excitatory neurotransmitter glutamate, the major neuromodulator dopamine and the main inhibitory neurotransmitter GABA to elucidate their role for sleep-dependent memory consolidation. The main points of the three studies are visualized in Figure 3.1. Study 1 revealed that NMDA receptor related plasticity is relevant for sleep’s impact on memory, probably by translating glutamatergic reactivation of memory traces into plastic changes within the hippocampus. Interestingly, blocking NMDA or AMPA receptors had no effect on the declarative word pair memory task. Nevertheless, increasing glutamate’s efficacy at the NMDA receptor using DCS increased the amount of retained word pairs only if the participants were allowed to sleep in the retention interval. This seemingly contradictory result is explained by the higher efficacy of DCS at NMDA receptors containing the NR2A subunit. Study 2 suggests that dopaminergic circuits are reactivated during post learning sleep. During a sleep containing retention interval pramipexole, a dopamine agonist, wiped out differences between high and low rewarded items learned before sleep, which was either because of interference at dopamine receptors within the hippocampus or because of inhibition of reward centres by autoreceptor activation. In study 3, tiagabine was used to strongly increase SWS, but this failed to enhance memory. This was probably due to disturbed spindle to slow oscillation coupling. Together these findings indicate that the major players in neuronal communication also participate in sleep-dependent memory consolidation. Specifically, glutamatergic plasticity is involved in the transformation of reactivation into plastic changes. Dopamine signalling seems to be important to distinguish between more or less important information and, thus, modulates sleep-dependent memory consolidation. The tiagabine results highlight that the effects of sleep’s oscillatory EEG pattern on memory relies on the intricate interplay of different frequency bands.
3.2 Consequences for the active system consolidation hypothesis

3.2.1 Initial consolidation within hippocampal networks

The active system consolidation hypothesis in its latest form (Rasch & Born, 2013) establishes a model, where memory traces encoded into hippocampal ensembles during prior wakefulness are redistributed to cortical stores during SWS. This theory, based on the system consolidation concept (Dudai, 2004), constitutes a valid framework fitting a variety of findings of sleep’s effect on memory that can explain findings like insight after sleep (Wagner et al., 2004) or increased false memories (Diekelmann et al., 2010; Payne et al., 2009). Nevertheless, there are also accounts of initial hippocampal strengthening that could take place before memory traces are transferred to the cortex (Inostroza & Born, 2013). And while there is evidence for fast hippocampal disengagement if information relates to an existing schema (Tse et al., 2007; Tse et al., 2011), Gais et al. (2007) show that hippocampus activation is increased at retrieval if sleep was allowed directly after encoding. The latter is also in keeping with evidence for hippocampal contribution to retrieval five days after acquisition that disengages within 25 days (Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999).

An important aspect for researching memory that is thought to rely on different systems depending on its age is to consider if different plastic mechanisms are at work.
work in the anatomically different brain regions. One hint in this direction is that the present data were unable to replicate the findings that sleep-dependent consolidation of sensory memory can be blocked by AMPA and NMDA receptor antagonists in our hippocampus dependent memory task (Gais et al., 2007). In fact, the acquisition of sensory memory tasks relies on many repetitions of the same material during learning that are necessary to form connections between sparsely connected cortical neurons (Chklovskii, Mel, & Svoboda, 2004; Frankland & Bontempi, 2005; Trachtenberg et al., 2002). In the hippocampus encoding is thought to take place by strengthening already existent connections and it has been proposed that the transfer of memory from the hippocampus to the cortex may rely on a similar process as encoding of sensory information that is driven by the repeated reactivation of the trace (Frankland & Bontempi, 2005), which should make this process of system consolidation similarly susceptible to AMPA or NMDA receptor blockade as consolidation of the perceptual task. Hence, while, our finding that DCS increased sleep-dependent declarative memory consolidation shows that glutamatergic neurotransmission is important for consolidation of the declarative task, the missing effect of AMPA or NMDA blockade, indicates that some of the glutamatergic processes involved may be different from those responsible for plastic changes in the cortex therefore arguing against system consolidation being solely responsible for the effect of sleep on memory consolidation.

In this framework it seems quite uncertain whether the transfer of memory is completed during the first post encoding night (Inostroza & Born 2013). As stated above, hippocampal activity is increased two days after encoding if participants are allowed to sleep and this effect is absent 6 months later (Gais et al., 2007). Therefore, initial synaptic consolidation after reactivation during sleep within the hippocampus may be a prerequisite for lasting memories to be transferred to the cortex over a longer period. However, theories of sleep-dependent memory consolidation must additionally to a mere strengthening of memory explain the fast extraction of insight in some studies (Wagner et al., 2004) and the development of false memories (Diekelmann et al., 2010; Diekelmann, Landolt, Lahl, Born, & Wagner, 2008; Payne et al., 2009), thus, it may turn out that traces are not only strengthened within the hippocampus, but that they are also transformed within it.

One reason for initial consolidation within the hippocampus may be the extraction of adaptive behaviour patterns. The finding that the ventral striatum is reactivated in
response to hippocampal reactivation (Lansink et al., 2008; Lansink et al., 2009) indicates that reward circuitry is available during sleep. The present work shows that dopaminergic signalling can influence the strengthening of memory across sleep, indicating that depending on the potential use of a memory its strength can be manipulated during offline periods. Possibly, the merit of some new information can only be assessed after a certain period, which would make it opportune to have this information available for transfer at a later time. However, this point will have to remain for further investigation.

The finding that increasing slow oscillations by tiagabine did not change declarative memory performance may also be attributed to concurrent effects of consolidation within the hippocampus and system consolidation. Slow oscillation up-states have been shown to increase reactivation within the hippocampus (Wilson & McNaughton, 1994) and may have done so in the current study leading to increased intra-hippocampal consolidation. The disruption of spindle to slow oscillation coupling may have disrupted system consolidation. Both effects together may have created the null effect.

3.2.2 Metaplasticity & homeostasis

The encoding of memory can be achieved by plastic changes of connections between neurons. A popular theory of sleep function assumes that sleep is crucially involved in rebalancing synaptic weights that have been unbalanced by learning this net synaptic-downscaling is thought to occur to achieve synaptic homeostasis thus countering up-scaling during wakefulness (Tononi & Cirelli, 2003, 2006). This synaptic homeostasis hypothesis opposes the views of active systems consolidation, which assumes synaptic potentiation also occurs during sleep, and in its first conceptualisation explained memory benefits through sleep to originate from a better signal to noise ratio generated by synaptic-downscaling. The theory assumes that learning in the cortex is primarily achieved by potentiation during wakefulness and that this potentiation leads to increased demands in space and energy and, if it remains unchecked, to a saturation of learning ability. Accordingly, markers of synaptic potentiation, such as gene or receptor expression, increase over wake and are reduced by sleep (Vyazovskiy, Cirelli, Pfister-Genskow, Faraguna, & Tononi, 2008). Also, in adolescent rats spine growth is greater during wake and lower during sleep, while the opposite is true of spine shrinkage (Maret, Faraguna, Nelson, Cirelli, & Tononi,
Consequences for the active system consolidation hypothesis

Renormalization and thus sleep is thought to be the price we pay for plasticity during wakefulness (Tononi & Cirelli, 2014).

In an attempt to incorporate the compelling evidence in favour of sleep’s role for active memory consolidation the original theory has recently been updated from a theory of general downscaling to a down selection hypothesis (Tononi & Cirelli, 2014). In this outline the authors assume that each night the brain samples all memory available to the long-term memory system and down-scales those memories that have been potentiated but are no longer needed, sparing those memories that are of future use. Another plausible reconciliation is to assume that local processes of specific potentiation can occur during sleep that can strengthen memory traces, but that general downscaling leads to a net depotentiation of synapses (Diekelmann & Born, 2010). This theory is very much in line with the results concerning NMDA receptor related plasticity. NMDA receptors can permit small currents of Ca\(^{2+}\) even without depolarization exceeding the threshold to remove the Mg\(^{2+}\) block (Espinosa & Kavalali, 2009). This mechanism could favour depotentiation due to low concentrations of Ca\(^{2+}\) (Berberich, Jensen, Hvalby, Seeburg, & Kohr, 2007). Interestingly, this permeability was found during states corresponding to the slow oscillation up state (Espinosa & Kavalali, 2009). In this scenario, as mentioned above, LTP after reactivation is responsible for local strengthening. Low concentration Ca\(^{2+}\) induced by slow oscillation up states, on the other hand, is involved in depotentiation and, therefore, renormalisation of synaptic weights. In fact, in line with this argument, it has been shown that hippocampal synapses that have lately expressed LTP cannot express LTD within the next hour (Peineau et al., 2007).

Another highly discussed form of shifting plasticity is termed metaplasitcity (Hulme, Jones, & Abraham, 2013). It concerns the notion that plasticity is not uniform at a given synapse but can vary depending on its short or long term experience. Regarding this background, the fate of new presentations may be determined by the composition of receptors that are included into the active zone during prior wakefulness. For example the metabotropic glutamate receptor 5 is involved in hippocampal plasticity and has been shown to increase across periods of sleep deprivation (Hefti et al., 2013; Tadavarty, Rajput, Wong, Kumar, & Sastry, 2011). This makes it quite possible that it transfers metaplastic information to synapses that have encoded new information. Another candidate for sleep related metaplasticity is the increase of NMDA subunit N2A after a novel experience (Baez et al., 2013). In this case the sub-
Applications of the current work

unit change could be interpreted as a tag that indicates which synapses should survive renormalization and which should be deleted.

3.2.3 A role for forgetting

The idea of synaptic homeostasis has a second appealing consequence. If memory is formed irrespective of later use and only afterwards the relevant information is extracted, there may be need for a process of active decay of already formed traces (Hardt et al., 2013). The hippocampus is assumed to have a finite capacity and consolidation has been shown to only affect those traces that have been identified as being of future use by instruction (Wilhelm, Diekelmann, et al., 2011) or reward (Fischer & Born, 2009). The present data showed that dopaminergic signalling may be used to distinguish the adaptive value of already formed traces. Together the neurochemical and metaplastic tags may instruct the sleeping brain, which memory to preserve and which to erase, thereby freeing capacity for new learning. In fact it has been shown that learning is improved if it occurs after an interval of intensive slow wave sleep (Antonenko et al., 2013; Van Der Werf et al., 2009).

3.3 Applications of the current work

While the present research is focused on generating basic scientific insights, its pharmacological character offers applications in patient populations as well as the potential of misuse. The enhancement of cognitive functions has received considerable attention in last years, even though ethical reasons against such strategies remain (Buchanan, 2011; Farah et al., 2004). Considering the enhancement of memory by drugs, two basic strategies exist: (1) the facilitation of encoding and (2) the facilitation of consolidation. The drugs used in the present experiments may offer tools for manipulations of specific behaviour. While in the case of DCS the unresolved question of what happens to forgetting must be considered, it may offer the possibility of improving psychological treatment that relies on learning, such as cognitive behaviour therapy. Indeed, there exist a row of studies that have been evaluated in a meta-analysis to show a beneficial effect of DCS on behaviour therapy (Bontempo, Panza, & Bloch, 2012). However, it remains unclear, if this benefit is produced during encoding or during consolidation (Vervliet, 2008). The current data suggest that the best strategy could be, only to boost those sessions that were successful (e.g., only exposure sessions where patients experienced a reduction in anxiety at the end) by giving
Critical appraisal and future directions

DCS before subsequent sleep. Regarding pramipexole it could be interesting to use this agent to directly counter the effects of relapse (e.g., for pathological gambling). This could be done by giving the drug directly the night after the patient's engagement in maladaptive behaviour occurs thereby disrupting the preferential consolidation of highly rewarding stimuli. In both cases chronic administration of the drugs cannot be advocated as the facilitating effect of DCS only works acutely, probably due to receptor adaption (Lanthorn, 1994; Quartermain, Mower, Rafferty, Herting, & Lanthorn, 1994). On the other hand, pramipexole has been shown to elicit adverse effects such as compulsive behaviour in patients with RLS or Parkinson’s (Pourcher et al., 2010; Weintraub et al., 2010).

The search for drugs that can improve sleep efficiency has produced a variety of compounds. Especially, as memory deficits of old age are slowly beginning to be related to SWS (Mander et al., 2013; Van Der Werf et al., 2009), methods to increase slow wave activity are being investigated. Tiagabine was hoped to become available in this indication for some time, but the current results advise against this step. Another GABA agonist zolpidem, however, has been shown to have beneficial effects on declarative memory consolidation in young adults in some studies (Kaestner et al., 2013; Mednick et al., 2013) and has only minimal residual effects the next day (Unden & Schechter, 1996), which makes this substance a pharmacological candidate for improved cognition in the elderly. Nonetheless, a number of studies shows effective manipulation of slow wave sleep can be achieved without having to recur to invasive methods (e.g., Marshall et al., 2006; Ngo, Martinetz, Born, & Molle, 2013).

3.4 Critical appraisal and future directions

The present experiments were carried out to a high standard. Nevertheless, future experiments will be able to correct some shortcomings and extend on the results. Generally, future research should go beyond the major players identified in the present studies by identifying auxiliary roles of neurotransmitters and/or receptors that have been shown to influence plasticity in the hippocampus, e.g., endocannabinoids, ghrelin, metabotropic glutamate receptors or gap junctions,

Specifically, Study 1 summarizes data where retention intervals of different length are used. As the ketamine and caroverine conditions had retention intervals only during the early SWS rich half of the night it would be desirable to rule out that the second half of the night is important to produce effects in the glutamatergic system. Also
Critical appraisal and future directions

it will be important to experimentally prove the influence of subunit composition of NMDA receptors on the effect of sleep on forgetting and consolidation, e.g., by applying specific blockers in a rat model. This would also allow establishing whether consolidation is happening in the hippocampus or the cortex, by infusing directly into the structure of interest.

Further, Study 2 found effects of a d2-like receptor agonist on reward memory. Due to the drug applied there is no possibility to extract if inhibition of reward circuitry or interference led to the effects. Therefore, it would be important to modulate other dopamine receptors to see if a similar effect can be observed. Additionally it would be interesting to see if other inputs to the dopaminergic system, e.g., by the prefrontal cortex, also contribute to sleep-dependent consolidation. This could be done by combining the pharmacological approach with psychological manipulations of relevance.

Finally, Study 3 showed that pharmacologically increasing slow wave activity does not improve memory. To rule out that the GABAergic agent had a disruptive influence on LTP, which was countered by increased slow wave activity it would be of interest to directly infuse tiagabine into the different brain areas, which could be done in an animal model.
4 Abstract

The beneficial influence of sleep on memory has received considerable support during the last decade. The most widely accepted mechanism for the sleep-dependent strengthening of memories acquired during preceding wakefulness is that of their reactivation during slow wave sleep. The present thesis focused on pharmacological manipulations of sleep-dependent memory consolidation to elucidate neurochemical mechanisms that translate this reactivation into plastic changes. To this end, participants learned memory tasks before a retention interval, during which an active agent was administered.

The most abundant excitatory neurotransmitter in the human brain glutamate participates in many forms of plasticity. The best studied form of plasticity is that of the glutamatergic synapse and relies on AMPA and NMDA receptor interaction. However, it is unclear if glutamatergic neurotransmission mediates the sleep-dependent consolidation of declarative memories. Hence, altering the action of these receptors during sleep, promises insights into the neurochemical mechanisms of plasticity that lead to sleep-dependent memory consolidation. Using AMPA and NMDA receptor blockers during sleep did not influence the consolidation of a declarative word pair associates task. Participant’s performance on the same task, however, was significantly increased, if d-cycloserine, a NMDA receptor co-agonist, was given during sleep. This result indicates that NMDA receptor mediated plasticity is important for sleep-dependent declarative memory consolidation and that the processes involved may differ from those observed during wakefulness.

Dopamine is an important neuromodulator that can influence memory strength by facilitating synaptic plasticity. Activity of dopaminergic reward circuitry leads to better learning of rewarding information. The goal of the second study was to elucidate if dopaminergic circuitry is also involved during the reactivation of reward memory during sleep. Participants had to learn pictures, for which they were promised a high or a low reward at retrieval. Giving pramipexole, a D2-like receptor agonist, during the sleep retention interval wiped out the high over low reward benefit observed under placebo. Importantly, this effect was independent of encoding depth and thus speaks
for reactivation of dopaminergic reward circuitry during sleep influencing the fate of memory encoded during prior wakefulness.

Inhibitory neurotransmission in most cases involves the neurotransmitter GABA. GABA has also been shown to be involved in switching between states of arousal and sleep. It is important for the generation of the slow frequency EEG oscillations characteristic of slow wave sleep that have been shown to benefit sleep-dependent consolidation of declarative memory. In the third study administration of the GABA reuptake inhibitor tiagabine during retention sleep was used to manipulate this generating mechanism of slow wave sleep with the aim of boosting declarative memory retention. As expected, this manipulation highly increased the amount of slow wave sleep and slow oscillations that participants displayed, but the associated sleep spindle activity was dampened. As declarative memory was unexpectedly not enhanced by this treatment, it seems probable that effective slow wave sleep must also be accompanied by sleep spindle activity phase-locked to slow oscillations.

Together these studies demonstrate how the pharmacological approach can yield information about the neurochemical underpinnings of sleep-dependent memory consolidation and identified neurotransmitter systems that are involved in this process. Future pharmacological experiments, however, also in animal models, will have to specify the mechanisms we are only beginning to understand.
5 Abstract (German)


Dopamin ist ein wichtiger Neuromodulator, der die Gedächtnisstärke durch die Manipulation von synaptischer Plastizität beeinflussen kann. Die Aktivierung von dopaminergen Belohnungsschaltkreisen führt zum besseren Lernen von belohnter Information. Das Ziel der zweiten Studie war es herauszufinden, ob dopaminergen Schaltkreise von der Reaktivierung belohnter Informationen im Schlaf betroffen sind. Teilnehmer lernten Bilder, für die Ihnen eine hohe oder niedrige Belohnung versprochen wurde. Die Einnahme von Pramipexol, einem d2-like Rezeptoragonisten, wäh-

Inhibitorische Neurotransmission wird meist durch den Neurotransmitter GABA vermittelt. GABA ist auch am Wechsel zwischen Stadien der Aufmerksamkeit und Schlaf beteiligt. Der Neurotransmitter ist ebenfalls wichtig für die Generation der langsamen EEG-Oszillationen, die den Tiefschlaf charakterisieren und die sich günstig auf die schlafabhängige Gedächtniskonsolidierung auswirken. In der dritten Studie wurde die Gabe des GABA-Wiederaufnahmehemmers Tiagabine während Schlafs genutzt, um die tiefschlafigenerierenden Mechanismen zu aktivieren und somit deklarative Gedächtnisleistung zu verbessern. Wie erwartet, wurde die Menge an Tiefschlaf und langsamen Oszillationen stark erhöht, aber assoziierte Spindelaktivität wurde unterdrückt. Da deklaratives Gedächtnis unerwarteterweise nicht beeinflusst wurde, scheint es wahrscheinlich, dass effektiver Tiefschlaf mit der Phasenkopplung von Schlafspindeln und langsamen Oszillation einhergehen muss.

Zusammen demonstrieren diese Studien, wie die pharmakologische Herangehensweise Einblicke in die neurochemischen Vorgänge der schlafabhängigen Gedächtniskonsolidierung liefern kann und wie dadurch Neurotransmittersysteme identifiziert werden können, die an diesem Prozess beteiligt sind. Zukünftige Experimente, auch in Tiermodellen, werden die identifizierten Mechanismen, die wir gerade erst zu verstehen beginnen, weiter spezifizieren müssen.
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