Aus dem Institut für medizinische Psychologie der
Universität Tübingen

Influence of the N-Methyl-D-Aspartate Receptor coagonist, D-Cycloserine, on Memory Consolidation and subsequent Learning under Sleep Deprivation

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Medizin

der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen

vorgelegt von

Braganza, Elena Marie

2020

Dekan: Professor Dr. B. Pichler

1. Berichterstatter: Professor Dr. J. Born
2. Berichterstatter: Professor Dr. T. Leyhe

Tag der Disputation: 23.10.2020

Contents

List of Figures

List of Tables

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid
ANOVA	Analysis of variance
ASC	Active system consolidation theory
Ca2+	Calcium
DCS	D-cycloserine
EPSP	Excitatory post synaptic potential
fMRI	Functional magnetic resonance imaging
GLM	General linear model
HC	Hippocampus
IPSP	Inhibitory post synaptic potential
LTD	Long-term depression
LTM	Long-term memory
LTP	Long-term potentiation
Mg2+	Magnesium
MTL	Medial temporal lobe
MDMQ	Multidimensional mood questionnaire
MTT	Multiple trace theory
NC	Neocortex
NMDA	N-methyl-D-aspartate
NR2A	NMDA receptor subunit (equivalent to GluN2A)
NR2B	NMDA receptor subunit (equivalent to GluN2B)
PAL	Paired association task (word pair task)
PVT	Psychomotor vigilance task
SCT	Standard consolidation theory
SEM	Standard error of the mean
SHY	Synaptic homeostasis hypothesis
SSS	Stanford sleepiness scale
SWR	Sharp-wave ripples
TTT	Trace transformation theory
WFT	Word fluency task

1 Introduction

1.1 General Introduction

An insight into the mechanisms of memory and the dynamics within neurological networks serves as a powerful tool for a better understanding of human behaviour. It also elucidates our understanding of the pathology of memory deficits. A distinguishing feature of human consciousness is the capacity for explicit perception and reflection. The ability to form lasting memories which are flexibly modified by new input and rearrangement of pre-existing information is essential for these cognitive capabilities. Since the discovery of the double helix by Watson and Crick (1953) the genetic and biochemical nature of these processes has been elucidated with a growing pool of methods. The manipulation of these processes using pharmacological agents is becoming better understood. Specific neurotransmitters including glutamate, dopamine, acetylcholine, serotonin and noradrenaline have been shown to mediate processes of memory formation and are thus a promising objective for investigative studies about memory processes. Especially promising candidate mechanisms for long term memory formation as targeted in the current study are long-term potentiation (LTP) and long-term depression (LTD). The mechanism of LTP was first demonstrated at the glutamatergic input in the hippocampal formation (Bliss and Lomo, 1973). Specifically the glutamatergic N-methyl-D-aspartate (NMDA) receptor plays a crucial role for LTP induction and memory formation (Collingridge and Bliss, 1987, Malenka and Nicoll, 1999, Park et al., 2014). The role of this receptor for synaptic plasticity was extensively investigated by application of NMDA receptor agonists and antagonists (Collingridge et al., 1983, Wigstrom et al., 1986, Rosenzweig et al., 2002, Gais et al., 2008, Feld et al., 2013).

By pharmacological manipulation of the NMDA receptor by application of the receptor coagonist d-Cycloserine (DCS) the present work attempts to elucidate its role during memory consolidation for subsequent learning under sleep deprivation. The data presented in this work have recently been published under my co-authorship together with the data of a related sleep study (Alizadeh Asfestani et al., 2018). The article

presents the results of a combined data analysis of the two studies, whereas in the current work the results for the wake setting alone are presented.

1.2 Memory

1.2.1 A brief History

The plasticity of the brain was inferred by neurologists and psychologists to explain the manifold cognitive abilities and behaviours of animals and humans decades before these ideas were first experimentally examined. Ribot thought of memory as an alternation in activity of cells in the cerebral cortex (Ribot and Smith, 1882). In his engram theory Richard Semon proposed that the excitement of irritable substance by a stimulus may lead to lasting modifications in this substance even after the excitement had ceased (1921). Although his proposition is explicitly not restricted to the nervous system, the word engram has in the subsequent literature become a term with a meaning equivalent to memory trace (Schacter et al., 1978, Polster et al., 1991, Poo et al., 2016). This term reflects the simple concept of new information being stored in our nervous system. The means by which this is achieved is not generally defined, as there are a multitude of candidate mechanisms underlying the formation of a memory trace or engram (McGaugh, 1972). A generally accepted concept was introduced by Donald Hebb in his *neurophysiological postulate.* He formulated the idea, that if *'an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased'* (Hebb et al., 1949, p. 62).
He also puts forward the potential '*lasting or decay of synaptic knobs'* (Hebb et al., 1949, p. 228) as the probable underlying mechanism for this increase in efficiency thus emphasising the importance not only of neural but specifically synaptic plasticity as the underlying mechanism of memory formation. To the present day Hebbian plasticity is a widely used term to address cells or cell assemblies that fulfil the described postulate.

A further significant advance in our understanding of memory processes resulted from the observations on the patient Henry Molaison, generally referred to as H.M. (Scoville

and Milner, 1957, Squire and Wixted, 2011). H.M. received a bilateral resection of the hippocampus (HC) and adjacent structures in the attempt to relieve his severe epileptic symptoms. He subsequently showed a severe and persistent anterograde amnesia and a temporally graded retrograde amnesia. His general intelligence was not reduced (W. B. Scoville, 1954, Scoville and Milner, 1957). As will be further illustrated (see sections 1.2.2 – 1.2.4), this case study gave essential impulses for the differentiation of the stages of memory formation, the distinction between various types of memory and the theories about multiple memory systems partly attributable to specific localizations within the brain (Squire and Wixted, 2011). Together with various other human case studies (Scoville and Milner, 1957, Penfield and Milner, 1958) and animal studies (Malamut et al., 1984, Zola-Morgan et al., 1994), it substantially contributed to currently widely recognised paradigms.

1.2.2 Types of Memory

H.M.'s memory abilities were continuously reassessed, together with similar case studies (Milner et al., 1968, Zola-Morgan et al., 1986). A variety of different tasks and test paradigms were conceived to analyse the nature of their memory deficits, thus accumulating evidence that the amnesia in these cases is not a global but a selective memory deficit (Graf et al., 1984, Squire and Wixted, 2011).

This resulted in the functional classification of memory. As mainly perceptual motor skills were initially shown to be spared the term *procedural memory* to contrast the declarative memory deficits seen in these patients was introduced (Corkin, 1968, Cohen et al., 1985). In 1980 Cohen and Squire defined the word declarative as a *fact-and-event memory* that can be brought to mind actively and can be declared. The term procedural memory does not encompass all types of memory, that don't constitute declarative memory. They suggest the division of declarative or *data-based* memory and procedural or *rule-based* memory, following the former distinction of *knowing that* and *knowing how* by Ryle (Ryle, 1949, Cohen and Squire, 1980). In 1988 Squire and Zola-Morgan introduced the broader term non-declarative memory as an umbrella term for the diverse memory systems. Non-declarative types of memory comprise a very heterogeneous group of memory abilities, generally considered to be independent of the HC. The terms

declarative and non-declarative with their respective subcategories are now part of the canonical memory classification scheme (see figure 1, adapted from Squire et al., 2004).

Declarative memory is again subdivided into the two categories of semantic and episodic memory (Tulving, 1972). Semantic memory is thought to encompass factual information such as words, symbols, rules, relational information, categories and concepts. This is contrasted with episodic memory consisting of information about single events that were experienced by the individual. It is thought to include information about temporal-spatial relations (Tulving, 1972).
Tulving also puts forward the idea, that episodic memory changes with every time it is accessed, while semantic memory is less susceptible to transformation and loss.
This notion is compatible with later theories which postulate that semantic memory is derived from episodic memory via abstraction and in this process also become less susceptible to interference (Moscovitch et al., 2006, Winocur et al., 2010, Nadel and Hardt, 2011; section 1.4.3).

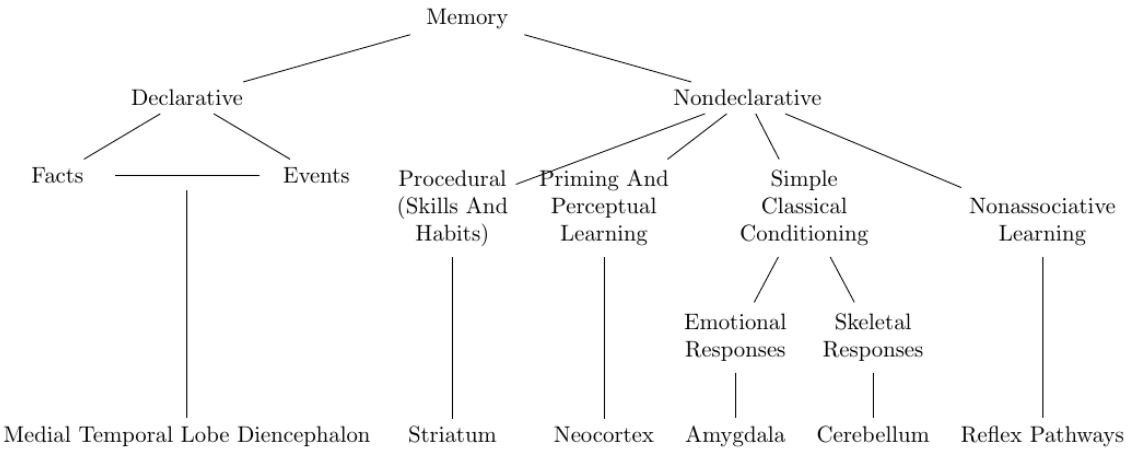

Figure 1 Taxonomy of Types of long-term memory *- including the brain structures thought to be most significant for each form of memory. Adapted from Squire et al. (2004)*

The described types of memory all refer to mnemonic functions that may be learnt and remembered for long periods of time. They represent the different forms of long-term memory (LTM) within a classification that originally divides memory into three separate memory stores. Hereby memory is divided into a sensory register, a short-term store and a long-term store. New stimuli or inputs first arrive in the sensory register,

where all information remains only for milliseconds and is processed and transferred to the short-term store. The short-term store is thought to lose the information within about 30 seconds unless it is actively maintained e.g. by rehearsal. Here again the information is thought to be processed and in part transferred to the long-term store, where it is permanently saved and never lost (Shiffrin and Atkinson, 1969). This model by Shiffrin and Atkinson is still much cited and the basis for current theories on memory, though it has been revised in diverse respects. The proposition of absolute permanence of the long-term store has been strongly challenged and is no longer tenable. There is now consensus that LTM also remains dynamic (see sections 1.4.4 – 1.4.7). The concept of the short-term store was refined and the term *working memo*ry was introduced. Working memory is still considered to be a store, in which new input is stored and processed within the first seconds and in which information can actively be retained for minutes. In the original understanding of short-term memory, it was considered as short-term store which is responsible for the formation of long-term memories. By contrast working memory is seen as a memory system fulfilling distinct functions separate from the LTM system (Baddeley, 1992). The human case studies including H.M. also helped to establish this separation between working memory and LTM. Even patients with severe amnesia could remember three-figure numbers for several minutes as long as they were not disturbed (Scoville and Milner, 1957). This implies that the working memory was unimpaired in these patients, who could no longer form new long-term memories.

1.2.3 Memory Formation

LTM is generally regarded as a dynamic process within a neurological network, that is split up into the three following stages:

Encoding – Consolidation – Retrieval

When a new stimulus or input reaches the nervous system, leading to processes of memory formation, this is termed encoding. In this process a memory-trace or engram is formed. The memory-trace is determined by the neurophysiological changes within participating neurons (see section 1.3). The later reproduction of a previously learnt

trace is termed retrieval. These two stages describe processes by which we commonly experience memory in everyday life. They generally take place via interactions with our surroundings. Ebbinghaus was the first to introduce test-paradigms in the attempt to scientifically measure the processes of learning and retrieval (Ebbinghaus, 1885).
Ideas about the need of an active process, consolidation, between these apparent stages, were prompted by attempts to explain retrograde amnesia after cerebral trauma. Information which had been encoded shortly before the trauma, could not be reproduced. As a possible explanation for this observation Ribot proposed that freshly encoded memory must be unstable for a certain period of time after encoding (Ribot and Smith, 1882). In 1900 Müller and Pilzecker described the vulnerability of newly learnt memory traces to similar contents shortly after encoding. If a certain timespan had passed between learning and the exposure to the similar contents, this interfering effect could no longer be found. Both observations imply the need for a mechanism by which initially unstable memory traces are stabilized, and that this process occurs within a circumscribed timespan after encoding. Müller & Pilzecker were also the first to use the term consolidation in order to describe this process (cited in Lechner et al., 1999).
A more elaborate concept for consolidation was introduced by Marr (1971). With a calculational line of argumentation based on histological neuroanatomical knowledge he attempts to predict the probable qualities and functions of the HC and neocortex (NC). He assumes that the HC as fast learner with low storage capacity has a time-limited role, whereas the NC as slow learner with high storage capacity serves as a long-term store (Marr, 1971, Frankland and Bontempi, 2005, Battaglia et al., 2011). This general concept served as a basis for various consolidation theories. To this date there is no clear-cut definition of consolidation (Polster et al., 1991, Battaglia et al., 2011). The diversity of different theories about consolidation and its underlying mechanisms reflect this ambiguity.
In section 1.4 a selection of widely debated and acknowledged consolidation theories will be described in further detail. A basic definition and subdivision will however be given here: Generally consolidation describes a process by which initially fragile memory traces are transformed into more stable traces and thereby new input is converted into a long-term form (Bosshardt et al., 2005). A consolidated memory may be defined by its immunity to disruption by stimuli or substances, which are capable of

disrupting memory formation if applied directly after initial encoding (McGaugh, 1966, Misanin et al., 1968, Przybyslawski and Sara, 1997). A major subdivision on a neurophysiological level distinguishes synaptic consolidation from system consolidation (Frankland and Bontempi, 2005, Dudai, 2012).

Synaptic consolidation describes a cellular or local process, also termed *fast consolidation* that takes place within minutes to hours, its purpose being mainly the stabilisation and enhancement of memory traces (Dudai, 2004). These cellular processes include the formation of new and rearrangement of existing synapses (see section 1.3). In a variety of sources synaptic consolidation is distinctly attributed to the phase shortly after initial encoding induced by an external stimulus. In this work the term is used on a mechanistic level as proposed by Dudai (2012). According to his definition it includes every local consolidation process, thereby also encompassing reconsolidation or the local consolidation processes that are triggered by reverberations between brain networks in the context of system consolidation.

System consolidation describes the reorganisation of memory traces within and between different memory systems and brain regions. It is also termed *slow consolidation* as it takes place over longer periods of time reaching from hours and weeks to years (Dudai, 2004, Frankland and Bontempi, 2005). In the context of their *law of regression* Ribot and Smith (1882) already described cases of temporally graded retrograde amnesia and presented the concept of an organisation of memory after encoding as prerequisite for the stabilisation of a new memory trace. An extensive discussion and experimental approach to the subject was greatly stimulated by the human case studies. The fact that no new episodic or semantic memory could be acquired by H.M. after the operation revealed that these memories must be at least initially HC-dependant. From this the necessity for a process that changes memory traces and in particular the included sites was recognised (Zola-Morgan and Squire, 1990, Frankland and Bontempi, 2005). The observation that the retrograde amnesia was temporally graded already implied, that the process of system consolidation is a gradual one (McClelland et al., 1995). The concept about distinct functions of HC and NC as proposed by Marr and the lesion studies by Squire and Milner reflect possible mechanisms and substrates for system consolidation.

An additional important property of system consolidation is that it may lead to a change in the quality of the memory trace. It may for example lead to insight and gist abstraction (see section 1.4.4).

1.2.4 The role of the Medial Temporal Lobe and Hippocampus

The example of an amnesic patient, where a post mortem autopsy had revealed a softening of medial temporal lobe structures including the HC led to the suggestion of a relationship of amnesia and this part of the brain (Bechterev, 1900, cited in Zola-Morgan et al., 1986). A major breakthrough in confirming this connection came with the case studies mentioned above (Scoville and Milner, 1957). Memory deficits could only be seen in the patients with bilateral hippocampal lesions and the severity of the memory deficits showed a positive correlation with the extent of hippocampal damage. This demonstrated the association of recent declarative memory with the HC and the medial temporal lobe (MTL). Because the lesions in H.M. included various parahippocampal areas, the hypothesis derived from this case was not adequate to make statements specific to the HC. Further research in part indicated the HC specificity of the declarative memory impairments (Zola-Morgan et al., 1986), but in part also emphasized the importance of adjacent structures such as the anatomically linked cortices (Zola-Morgan et al., 1994). Eventually the terminology of a medial temporal lobe memory system that comprises the HC and adjacent cortices as crucial region for declarative memory formation and consolidation was suggested (Squire and Zola-Morgan, 1991). The medial temporal lobe memory system served as clear example for the dependency of certain memory abilities on specific brain regions. Further theories and studies lead to the categorisation of the HC as fast learner which serves as *hub of the brain* in a time-limited manner with a crucial role for the declarative domain (Battaglia et al., 2011; see sections 1.4.1 - 1.4.5)**.**

1.3 Synaptic plasticity

Memory formation is thought to be implemented by neural plasticity. Synapses are a main site of this array of mechanisms to modify neuronal connectivity (Hebb et al., 1949, Poo et al., 2016, Kandel, 2001). The following section will give an introduction to the concept of synaptic plasticity and the most well researched anatomical, physiological and molecular correlates of synaptic plasticity and memory formation.

1.3.1 Synapse structure and synaptic signal transmission

A synapse is comprised of a presynaptic axonal bouton and a post synaptic density which are separated by a synaptic cleft. Signal transmission across the thin synaptic cleft is realised by neurotransmitters. These are small molecules which are released from the presynaptic membrane into the synaptic cleft. They then bind to receptors in the postsynaptic membrane where they can induce a range of excitatory, inhibitory or modulatory actions. The main mechanism of action is to alter the permeability of ion channels. The resulting ion flux may induce a depolarisation leading to an excitatory postsynaptic potential (EPSP), or a hyperpolarisation leading to an inhibitory postsynaptic potential (IPSP).

The quality and intensity of synaptic signal transmission is influenced by several mechanisms that cooperate to achieve synaptic plasticity. These include changes in the quantity of neurotransmitters released into the synaptic cleft, dynamics of removal of transmitters from the synaptic cleft and changes in how effectively cells respond to the neurotransmitters. The latter is e.g. influenced by the number and type of receptors on a synapse. Synaptic signal transmission influences these mechanisms via a diversity of molecular pathways that can lead to long lasting modifications in the synapse. Such long lasting synaptic modifications may include influences on e.g. recruitment of receptors into the cell membrane, gene transcription, protein synthesis, and even sprouting of new synapses.

1.3.2 Glutamatergic receptors and long-term plasticity

There are two main categories of receptors that mediate synaptic plasticity, ionotropic and metabotropic receptors. Ionotropic receptors contain ion channels, i.e. small pores by which specific ions can pass the cell membrane. The opening probability of these ion channels is regulated by receptor activation through the specific binding of a dedicated neurotransmitter, as well as other ligands. By contrast metabotropic receptors act through intracellular signal transduction and do not contain ion channels. They may however indirectly influence the opening probability of ion channels as well as a diversity of other intracellular processes (Valbuena and Lerma, 2016). The most well understood forms of long-term synaptic plasticity rely on the NMDA and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid (AMPA) receptors which are both ionotropic glutamate receptors (Coyle and Tsai, 2004, Malenka and Nicoll, 1999). Glutamate is the main excitatory neurotransmitter in the human brain (Petroff, 2002). Metabotropic receptors have also been shown to influence synaptic plasticity but seem to mostly play modulatory roles (Malenka and Nicoll, 1999, Lynch, 2004). The AMPA receptor has faster activation kinetics and shorter opening times than the NMDA receptor. It allows a high ion flux and is thought to be the principal mediator of neuronal excitation (Valbuena and Lerma, 2016). The NMDA receptor has specific properties which allow it to act as coincidence detector, enabling Hebbian plasticity (Tsien, 2000, Lynch, 2004, Valbuena and Lerma, 2016). At resting membrane potential the NMDA receptor pore is blocked by Magnesium (Mg^{2+}). However, when the cell is sufficiently depolarised, e.g. by the previous excitation from the same or other synapses, then the Mg^{2+} block is removed, allowing ions to pass. Receptor activation thus results in opening of the ion channel only if presynaptic activity (neurotransmitter release) and postsynaptic depolarisation coincide. The activation of the NMDA receptor leads to an influx of Calcium (Ca^{2+}), a powerful intracellular signalling molecule. Ca^{2+}, for instance, may activate kinase cascades which are important for the regulation of gene expression and lead to recruitment of AMPA receptors into the synaptic membrane (Coyle and Tsai, 2004). Indeed, Ca^{2+} influx into the postsynaptic cell has been shown to be necessary and sufficient to induce LTP, the most well described form of long-term plasticity (Bliss and Collingridge, 1993, Lynch, 2004, Malenka and Nicoll, 1999). This form of synaptic plasticity was first demonstrated in mammals by Bliss and Lomo

(1973). The induced long-term changes within the synapse include the activation of genes, transcription and protein synthesis (Bading et al., 1993, Malenka and Nicoll, 1999). These processes can lead to the potentiation of existing synapses, the emergence of new synapses, or the activation of previously silent synapses (Poo et al., 2016). NMDA activity even seems to have trophic effects on the nerve cells. Thus, NMDA receptor antagonists have been shown to induce widespread neuronal apoptosis in young rats (Olney, 2003).

In addition to the glutamate binding site the NMDA receptor has a modulatory glycine binding site. The binding of a ligand to both sites is necessary for the ion channel to open (Kleckner and Dingledine, 1988, Bergeron et al., 1998). The glycine binding site is not saturated in physiological conditions and increasing glycine levels enhances NMDA activity (Johnson and Ascher, 1987, Bergeron et al., 1998). The regulation of synaptic glycine concentrations via glycine transporter proteins appears to be a further means by which NMDA receptor activity is modulated in physiological conditions (Bergeron et al., 1998).

1.3.3 The complex role of the NMDA receptor

Notably, the activation of NMDA receptors does not necessarily lead to LTP.
An active form of long-term synaptic depression (LTD) also depends on NMDA receptor activation. Specifically, a high influx of Ca2+ leads to LTP, but moderate levels of Ca2+ influx can induce LTD, including the removal of AMPA receptors from the synaptic membrane (Malenka and Nicoll, 1999, Rosenzweig et al., 2002, Yashiro and Philpot, 2008). This is supported by animal studies showing that blocking NMDA receptors can prevent forgetting (Villarreal et al., 2002, Sachser et al., 2016). The underlying process may have been the inhibition of LTD. Lee et al. (1998) demonstrated chemically induced LTD by NMDA application. They furthermore showed that after saturating LTP with high frequency stimulation, chemically induces LTD via NMDA partially restored the capacity for subsequent LTP expression. Furthermore, there is evidence that the exact timing of firing of the pre- and postsynaptic neuron can influence the direction and amplitude of plasticity, a phenomenon termed spike timing dependent plasticity (Poo et al., 2016).

Finally, NMDA receptors include different subunits, which may lead to different forms of plasticity. Specifically the inclusion of NR2A or NR2B subunits respectively have been suggested to influence the direction of plasticity (Liu et al., 2004, Yashiro and Philpot, 2008, Hardt et al., 2013, Sachser et al., 2016).

1.3.4 Manipulation of NMDA receptors via DCS

In humans, the role of NMDA receptors can be investigated through the partial NMDA receptor agonist DCS, which occupies the glycine binding site. In line with the animal studies, DCS has been shown to enhance memory encoding in humans (Onur et al. (2010). A study by Feld et al. (2013) attempted to clarify the role of NMDA receptors on memory consolidation. The authors examined the long-term effect of NMDA receptor agonists and antagonists on paired association learning. To specifically assess consolidation, DCS was administered immediately following paired association learning, but recall was assessed only after DCS had reached negligible plasma levels. The authors found a significant improvement in recall performance when DCS was administered before an interval containing sleep. However, in a control group without a sleep interval this effect was not observed. The authors conclude that DCS specifically enhances sleep dependent memory consolidation processes. They specifically propose that this may rely on intrahippocampal synaptic enhancement by DCS.

1.4 Consolidation Theory and Sleep

There are many consolidation theories, in part complementing in part contradicting each other. This overview will only describe widely accepted theories. Many of these consolidation theories posit an important role of sleep and specific sleep stages. Sleep stages are categorised according to differences in electric brain signals measured by electroencephalogram (EEG), muscle tone measured by electromyogram (EMG) and eye movement measured by electroocculogram (EOG) according to criteria by Kales and Rechtschaffen (1968). They are divided into wakefulness, rapid eye movement (REM) and non-REM sleep. The EEG of the waking brain is dominated by alpha activity (8 – 13 Hz). REM sleep shows mixed EEG frequency, eye movements and

absence or near absence of muscle tone. Non-REM sleep is separated into 4 stages and characterised by low or missing alpha activity. Sleep stages 3 and 4 are generally summarised by the term slow wave sleep (SWS) and are characterised by delta waves (<4Hz), including slow oscillations (<1Hz). Non-REM sleep is also accompanied by hippocampal sharp-wave ripples (SWR, ~200Hz), and thalamo-cortical spindles (10-15 Hz). Temporally coordinated slow oscillations, spindles and SWR during SWS are thought to be optimal for sleep dependent consolidation processes (Marshall and Born, 2007; see section 1.4.5).

1.4.1 Stability-Plasticity Dilemma

The stability-plasticity dilemma addresses the issue of a system needing to be stable enough, so that information is not lost and at the same time plastic enough for new learning to be possible. It has its origins in computer science where it describes a design problem in intelligent systems that are capable of adapting to exterior influences via sequential learning. Initially a major problem in such systems was, that new learning led to forgetting of all previously learnt material. Based on these computational systems, theories were derived about how a network, be it neural or computer-based, would have to be organized in order to prevent such catastrophic forgetting or interference (Carpenter and Grossberg, 1988, McClelland et al., 1995, Abraham and Robins, 2005, Ans and Rousset, 1997). Abraham and Robins (2005) extensively argue that the retention of memory traces is most likely an active process which requires ongoing plasticity, rather than a static preservation of memory traces. McClelland et al. (1995) describes how a fast learning store, which acts as a buffer for newly learnt items and allows long-term changes in the larger long-term store to take place very gradually can be a solution to the problem of catastrophic forgetting in computational models and suggests that the same mechanisms also apply to neural networks. Incorporating the findings by Scoville and Milner (1957) and the initial proposal by Marr (1971), he suggests that the HC might function as such a buffer for the NC (McClelland et al., 1995, Alvarez and Squire, 1994). This two-store model would explain the temporary but crucial role of the HC for declarative memory. He also states that declarative memory must be very flexible and vulnerable, as it is often formed after single episodes rather than requiring repetition. On this assumption he reasons that the HC as a buffer system,

that allows a gradual incorporation of new memory into existing frameworks, might be crucial specifically in the declarative domain. In their *dual-system approach* Ans and Rousset (1997) also describe a computational model in which catastrophic forgetting can be prevented via rehearsal mechanisms between two reverberating networks in accordance with McClelland's theory.

1.4.2 Standard Consolidation Theory

The two-store model described above forms the basis of the standard consolidation theory (SCT) which proposes that in declarative memory formation, encoding leads to combined hippocampo-neocortical representations. Gradually, via system consolidation the memory traces are thought to become strengthened within the neocortex and simultaneously independent from the HC, eventually reaching a permanent state (Frankland and Bontempi, 2005, Dudai, 2004, Moscovitch et al., 2006, Takashima et al., 2009). The consolidated HC-independent memory trace is thought to be qualitatively similar to the initial HC-dependent memory trace. Episodic and semantic memory are not differentiated with this theory (Moscovitch et al., 2006, Winocur et al., 2010).

1.4.3 Multiple Trace and Trace Transformation Theory

A development of the SCT led to the multiple trace theory (MTT) which postulates that only semantic memory becomes truly independent of the HC. In both theories, stimuli form bound memory traces in the HC and NC. The MTT then argues that via repetitive stimuli, multiple overlapping memory traces are formed. Semantic memory is then abstracted from the overlapping memory traces, losing the details of the episodes. The emerging context free semantic memory is thought to be saved solely in the NC while the more detailed episodic memories don't become independent from the HC (Nadel and Moscovitch, 1997, Frankland and Bontempi, 2005, Moscovitch et al., 2006). Functional magnetic resonance imaging (fMRI) studies corroborate this idea by showing hippocampal reactivations during recall of not only recent but also remote episodic memories (Gilboa et al., 2004, Viard et al., 2010). The trace transformation theory (TTT) evolved as a refinement of the MTT. The notion that episodic memory may not be lost during consolidation, but that an episodic memory and the semantic

memory derived thereof may co-exist, was put forward (Winocur et al., 2010, Nadel and Hardt, 2011). Which type of memory is preferentially reactivated during recall then depends on the context of retrieval. Furthermore, the TTT emphasizes the qualitative difference between contextual episodic memories and the transformed context-free semantic memories. Winocur et al. (2010) repeatedly use the term schematic memory synonymously with context-free or semantic memory. And in fact the described process of abstraction derived from information overlap is central to theories about schema formation and gist-learning (Lewis and Durrant, 2011, Nadel and Hardt, 2011).

1.4.4 Abstraction, Gist-Learning, Insight and Schema Formation

There are various terms for abstract associative learning, such as gist learning, schema formation, insight and abstraction. They have in common a derivation from memory trace overlap. They furthermore generally depend on reorganisation and qualitative changes of memory (Wagner et al., 2004, Inostroza and Born, 2013). The term insight may e.g. be defined by a qualitative change of a memory trace which includes the sudden gain of explicit knowledge (Sternberg and Davidson, 1995, Wagner et al., 2004).

Prior to the development of the SCT and MTT, fundamental ideas about schema formation were introduced by Bartlett (1932, Chapter X). He described schemata as *organized settings* of neurons, influenced by every sensory input that has an association to them. He describes how this mutable *active organized setting* may be the prerequisite for inference and prediction. Furthermore Lewis and Durrant (2011) described the integration of new input into existing schemata as basis for gist abstraction and emphasised the sleep dependency of this process. According to Lewis and Durrant information overlap during memory replay in the sleep state is necessary for gist abstraction and formation of cognitive schemata, as the repetition of similar contents leads to the incorporation of shared aspects. Diverse studies corroborate this notion by showing an enhancement of inference and insight by sleep (Fischer et al., 2006, Ellenbogen et al., 2007, Wagner et al., 2004). In their discussion of the stability-plasticity dilemma McClelland et al. (1995) state that abstraction is only possible if very small adjustments to the connection weights are made at each step. This requires multiple repetitions, which may be fulfilled by memory replay during sleep (see section

1.4.5). System consolidation is thought to take place over weeks, months or years. The *schema assimilation model* describes an exception to this rule. It has been shown that system consolidation can be achieved within 24-48 hours, if the memory trace in question can be integrated into a pre-existing schema (Tse et al., 2007).

1.4.5 Active System Consolidation Theory

The active system consolidation theory (ASC) states that memory traces are selectively reactivated during sleep to be transferred into long-term memory via reorganisation and gradual neocortical strengthening. Qualitative changes occur during this process (Born and Wilhelm, 2012, Rasch and Born, 2007, Rasch et al., 2007). This concept is principally applicable to any memory system, but most extensively researched and discussed for the declarative domain. Building on the SCT, the ASC specifies the processes by which the fast learning HC helps to re-enforce long-term memories in the NC. In 1994 Wilson & McNaughton showed that hippocampal place cells that fired together during learning tended to fire together during subsequent sleep. These hippocampal reactivations coincide with hippocampal SWR (Buzsaki, 1998). It has been suggested that hippocampal SWR may coordinate the reactivation and reinstatement of memory traces in cortical circuits (McClelland et al., 1995, O'Neill et al., 2010). Siapas and Wilson (1998) show a temporal correlation between hippocampal SWR and neocortical spindles in rats which are termed *spindle-ripple episodes* as further evidence for the hippocampo-neocortical communication. The typical low acetylcholine levels during SWS, together with coordinated neocortical slow oscillations, hippocampal SWR and thalamo-cortical spindles are thought to be optimal for the described hippocampo-neocortical dialogue (Bazhenov et al., 2002, Hasselmo, 1999, Marshall and Born, 2007).

Taking into account a selection of experimental data Buzsaki (1989) proposes that sharp wave bursts may involve the endogenous activation of NMDA receptors and extensively discusses them as candidate mechanism for LTP. The induction of long-term synaptic plasticity in the context of the hippocampo-neocortical dialogue and the described oscillation patterns is also suggested by various further studies. Thus, in an animal study by Chauvette et al. (2012), LTP induction by the thalamo-cortical oscillations could be demonstrated. Laroche et al. (1990) showed how LTP in the

prefrontal cortex of rats could be induced by hippocampal stimulation. Boosting slow oscillations during sleep was shown to potentiate memory performance in humans (Marshall et al., 2006).

1.4.6 Reconsolidation and Labilisation

According to Bartlett (1932) memory recall should be viewed as an active reconstruction rather than the mere reproduction of information. During a certain experience, different schemata may be involved with new interrelations forming within and between them. Bartlett also described, how the attitude towards a certain memory greatly influences the construction of the memory during recall. The constructed memory then fulfils the goal of justifying the initial attitude. Memory recall is thereby not just the reproduction of a memory trace, but rather an active process during which the trace is engaged and changed. This observation is confirmed by Sara (2000). These considerations imply the necessity for renewed consolidation after every activation of a memory trace. An experimental approach to this notion of modifications after every memory reactivation followed decades later. Misanin et al. (1968) show that disruption of memory via retroactive interference using electric shocks in rats is not restricted to the timespan shortly after initial learning of the memory trace. If the consolidated memory trace is reactivated prior to the exposure to a disrupting input (e.g. electric shock), it is again rendered susceptible to retroactive interference. This indicates that a consolidated memory may be labilised by reactivation. Thereby the resistance to induced amnesia is not merely determined by the recency of a memory trace, but rather by the state of the trace in the moment of interference. Przybyslawski and Sara (1997) show that the reactivation of a memory trace in rats renders it susceptible to interruption via NMDA receptor antagonists and that this process shows a temporal gradient just as seen in initial consolidation. They therefore assume that NMDA receptor dependent intracellular events after initial encoding, must be at least partially recapitulated after every reactivation of a memory trace. Debiec et al. (2002) show how HC-independent memory traces are rendered susceptible to hippocampal damage via intrahippocampal infusion of protein synthesis inhibitors, if reactivated shortly before the infusion. In contrast to initial consolidation of new memory this susceptibility is restricted to 2 days

rather than several weeks, indicating that HC-dependent reconsolidation processes are recapitulated in a much shorter time than initial consolidation.

1.4.7 Processes of Forgetting and the Synaptic Homeostasis Hypothesis

1.4.7.1 Necessity of forgetting

Forgetting is a universal process that must be associated with an evolutionary benefit. Various authors describe metabolic, physiological and psychological advantages of the forgetting process. Thus, in their synaptic homeostasis hypothesis (SHY) Tononi and Cirelli (2014) review multiple studies about energy consumption of brain tissue, especially after synaptic enhancement. They conclude that it would be wasteful not to downscale. Learning capacity would be saturated. They argue that a memory trace can be better learnt or remembered the lower the *signal-to-noise ratio*. The term noise in this context refers to neuronal activity that does not contribute to the specific memory trace. This may be simple stochastic activity or the activity of competing memory traces. If synaptic enhancement were never counteracted by synaptic depotentiation, there would arguably be ever growing synaptic noise. The ability to capture *suspicious coincidences* between all the other *synaptical noise* is delineated as a prerequisite for the formation of a new memory trace. They argue that downscaling towards a baseline equilibrium, while preserving the relative differences in synaptic strength of previously induced learning, would be an energy efficient way to decrease the signal-to-noise ratio and store a new memory trace. This could be achieved if synaptic strength were proportionately reduced in all synapses (Tononi and Cirelli, 2014). Others have proposed reduced competition among representations as an important adaptive function of forgetting. Kuhl et al. (2007) show how brain areas which are thought to function as control regions that detect and resolve mnemonic competition, are less engaged after targeted forgetting. They interpret this as an important adaptive function of forgetting, for targeted remembering. In interference theory the repeatedly demonstrated retroactive interference of recently encoded memories is attributed to a limited learning capacity of the HC (Wixted, 2004). Furthermore, during the process of system level consolidation, where traces are thought to be transferred from one system to another, downregulation within the initial store must constitute an integral part of this process.

1.4.7.2 Correlates of forgetting: Interference and Decay

There are two main concepts for the processes underlying forgetting, interference and decay. According to interference theory new incoming input compromises previously formed memory traces, thereby leading to forgetting (retroactive interference). This idea is already expressed by the stability-plasticity dilemma (section 1.5.1). Interference theory furthermore correlates to the concept of the signal-to-noise ratio necessary for memory formation and preservation as described in the SHY. New input enhances synaptic noise which is detrimental to the memory trace. The first study showing sleep dependent benefits on memory ascribe this effect to the missing interference by new stimuli during sleep (Jenkins and Dallenbach, 1924). Previously learnt material is also thought to interfere with subsequent learning. Notably this proactive interference was seen if prior learning trials were massed, but not if they were spaced over several days (Wixted, 2004). This may be integrated with the later proposition, that processes of memory consolidation and forgetting are necessary to recuperate the capacity for new learning (Wixted, 2004).

There are also multiple accounts for forgetting by decay. This may be a passive process due to a successive fading of the previously stimulus induced metabolic changes. Such a passive rundown was postulated by Thorndike's law of disuse and is also corroborated by studies that show time dependent decrease in synaptic varicosities in low stimulus environments (Bailey and Chen, 1989, Wixted, 2004, Hardt et al., 2013). There are however several accounts for active forms of memory decay. Thus, LTD has been shown to depend on NMDA receptor activation (Villarreal et al., 2002, Rosenzweig et al., 2002, Sachser et al., 2016, section 1.3.3). Hardt et al. (2013) propose that forgetting mainly takes place by well-regulated active decay processes. This is corroborated by studies reporting a selective decay or facilitation of items that have previously been cued to be forgotten or remembered, respectively (MacLeod, 1999, Saletin et al., 2011, Wilhelm et al., 2011). Such a selectivity of forgetting is hard to integrate with a simple fading of synaptic strength or interference theory alone.

1.4.7.3 Occurrence of forgetting

The processes of forgetting can occur at every stage of memory formation.
The above-mentioned influence of the intention to remember or forget on subsequent memory performance suggests that factors affecting the forgetting-mechanism must be activated during encoding. This could occur by direct modulation of synaptic plasticity or indirectly e.g. by tagging for later differential consolidation (Morris, 2006). Reduced memory performance may in part also be accounted for by restricted availability at the moment of recall. This cannot however explain all types of forgetting. There is substantial evidence that the process of consolidation and especially sleep dependent consolidation, with the qualitative changes it evokes, plays an important role for selective memory enhancement and forgetting. The beneficial effect of sleep on subsequent new learning has been repeatedly attributed to a freeing of capacity subsequent to forgetting via downregulation of synaptic weights (Van Der Werf et al., 2009, Hardt et al., 2013, Antonenko et al., 2013). This is supported by the SHY, which attributes the beneficial effect of sleep on memory to a net downscaling of synaptic weights during slow wave sleep (Tononi and Cirelli, 2003). This is thought to counterbalance a net upscaling during prior wakefulness. They suggest that the decrease of certain neuromodulators, such as noradrenaline, histamine, and serotonin during sleep may regulate synaptic downscaling. They emphasise, why downregulation may not be compatible with the Hebbian plasticity processes which take place during new learning, thereby indicating why an offline state would be necessary for synaptic downscaling. They underline this by reviewing studies showing an upregulation of LTP related molecules during wakefulness and downregulation during sleep.
Nevertheless they point out, that this theory and theories based on the idea of active synaptic enhancement such as the active system consolidation theory are not mutually exclusive but may complement each other. Diekelmann and Born (2010) also argue that qualitative changes of memory traces promoted by sleep cannot be explained by the SHY alone. The active system consolidation theory offers an explanation.

1.5 Objectives and Hypotheses

1.5.1 Main hypothesis:

The objective of this study was to explore the effect of NMDA receptor enhancement via the partial agonist DCS on memory consolidation and memory capacity during sleep deprivation. In order to investigate this, DCS was administered after an initial learning task before a sleep deprivation interval and the effect on a subsequent learning task was measured. I hypothesized that NMDA receptor activity could play a role for synaptic enhancement and downregulation during the consolidation phase thus influencing the capacity for new learning. Notably, the new learning task was conducted after DCS had reached negligible plasma levels, so that preclusion of direct DCS effects during the new learning task is assumed. A previous study suggests that DCS increases synaptic plasticity by enhancing LTP within the HC in a sleep but not wake setting (Feld et al., 2013; see section 1.3.4). The described sleep dependent synaptic enhancement is thought to disturb subsequent learning due to enhanced proactive interference. I hypothesise that post-learning DCS administration during an interval of sleep deprivation should not lead to a decrease in new learning, as would be expected in a sleep setting.

1.5.2 Secondary hypothesis 1:

I wanted to see if the use of overlapping and thereby potentially more interfering material influences the strength of the DCS effect. As stated above (section 1.5.1), only in the sleep setting proactive interference of the initial task on subsequent learning would be expected and this effect was assumed to be increased by use of overlapping material. In a sleep deprivation setting no or only marginal differences between the interference and no-interference list were expected.

1.5.3 Secondary hypothesis 2:

The new learning task (including learning and recall) was executed three consecutive times with the same word pair lists, to ensure that the participants show an adequate overall recall performance for reliable detection of performance differences. Furthermore, in the initial learning task multiple learning and recall sessions are usually

executed before the required criterion of 60% was reached, so repeated sessions in the new learning session were chosen for higher consistency. I expected an increase of learning performance over the three time points.

1.5.4 Secondary hypothesis 3:

In order to assess direct enhancing effects on consolidation of the initial memory task, I carried out a recall session of the initial task after the new memory task. Here I expected to confirm the results by Feld et al. (2013) where DCS applied during the consolidation phase in a sleep deprivation setting did not affect later recall.
Furthermore, as the intention to remember affects memory performance (Saletin et al., 2011, Wilhelm et al., 2011), the recall of the initial task was important for our main task, which targets proactive interference. Without the described final recall session, the participants may not have formed an intention to remember the initial task, thereby also diminishing the targeted effect.

2 Materials and Methods

2.1 Study Population

2.1.1 Recruitment

The study population was recruited by public displays and by e-mail sent via the university server of the Eberhard Karls University, Tübingen. On request, participants received detailed information in the form of an attached file via e-mail.

2.1.2 Informed consent and data protection

During the first meeting written informed consent was obtained from all participants. A data protection form and a form relating to previous participation in other studies was filled out and signed by the participants and investigator. To ensure data protection, the experimental and personal data were only examined by directly involved scientific staff and not disclosed to third parties. The collected experimental data are stored under a pseudonym. As recommended by the German Research Foundation to ensure good scientific practice, all data is stored in the premises of the institute for a period of ten years. The provisions of data protection law are met.

2.1.3 Financial compensation

Each participant received 370 euros compensation for complete participation. For incomplete participation a correspondingly lower amount was paid. Participants had the option to drop out of the study at any time, without being obliged to name a reason. In case of an early termination due to adverse effects of the medication, the study physician and ethics committee would have been informed. This was not necessary in the described study.

2.1.4 Participant Criteria

According to the ethics proposal, project number: 136/2015BO1

2.1.4.1 General criteria

The study was conducted with healthy male participants with an age-range from 18 to 30 years. These were all non-smokers, german native speakers, with the previous minimal educational level of the german general qualification for university entrance (Abitur). Participants were generally excluded if they had previously taken part in any experiments with a word pair association task (PAL). After cross continental flights with a time difference of > 4 hours, after shift-working and in the case of prior participation in studies containing medication or medication intake for other reasons a minimal interval of 6 weeks was maintained before the first experimental session. The ingestion of nonsteroidal anti-inflammatory drugs (NSAID) was not regarded as exclusion criterion, unless it was consumed on the experimental day or in high amounts in the previous days. Excessive alcohol consumption was a further exclusion criterion. As reference point I used the official guidelines of the German nutrition society. These guidelines recommend a consumption of less than 20 gram/day. Participants with sleeping problems or high variability in their bed and awakening times, were excluded.

2.1.4.2 Medical history and examination

A standardised history and medical examination was conducted by the investigator with all potential participants prior to the experiments. The exclusion criteria involved known psychiatric, neurological or endocrine diseases, allergies to medication or food and chronic or current use of medication. DCS specific, participants were excluded who reported a history of hypersensitivity to DCS, substance abuse or addiction, fear disorder, psychosis, kidney or liver disease.

The physical examination included the measurement of the height and weight with subsequent calculation of the body mass index. The cardio-pulmonary system was assessed using auscultation and percussion of the chest, measurement of the heart rate, and blood pressure. The abdomen was examined via auscultation and palpation. A

neurological examination was performed. Blood was examined to exclude serious illness (appendix 1). In case of minor deviations from the norm, the study physician was consulted to estimate the pathological significance. If negligible significance was attested, the participant was not excluded.

2.1.5 Code of Conduct

Participants were given special instructions for the days prior to the experimental day, the experimental day itself and the following days:
The participants were asked to keep a regular sleeping rhythm especially in the week before the trial. The dates for the trial were chosen so that no stressful events would take place for at least one week before until at least one week after the experimental session. The participants were instructed to inform the investigator in case any unexpected stressful or emotionally charged event occurred, so that the experimental session could be rescheduled if deemed necessary. The participants were asked not to consume any, or only very moderate amounts of alcohol on the days before the trial. In case of ingestion of medication within 6 weeks of the experimental session the investigator was to be informed. On the day of the trial the participants were awake by 09:00 o'clock. After 12:00 o'clock no caffeine was consumed. The participants were also asked to refrain from excessive sports on the experimental days.

2.2 Study Medication

In the current study the NMDA receptor coagonist DCS was used to investigate the role of this receptor for subsequent learning. The glycine binding site is often preferentially targeted instead of the glutamate binding site as it has been shown to have similar enhancing effects on the NMDA receptor with lower excitotoxicity than glutamate (Coyle and Tsai, 2004).
A dose of 175 mg of DCS was selected. This dosage has been shown to be effective in facilitating sleep dependent declarative memory consolidation (Feld et al., 2013).
DCS has a plasma half-life of approximately 9-10 hours with a plasma maximum after about 1-2 hours (Zhu et al., 2001).

The study medication was produced by the Chao Center for Industrial Pharmacy & Contract Manufacturing, USA (Cycloserine Capsules®, 175 mg). Production of DCS and placebo capsules were carried out by the pharmacy at the University of Heidelberg. The study physician carried out balancing and ensured double-blind procedures.

2.3 Study Design

2.3.1 General overview

The data was acquired following a randomized double-blind, placebo-controlled, within-subject, crossover study design. All randomized factors, including treatment, list order, versions of control tasks and documentaries were performed in a balanced order. Each participant was required to appear at the sleep laboratory on three separate days. After selection of potential participants, a one-hour meeting was conducted. Inclusion criteria were assessed and necessary forms were filled in and signed as specified above (section 2.1.2). If all criteria for the experiment were met, two further appointments were arranged. The experiment comprised two sessions with a duration of 24 hours each, scheduled at least 14 days apart. Both sessions were identical but for the randomized factors.

The memory tasks, control tests and questionnaires were conducted according to a detailed protocol which always followed the same template (appendix 2). The exact time of the beginning of each new task and the time of ingestion of the DCS or placebo capsule were registered in the protocol.

2.3.2 Experimental Procedure in detail

The experiments began at 20:30 o'clock with the first test interval. Participant data concerning the experimental and previous day was elicited via a first questionnaire (appendix 3). After assertion that the answers to the questionnaire included no exclusion criteria for the present execution of the experiment the PAL was initiated. Two lists of each 40 word pairs of semantically related words were learnt and recalled. The learning

and recall of PAL were executed until a criterion of 60% was reached. Next, the psychomotor vigilance task (PVT) was conducted. Concluding the first test interval the participant filled out the second questionnaire with self-report measures on sleepiness and mood. At 22:30 the participant received placebo or 175mg DCS. Then the participant stayed awake during a retention interval of 20 hours, which represents approximately twice the biological half-life of DCS (Zhu et al., 2001). The participants were instructed to refrain from active rehearsal during the retention interval. They were constantly supervised to prevent unintentional sleep. In the morning, 8,5 hours after DCS ingestion the participants filled out the third questionnaire (self-report measures on sleepiness and mood). At 18:30 the second test interval with a duration of approximately two hours was initiated. It began with the new PAL. The participant learnt two new word pair lists of each 40 word pairs, one of the two lists being an interference list (see section 2.3.3). The new word pair lists were learnt and recalled three consecutive times. Then a recall session for the initially learnt word pairs from the previous evening was conducted. This was followed by the encoding and recall of numbers, a word fluency task (WFT), an autobiographic interview, the PVT and the fourth questionnaire (self-report measures on sleepiness and mood). At the end of the session the participants received a last questionnaire in which they were asked whether they believed they had received placebo or DCS. Figure 2 summarises the study design.

Figure 2 Study Design

2.3.3 Memoy tasks

The declarative paired association task in both test intervals required learning of two lists with each 40 semantically related word pairs. During learning the two lists were consecutively shown on a computer screen, each word pair was shown for 4 seconds, separated by an interstimulus interval of 1 second. After the presentation of both lists recall performance was measured with a cued recall procedure. The signal word was shown on the screen and the participant had to name the target word. The investigator

sat behind the participant and marked if the correct, wrong, or interference word was named and continued to the next signal word on the screen.
In the first test interval (initial PAL) learning and recall were repeated up to 5 times until at least 60% correct answers were reached for each list. In 2nd - 5th learning the word pairs were only shown for 3 seconds each. If only one list failed the criterion, only the according list was repeated. Dependent on the necessary number of PAL repetitions the session lasted 50-100 minutes.
In the learning task on the second evening (new PAL) one of the word pair lists constituted an interference list. The interference list showed identical signal words as one of the lists from the first test interval combined with new target words.
Learning and recall of both lists was repeated three consecutive times independent of recall performance. As during the first test interval in the first learning session, the word pairs were shown for 4 seconds. In the following learning sessions the word pairs were shown for 3 seconds.
After the learning and recall of new PAL was completed, the cued recall procedure as described above was executed for initial PAL.

2.3.4 Control measures – vigilance, sleepiness, mood ratings and test of Encoding

In questionnaires two, three and four sleepiness and mood were assessed using self-report measures. These included the stanford sleepiness scale (SSS; Hoddes et al., 1973).and the multidimensional mood questionnaire (MDMQ; Hinz et al., 2012).
Vigilance was assessed by a 5-min version of the PVT (Dinges et al., 1997). In this task the mean reaction time was measured. The participant was instructed to press a button as fast as possible as soon as a bright millisecond clock appeared and started counting upwards on a computer screen. After the button was pressed, the reaction time was shortly displayed. In order to test if there were general differences in LTM retrieval performance a word fluency task (WFT) was executed (Aschenbrenner et al., 2000). The Participants were instructed to generate as many words as possible belonging to a defined category (hobby or profession) or beginning with a certain letter (P or M) within two minutes. A numbers learning task, the ‘Eigenschaftswörterliste’ (Janke and Debus, 1978), and a standardised autobiographic interview were also performed in this

study**.** This ensured that the study protocol would be as similar as possible to the related sleep study (Schwidetzky, personal communication) for better comparability. Because these data offered no objective measurements relevant to the interpretation of the study goals, they have not been evaluated. At the end of the session the participants were asked if they believed to have received the active agent or placebo.

2.3.5 Interval between the experimental sessions

In order to ensure that the participants would not sleep between first and second test interval, the participants were continuously supervised by the investigator or a scientific assistant following standardised protocols. After ingestion of DCS or placebo at 22:30 o'clock the scientific assistant conducted a night-time protocol over the duration of eight hours (appendix 4). At 06:30 o'clock the day-time protocol (appendix 5) was initiated and continued by the investigator until the beginning of the second experimental session at 18:30 o'clock. The protocols included periods of documentary watching, intermittent walks on the premises of the Tübingen University Hospital, and defined periods for standardised meals and snacks.

2.3.6 Shifts of the experimental protocol / double-experiments

Due to external constraints I was forced to alter the initial protocol in order to ensure the timely completion of the study. As the timetable of the first protocol did not permit the simultaneous performance of two experimental sessions, a new protocol was elaborated. In this adjusted protocol the complete test intervals were shifted either 45 minutes forwards or 30 minutes backwards. The resulting 1 hour and 15 minutes constitute the minimal possible time interval, still allowing the conduction of the PAL as crucial task by the same investigator for every participant.

The execution of the night-time protocol was equally shifted. In this manner during the peak of DCS-blood-concentration ~2 hours after ingestion the meals were delivered to each participant with the same time-lag to DCS ingestion.

2.4 Data reduction and statistical analysis

2.4.1 Sample size

The study was initially planned for 20 participants. The experiment was eventually conducted with 24 participants in total. In three cases the experiments were not concluded. In one the study was terminated because of a mistake in the execution of the study-protocol. In the second case the participant had given false information concerning his prior participation in studies including memory tasks. In the third case the participant discontinued the experiment because of the onset of headache. It was subsequently noted, that this had occurred during the placebo session. Thus, the headache could not be an adverse effect of DCS as none had been administered. Having acquired 19 sets of evaluable data, it was decided to continue the experiment with two more participants to ensure a timely conclusion. As there was no further drop out the final number of evaluable data reached 21.

2.4.2 Statistical Analysis

Statistical analysis was conducted employing the analysis of variance with the general linear model (GLM) using the “Statistical Package for Social Sciences Statistics” (IBM Corp. SPSS Statistics) version 21.0.0. For the primary target-variable new PAL a three-way analysis of variance (ANOVA) was used, including the repeated measures for the within-subject factors treatment (DCS vs placebo), list (interference vs no-interference) and timepoint (1 vs 2 vs 3). Hereby the timepoints constitute the three successive recall sessions during the new learning phase. For the secondary target-variable, the consolidation of initial PAL, a two-way ANOVA was carried out using the differences of recall performance on the second evening in comparison to the performance on the first evening. Here the repeated measures for the within-subject factors treatment and list were included. Greenhouse-geisser corrections of degrees of freedom were applied where necessary. Interaction trends were followed up by lower level ANOVAs or t-tests. Control measures were evaluated via t-tests. For all tests the significance-level is defined at $p \leq 0.05$.

3 Results

The main target of this study was to assess if DCS would affect the freeing up of capacity for new learning following initial learning of similar content (see section 1.5). To this end I performed an initial PAL followed 20h later by a new PAL, where the new PAL performance was assessed to measure new learning. Participants stayed awake during the entire timespan. DCS was administered shortly after the initial PAL, such that plasma DCS should be negligible during new PAL, precluding direct DCS effects on new learning and thus isolating the indirect long-term effect of interest. Accessing the indirect DCS effect on new PAL, allows to infer a direct DCS effect on consolidation of initial PAL. New PAL was performed with either the same signal words as the initial PAL (interference list) or new signal words (no interference list), and both lists were learned and recalled three consecutive times (time points).

3.1 New learning, PAL

Over all pooled time points new learning was enhanced by DCS (treatment: $F_{(1,20)} = 5.218$, $p = 0.033$). As expected, performance increased across the three time points (time point: $F_{(1,20)} = 174.362$, $p = 0.000$), which represent the three consecutive times of learning and recall of the same contents. There is an overall trend for an interaction of treatment and timepoint (treatment*time point: $F_{(1,20)} = 2.818$, $p = 0.085$). A separate repeated-measures ANOVA was executed for each time point. At time point one no significant treatment effect could be seen ($treatment_{(timepoint1)}$: $F_{(1,20)} = 1.969$, $p = 0.176$). A significant effect of DCS on new learning could be seen in the second and third time point (treatment(timepoint2): $F(1,20) = 8.295$, $p = 0.009$; treatment(timepoint3): $F(1,20) = 4.420$, $p = 0.048$; Alizadeh Asfestani et al., 2018), which accounts for the treatment x timepoint interaction trend and also for the reported overall effect of DCS (see figure 3; table 1).

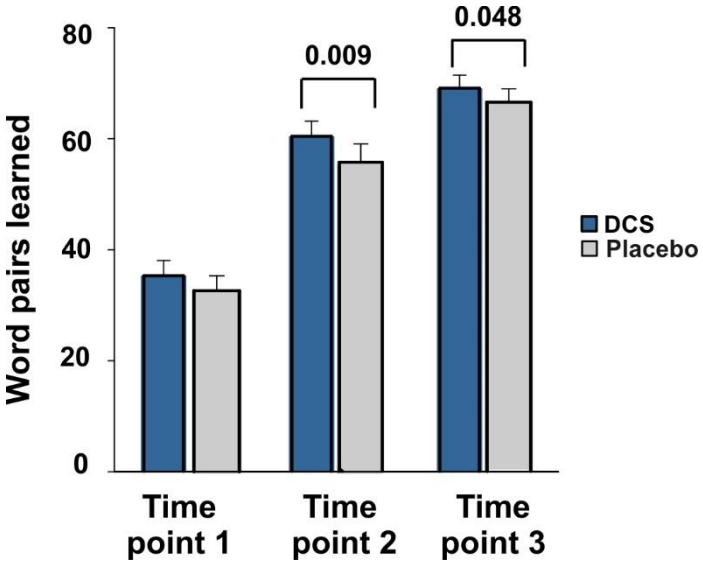

Figure 3 New learning performance by timepoint and condition, pooled over both lists: *Mean and standard error of the mean (SEM) of the amount of correctly recalled word pairs during new PAL for time point 1, 2 and 3 respectively, with the relevant significance-levels (numbers above bars). Adapted from Alizadeh Asfestani et al. (2018).*

Table 1 New learning performance by timepoint, condition and list*: Mean (SEM) of correctly recalled word pairs in the new learning phase for the interference and no-interference list, for DCS and placebo condition and for the separate time points (Alizadeh Asfestani et al., 2018).*

	D-Cycloserine		Placebo	
Interference				
Time point 1	19.38	(1.32)	16.67	(1.49)
Time point 2	30.76	(1.25)	27.57	(1.57)
Time point 3	35.10	(1.13)	33.00	(1.22)
No-interference				
Time point 1	15.95	(1.61)	16.00	(1.62)
Time point 2	29.67	(1.62)	28.19	(1.97)
Time point 3	34.00	(1.34)	33.57	(1.26)

Overall our results suggest a significant DCS effect which appears to be driven by the interference list. Firstly while the interaction effect shows no formal significance, it was

strongly suggestive of an increased DCS effect for the interference list (treatment*interference: $F_{(1,20)} = 3.269$, $p = 0.086$). Secondly there were significant effects in the post hoc tests. The interference list showed a better performance over the no-interference list in DCS condition, but not placebo condition ($t_{(20)} = 2.573$, $p = 0.018$, $t_{(20)} = -0.172$, $p = 0.865$ for DCS and placebo respectively; post-hoc test; see figure 5; table 2).

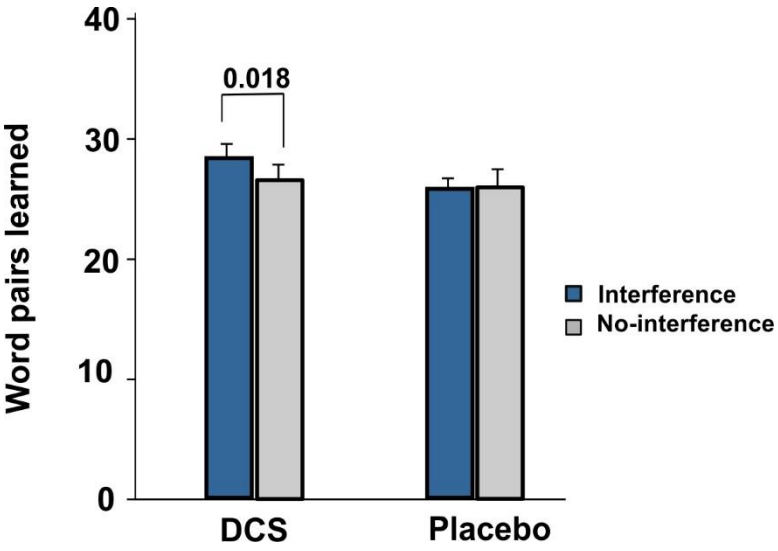

Figure 4 New learning by list and condition: *Mean number of word pairs learned for new learning of the interference and no-interference list and for DCS and placebo condition respectively, with the relevant significance level (number above bar).*

Table 2 New learning by list and condition*: Mean and SEM*

	DCS		Placebo	
Interference	28.41	(1,12)	25,75	(1,31)
No-interference	26.54	(1,41)	25.92	(1,55)

Regarding the number of learned word pairs and necessary trials to reach the criterion during learning, no significant differences between placebo and DCS conditions were found (all $p \geq 0.833$). This reaffirms that no random differences in performances influenced our results.

3.2 Consolidation of initial PAL

In order to replicate the previous results by Feld et al. (2013) where DCS applied during consolidation phase in a sleep deprivation setting did not affect later recall, I carried out a recall session of initial PAL after the new learning task.

As expected, treatment showed no significant effect on recall of initial PAL. The recall performance for the interference list was significantly lower than for the no-interference list ($F_{(1,20)} = 13.078$, $p = 0.002$, see table 6 and 7). This could easily be explained by proactive interference from the new PAL. An interaction was not seen ($F_{(1,20)} = 0.059$, $p = 0.811$).

Table 3 Consolidation of old Word Pairs: *Mean (SEM)*

	DCS		Placebo	
Interference	-8.43	(0.70)	-8.43	(1.02)
No-interference	-5.67	(0.77)	-6.00	(0.68)

3.3 Control Measures

Controls for vigilance, general retrieval performance, sleepiness and mood were performed, in order to ensure that the measured effects are due to DCS effects. These controls are particularly important in our setting, since sleep deprivation could strongly and variably affect performance by itself.

All control measures refer to differences between the two treatment conditions (DCS vs placebo) and were performed after each PAL learning session (initial PAL and new PAL). The standford sleepiness scale (SSS) and multdimensional mood questionnaires (MDMQ), used to assess the subjective measures of sleepiness or mood, were additionally executed in the morning, approximately 8,5 hours after treatment.

The SSS did not notably differ between conditions at any time (all $p \geq 0.167$).

No significant differences between the two conditions for mood, tiredness and calmness were found, as was accessed with the MDMQ. However, in the morning after DCS ingestion, the participants showed a trend for higher good mood and less tiredness in comparison to placebo (tiredness: $t(20) = 1.910$, $p = 0.071$; good mood: $t(20) = 1.805$, $p = 0.086$; Alizadeh Asfestani et al., 2018; see table 8). General vigilance was assessed via a PVT. It showed no differences between conditions at any time (all $p \geq 0.196$). In order to test if there were general differences in retrieval performance the WFT was executed. In this word generation task no significant differences in performance between the two conditions was found (all $p \geq 0.593$). Additionally to reaffirming that no random differences of general retrieval performance affect our results, this confirms that there is no increased performance due to residual DCS in the treatment condition.

At the end of each experiment the participants were asked to estimate which session they had just completed (DCS vs. placebo) in order to ensure that blinding was effective. The data suggest that participants were not able to discriminate between DCS and placebo condition (McNemars' exact test: $p \geq 0{,}774$).

Overall the results of our control measures suggest that the seen effect is not influenced by random factors or adverse drug effects, thereby affirming that the detected performance differences for new learning are DCS-induced.

Table 4 Control measures: *Mean (SEM). Alizadeh Asfestani et al. (2018)*

	D-cycloserine		Placebo	
Stanford Sleepiness Scale				
Learning	2.81	(0.25)	2.95	(0.26)
Morning	5.05	(0.23)	5.38	(0.22)
Retrieval	5.43	(0.28)	5.14	(0.29)
Psychomotor vigilance task				
Learning	3.73	(0.09)	3.74	(0.09)
Retrieval	3.60	(0.09)	3.55	(0.08)
Multidimensional mood questionnaire				
Initial learning				
Positive mood	17.29	(0.44)	17.05	(0.41)
Tiredness	13.90	(0.64)	13.14	(0.60)
Calmness	16.00	(0.56)	15.57	(0.62)
Morning				
Positive mood	13.19	(0.65)	11.76	(0.73)
Tiredness	8.19	(0.74)	6.90	(0.64)
Calmness	13.19	(0.68)	12.67	(0.70)
New learning				
Positive mood	13.48	(0.75)	13.24	(0.70)
Tiredness	7.00	(0.69)	6.67	(0.64)
Calmness	12.00	(0.75)	12.24	(0.69)
Word fluency task				
Learning	16.71	(0.98)	17.24	(0.98)
Retrieval	15.14	(1.14)	15.67	(0.79)

4 Discussion

4.1 Main results

In this study I have demonstrated that the partial NMDA receptor agonist DCS administered during the consolidation phase of an initial declarative learning task, does not impede subsequent learning of similar content in a sleep deprivation setting. This result confirms the proposed hypothesis of unimpeded new learning (section 1.5.1). Unexpectedly there was in fact a significant improvement in new learning after DCS administration in a wake setting. Though statistically insignificant, our results showed a trend for the interaction of treatment and list (interference vs no-interference), suggesting that the enhancing DCS effect on subsequent learning may be driven by the interference list.

Further, I found that the recall of initial PAL was not influenced by DCS in the wake setting, in line with the previous results by Feld et al. (2013). The recall of the no-interference list was significantly better than the recall of the interference list for the initial PAL, which I attribute to proactive interference from the main task (new PAL).

In the following I will first review the evidence supporting the theoretical account underlying our initial hypothesis. I will then critically discuss this account in light of the enhanced memory performance found here as well as in a related study in a physiological sleep setting with otherwise identical methodology (Alizadeh Asfestani et al., 2018).

4.2 Experimental evidence for the initial hypothesis

Since the idea was first proposed by Hebb et al. (1949) synaptic plasticity has been well established as substrate for associative memory (see sections 1.2.1 and 1.3). On a neurophysiological level LTP and LTD have been strongly implicated in the enhancement or reduction of synaptic strength respectively (see section 1.3.2). NMDA receptors have been shown to play a crucial role for LTP and LTD (Collingridge and

Bliss, 1987, Collingridge et al., 1983). The manipulation of these receptors by agonistic or antagonistic agents has been demonstrated to induce or reduce LTP and LTD (Billard and Rouaud, 2007, Villarreal et al., 2002) and to influence memory on a behavioural level (Kochlamazashvili et al., 2012, Gais et al., 2008). In the current study I manipulated the NMDA receptor by the partial agonist DCS. Animal experiments demonstrated the enhancing effect of DCS on memory in rodents and rabbits (Monahan et al., 1989, Flood et al., 1992, Thompson et al., 1992). In 2010 Onur et al. demonstrated an enhancing effect of DCS on encoding in humans under physiological conditions including normal sleep patterns. Subsequently a study by Feld et al. (2013) investigated the influence of DCS on sleep dependent consolidation mechanisms by measuring subsequent recall performances after the administration of DCS. Importantly performance was measured approximately 22 hours after ingestion where DCS plasma levels should be negligible. They demonstrated an enhancement of recall performance by DCS if it was allowed to act during a physiological sleep cycle. However, the effect was not observed in a sleep deprivation setting. This is in line with our current results which showed no effect on consolidation of initial PAL in the wake setting.
Furthermore, I found a lowered recall performance for the interference list, which may easily be explained by proactive interference from the new PAL (Wixted, 2004).
Feld et al. (2013) suggested that the demonstrated enhancing effect of DCS on subsequent recall performance in their study was mediated by sleep dependent synaptic enhancement within the HC. In this case proactive interference on a subsequent task could be expected due to DCS mediated intrahippocampal enhancement, but this effect would not be expected in a wake setting.
This is consistent with the lack of a proactive interference effect, as found in the present study in a wake setting. Specifically, DCS did not reduce subsequent learning performance if administered in the consolidation phase during a wake interval.

It is important to note, that the above theoretical account, in its simplest form, is based on several assumptions:

1. DCS effects were thought to be mediated mostly by LTP, rather than LTD (section 4.2.1)
2. The HC has a limited learning capacity which is improved by sleep dependent renewal phases (section 4.2.2)
3. The effect of DCS within the first 24 hours was thought to rely mainly on intrahippocampal synaptic enhancement (see section 4.2.3)
4. The effect of DCS on consolidation was thought to be sleep dependent (see section 4.2.4)

These assumptions are supported by a wealth of evidence, which shall be reviewed in the remainder of this section.

4.2.1 LTP-specificity of DCS

A number of studies suggest that DCS primarily enhances LTP. Accordingly, I expected that DCS could lead to enhanced proactive interference in a subsequent task.
An animal study by Liu et al. (2004) indicates that NR2A receptors preferentially lead to LTP, while NR2B receptors are primarily engaged in the induction of LTD.
The selective blocking of NR2A receptors prevented the induction of LTP but not LTD, while the selective blocking of NR2B receptors abolished the induction of LTD without affecting LTP. Furthermore, an animal study by Sachser et al. (2016) demonstrated the blocking of LTP decay by infusion of a selective NR2B receptor antagonist.
As DCS has previously been shown to preferentially act on NR2A receptors (Kochlamazashvili et al., 2012, Sheinin et al., 2001, Feld et al., 2013), it appears reasonable to expect selective LTP induction. Further studies imply that ambient concentrations of glycine and d-serine are sufficient to saturate NR2B receptors but not NR2A receptors (Kutsuwada et al., 1992, Kochlamazashvili et al., 2012). This might explain why DCS as a partial agonist for the glycine binding site preferably activates NR2A receptors. In the study by Feld (2013), DCS applied during the consolidation phase of an initial task led to sleep dependent enhancement of subsequent recall. The inhibition of NMDA receptors via caroverine and ketamine was expected to lower subsequent recall performance. This however was not observed. Feld et al. suggested that DCS enhances LTP but not LTD, while ketamine and caroverine inhibit LTP and LTD similarly.

4.2.2 Capacity limit for HC-dependent learning

HC-dependent new learning of declarative information has been shown to benefit from prior sleep (Van Der Werf et al., 2009, Antonenko et al., 2013). This is attributed to a capacity limit of HC-dependent learning, which is alleviated if new learning is preceded by a sleep dependent renewal phase. According to the initial hypothesis, DCS should sleep dependently enhance intrahippocampal LTP, thus potentially interfering with this renewal phase including synaptic depotentiation. This was expected to enhance proactive interference and to lower subsequent memory performance.

A general capacity limit is already implied in interference theory. Retroactive interference by learning of new material has repeatedly been shown to induce forgetting, even if the new learning tasks are completely different from the originally learnt task (Wixted, 2004). If HC-dependent learning capacity were not limited, mental exertion should not induce forgetting of previously learnt material.

In the SHY (see section 1.4.7) the problem of synaptic saturation during wake is described. According to Tononi and Cirelli (2014) a limitation of synaptic strength is deemed necessary for effective new learning to be possible. They propose that homeostatic synaptic downregulation during sleep counteracts the saturating process, thereby enabling new learning.

A recent study explicitly addresses the question of a limited capacity of HC-dependent consolidation (Feld et al., 2016). If HC-capacity levels are exceeded, a capacity limit to this process should manifest itself in a limit to this sleep dependent benefit. The results of the study confirmed this capacity limit to sleep dependent consolidation benefit.

4.2.3 Intrahippocampal effect of DCS after 24 hours

Diverse studies have shown how new declarative memory traces gradually become independent of the HC, and how a hippocampo-neocortical dialogue is necessary for this process (see section 1.4.5). How fast the neocortical traces become strengthened and the hippocampal traces decay is still subject to debate, as is also reflected by the generally indicated wide time range for system consolidation (see section 1.2.3). Lesion studies indicate a range of weeks to years before a memory trace becomes independent of the HC (Zola-Morgan et al., 1986). Diverse studies show that one day after learning

the HC and intrahippocampal reactivations still play a central role. Thus, Bosshardt et al. (2005), using fMRI, demonstrated enhancement in the left HC at 24 hours but not 10 minutes after a memory task. He attributes this effect to a strengthening of synaptic connections in the HC within the first 24 hours. A strong gene expression in the HC one day but not 30 days after learning was found by Maviel et al. (2004) in an animal study. In the study by Feld et al. (2013) showing a sleep dependent increase of recall performance by DCS a similar time range was applied. This suggests that sleep dependent enhancement of subsequent retrieval performance was caused by strengthening of intrahippocampal connections by DCS.

This is corroborated by the results of a study by Gais et al. (2007), using similar word pair tasks in sleep and wake conditions. After the memory task the participants either slept for two nights (sleep condition) or were sleep deprived for one night and slept in the following night (wake condition). Using fMRI he demonstrates sleep dependent activity changes in different brain regions. Directly after learning no difference in brain responses were found between conditions, but at retesting 48 hours later a higher activity in the right HC was found in the sleep condition. This also indicates that in our word pair task sleep dependent consolidation mechanisms within the first two nights of post learning sleep should preferentially lead to an enhancement of intrahippocampal memory-traces. Furthermore, the demonstrated enhancing effect of DCS on encoding in the study by Onur et al. (2010) was accompanied by elevated activity in the right HC, measured by fMRI. A further study by Gais et al. (2008) demonstrated an effect of the NMDA receptor inhibitors caroverine and ketamine on a procedural visual texture discrimination task. Procedural memory is not affected by hippocampal lesions and is thereby thought to be mainly HC-independent (Corkin, 1968, Cohen et al., 1985).

In the study by Feld et al. (2013) a DCS induced effect was demonstrated in a declarative memory task. A procedural task however showed no difference between treatment groups. Therefore, DCS appeared to selectively affect only the declarative and thus HC-dependent tasks, again suggesting an intrahippocampal mechanism of action.

4.2.4 Sleep dependency of DCS-induced performance changes

In previous studies DCS-induced gains in performance were only present in sleep but not wake conditions implying that they rely on sleep dependent processes (Feld et al.,

2013, Gais et al., 2007). If the described DCS-induced effect on consolidation mechanisms is thought to impede subsequent learning, this effect should also be confined to the sleep setting.

4.3 DCS effect on subsequent learning after wake or sleep

Here I demonstrated, in line with the initial theoretical account, that new learning was not diminished after DCS administration during consolidation in a wake setting. However, I found an enhanced performance for new learning which may be driven by the interference list. Since the previous account of DCS action would arguably be most consistent with a null effect, this suggests DCS action may be more complex. Furthermore, while the present study addressed a sleep deprivation setting, a related study tested the effects of DCS on subsequent learning without sleep deprivation (Schwidetzky, personal communication). This study was otherwise methodologically identical to our study, facilitating a joint interpretation.

According, to our initial account (section 4.2), post-learning administration of DCS during an interval containing sleep should have led to increased proactive interference and decreased new learning. However, the related sleep study also showed an increase in performance for new learning (Alizadeh Asfestani et al., 2018).

The unexpected similar results for the DCS effect on subsequent learning in both sleep and wake setting, imply that the underlying mechanisms may be independent of sleep. An additional possibility is that the enhancement in the sleep condition was caused by a different mechanism then the enhancement in the wake condition. In this context it is important to note that a direct memory enhancing effect during new learning by residual DCS cannot be ruled out completely. However, given the pharmacokinetics of DCS, I deem this possibility unlikely. Together, these findings suggest that DCS action may be more complex than initially assumed, and our initial account needs to be revised.

4.4 Integration and explanation of the results

4.4.1 The action of DCS on LTP and LTD

According to the initial account DCS was assumed to selectively induces LTP (section 4.2.1). Our results however show enhanced learning performance after DCS, indicating that DCS applied during consolidation reduces proactive interference. A possible mechanism for this would be if DCS enhances LTD rather than LTP. Indeed, there is substantial evidence for a direct effect of DCS on LTD. For instance, Billard and Rouaud (2007) showed that DCS rescued LTP and LTD in mice with age-related impairment of NMDA receptor mediated plasticity. More recently a study by Kochlamazashvili et al. (2012) showed how the inhibition of NR2A and NR2B receptors may each separately inhibit the induction of LTD, thereby indicating that both receptors are necessary for LTD induction. Therefore, DCS may mediate LTD induction even if it selectively acts on NR2A receptors. Villarreal et al. (2002) showed that blocking NMDA receptors impaired forgetting of spatial memory in rats. This implies that NMDA receptors play a crucial role in forgetting and synaptic downregulation. Finally Hardt et al. (2013) argues, that because of the efficient pattern separation ability of the HC, interference is unlikely to account for forgetting in this brain region. Thus, NMDA receptor mediated active decay may be the main form of forgetting in the HC. Together, this suggests that DCS mediated activation of NMDA receptors may be able to enhance forgetting and synaptic downregulation, thereby reducing proactive interference and supporting new learning.

4.4.2 Limited learning capacity of the HC

The first assumption of a limited HC-dependent learning capacity can easily be integrated with the current results. As elaborated above, DCS may enhance new learning performance by inducing LTD, thereby lowering proactive interference with a subsequent task (section 4.4.1). This effect could not occur if hippocampal learning capacity were not limited and dependent on renewal phases.

4.4.3 Intrahippocampal enhancement vs system consolidation. The influence of sleep.

According to our initial account the DCS effect on consolidation, leading to subsequent better recall, should be due to intrahippocampal synaptic enhancement during sleep. However, this was expected to reduce new learning capacity rather than strengthening it. By contrast, DCS enhanced subsequent learning after an interval containing sleep, similar to the present findings in a sleep deprivation setting. A possible explanation for these results may be found in the SAM (Tse et al., 2007; see section 1.4.4). According to the SAM, a system consolidation process can be concluded within 24 – 48 hours if pre-existing schemata are available. This is corroborated by an fMRI study by Takashima et al. (2009). 24 hours after learning of an association task a decrease in hippocampal activity and an increase of neocortical activity is shown, in comparison to 15 minutes after the task. Accordingly, DCS may have enhanced the integration of initial memory into pre-existing neocortical schemata indirectly supporting the freeing of capacity within the HC (see section 1.4.4). Furthermore, in a study by Debiec et al. (2002) consolidated memory traces were shown to again become HC-dependent after reactivation. HC-dependent reconsolidation then took place much faster than initial consolidation. DCS has previously also been shown to enhance reconsolidation (Lee et al., 2006). Thus, in previous studies using similar memory tasks (e.g. Gais et al., 2008), the hippocampal activity change shortly after encoding could have been the manifestation of reconsolidation rather than primary consolidation, and the DCS effect could similarly have been an effect on reconsolidation.

Cued recall paradigms, as used in the current study, are thought to rely mainly on the formation of new associative connections, probing the hippocampal contribution to memory formation (Squire et al., 2007, Antonenko et al., 2013). It has however been suggested, that arbitrary word pairs would be optimal to test such a contribution (Graf and Schacter, 1985, McClelland et al., 1995). It cannot be ruled out completely that semantically related word pairs, as used in the current study, may have sufficient pre-existing memory representations for fast schema assimilation to take place. In further experimentation it might be interesting to evaluate if the use of arbitrary word pairs would lead to different results.

If fast system consolidation takes place, this may be accompanied by lower sleep dependency (Tse et al., 2007). SWR which are thought to involve mechanisms of information transfer from HC to NC are seen in SWS and quiet wakefulness (McClelland et al., 1995, O'Neill et al., 2010, Inostroza and Born, 2013). This hints towards a type of HC-dependent system consolidation during wakefulness. Notably, it was suggested that the high acetylcholine levels during active wakefulness would prevent the information transfer. However, in quiet wakefulness lowered acetylcholine levels compatible with system consolidation have been described (Hasselmo, 1999, Inostroza and Born, 2013). Considering the results by Feld et al. (2013) showing no DCS effect on consolidation in the wake setting, even if fast system consolidation plays a role, the process must be at least partially sleep dependent. However, the current results suggest that sleep independent processes, such as simple time dependence, may additionally contribute to this process.

If DCS supported schema formation or reconsolidation and reinforcement of schemata relating to the initially learnt word pairs, there may have been a strengthened neocortical schema available during new learning. This would allow enhanced new learning of schema related words (i.e. the interference list). As enhanced recall performance after DCS ingestion during consolidation was only apparent in the sleep setting, enhanced learning of the interference list should also be expected mainly in this behavioural state. Although this effect was not observed in the related sleep study (Schwidetzky, personal communication) and not statistically significant in the present study, I found a trend for enhanced learning of the interference list after DCS administration.

4.4.4 Interference and labilisation

It is also possible that the effect of DCS is not as sleep dependent as was initially considered, but that ongoing learning during wakefulness can obscure its effects.
As was shown previously DCS enhances encoding assumedly by LTP induction (Onur et al., 2010). DCS should then be expected to enhance LTP induction by new incoming stimuli in a wake setting, which would enhance retroactive interference on the consolidating memory. In the study by Feld et al. (2013), this may have counteracted

the concurrent enhancing effect of DCS on consolidation, so that the enhancement of recall performance only became apparent in the sleep setting.
In accordance with this, Villarreal et al. (2002) find that the application of an NMDA receptor antagonist preserved previously induced LTP and on a behavioural level lead to subsequent higher performance during recall. Enhanced recall of previously learnt material has also been described for anterograde amnesic agents such as alcohol or benzodiazepines (Wixted, 2004). As explanation for this observation Wixted (2004) suggests that NMDA receptor antagonists and the amnesic agents protect previously induced LTP from retroactive interference by impeding new LTP induction. He refers to this effect as retrograde facilitation.
Furthermore, reactivation during wake has been shown to induce labilisation of a memory trace (Diekelmann et al., 2011, Misanin et al., 1968, Przybyslawski and Sara, 1997). The application of an NMDA receptor antagonist prior to recall prevented the labilisation of the reactivated memory (Ben Mamou et al., 2006). The application of an NMDA receptor agonist should show the opposite effect, thereby indicating that DCS might enhance labilisation of previously learnt material, if reactivated. Notably, the present study was designed to minimize the chance of PAL reactivations. Thus, no written words were demonstrated to the participants until the second test interval, where DCS levels were assumedly negligible. Furthermore, participants were asked to refrain from active rehearsal. However, I cannot strictly exclude labilisation effects.
Overall, and as acknowledged by Feld et al. (2013), DCS induced enhanced interference and labilisation may account for the missing measurable effect of DCS on consolidation in the wake setting.

4.4.5 Influence of behavioural state and previous synaptic activity

Another intriguing possibility is that the behavioural state influenced the dynamics and proportions to which LTP and LTD were induced. If the mechanisms by which DCS enhanced LTP were sleep dependent while mechanisms enhancing LTD were time dependent, this would lead to the reported pattern of sleep dependent enhancement of consolidation and sleep independent enhancement of new learning performance. LTP enhancing effects of DCS, confined to sleep, could include homeostatic processes or active consolidation processes. The latter could e.g. occur in the context of sleep

dependent reactivations during active system consolidation. In fact, Feld et al. (2013) already noted, that DCS selectively enhanced memory traces that rely on hippocampal reactivations during sleep.

Notably, the effect of DCS on LTP or LTD as described in the SAM account (section 4.4.3) primarily considers potentiation or depression of the previously activated synapses or cell assemblies and is agnostic towards general homeostatic effects. The possibility that LTD induction was enhanced via DCS independent of prior activation by the task, thereby augmenting a homeostatic process of synaptic downregulation, may be considered. LTP enhancement by DCS may on the other hand depend on the reactivation of synapses during the sleep dependent hippocampo-neocortical dialogue. In this approach the direction of the DCS effect is not thought to depend on different localisations (NC vs HC), but rather on the physiological state and activity of the synapses which it encounters. This approach seems intriguing when considering that the activation of NMDA receptors may lead to LTD and that stronger activation at the same receptors may lead to LTP induction (section 1.3.3). Notably, the direction of plasticity seems to depend on the prior activity of the involved NMDA receptors (Huang et al., 1992). Diverse studies and accounts indicate an LTP induction during active system consolidation processes (Buzsaki, 1989, Laroche et al., 1990, Diekelmann and Born, 2010, Chauvette et al., 2012). In synapses and NMDA receptors that are already activated e.g. during the hippocampo-neocortical reverberations, possibly the agonistic effect at the NMDA receptors induce or prolong LTP. The previously described DCS induced enhancement of later recall could then rely on strengthened neocortical synapses as proposed in the SAM, but also by enhanced intrahippocampal synapses as initially proposed by Feld et al. (2013).

NMDA receptor activation alone does not suffice to induce LTP (Bliss and Collingridge, 1993). Thus, in synapses which are not close to or over the threshold of LTP induction, the presence of DCS may not suffice to activate the NMDA receptors sufficiently to produce LTP. Levels of NMDA receptor activation insufficient for LTP induction have been shown to even impair the ability to express LTP (Coan et al., 1989, Huang et al., 1992, Bliss and Collingridge, 1993). DCS may have augmented a homeostatic process of synaptic downregulation, independent of the behavioural state (sleep vs wake). Note that the synaptic homeostasis hypothesis postulates that sleep

rather favours LTD and wake favours LTP. However, according to Chauvette et al. (2012) SWS mediates an upregulation of excitatory postsynaptic potentials, rather than the downregulation postulated by the SHY (see also Born and Feld, 2012). Hengen et al. (2016) show how synaptic firing returns to a cell-autonomous set point during wake, while this effect is missing in the sleep setting. This indicates, that there must be processes of synaptic homeostasis that occur in wakefulness.
Currently it is difficult to infer if the direction of the DCS effect is dependent on prior synaptic activity, but it offers an intriguing possibility to explain a sleep dependency of LTP enhancement by DCS. However, other, possibly homeostatic processes confined to sleep may alternatively or additionally play a role.
An elucidation of the influence of prior synaptic activity on the direction of the DCS effects would help integrating and interpreting our current results.

4.4.6 Differing mechanisms in wake and sleep setting

It cannot be excluded that the DCS effects in sleep and wake setting were elicited by differing mechanisms. The better performance under DCS in wake condition could e.g. be exclusively due to LTP induction. An LTP-induced enhanced labilisation of the memory-traces, together with LTP-related enhancement of new interfering material may have led to forgetting, thus lowering proactive interference in the new learning task (section 4.4.4). The enhancement of new learning in the sleep condition may in turn be due to enhanced schema assimilation including intrahippocampal synaptic downregulation via LTD (see section 4.4.3). The DCS effect on new learning in the wake setting could thus be LTP-dependent, while the enhancement of new learning in the sleep setting is mediated by LTD. The differing dynamics of new learning in sleep and wake conditions, respectively, would corroborate such a view of different underlying mechanisms. In the current study DCS enhanced new learning during the second and third timepoint, while the effect was only apparent in the first timepoint in the sleep setting (Alizadeh Asfestani et al., 2018). The three timepoints represent the three consecutive times of learning and recall.
However, I believe that a more likely explanation for these different dynamics is a saturation effect of learning in the sleep setting. Indeed, in the sleep setting performance was substantially higher overall (Alizadeh Asfestani et al., 2018).

4.5 Conclusion

Together, this suggests that DCS enhanced new learning by processes independent of the behavioural state. This implies that our initial account of sleep and hippocampus dependent DCS action on LTP needs to be refined, suggesting a number of directions for future research.

I consider a DCS induced enhancing effect on LTD or related processes of synaptic plasticity as the most likely mechanism underlying enhanced new learning in the present study. This effect may be the physiological substrate of an enhancement of time dependent homeostatic synaptic depotentiation. This would corroborate accounts proposing sleep independent homeostatic processes of synaptic renormalisation (Hengen et al., 2016).

Irrespective of this and at the same time DCS may enhance LTP sleep dependently, e.g. by mechanisms that rely on the synaptic reactivations during active system consolidation, accounting for the findings by Feld et al. (2013). This proposition implicates that the direction of DCS effect may lead to LTD or LTP, respectively, dependent on the underlying physiological milieu. This is in line with various further studies and accounts. Thus, both LTP and LTD have been shown to depend on NMDA receptor activation (Malenka and Nicoll, 1999) and specifically DCS has been shown to affect both forms of plasticity (Billard and Rouaud, 2007). The direction of synaptic plasticity generally seems to be influenced by the previous and present receptor activity (Coan et al., 1989, Huang et al., 1992). Furthermore, diverse accounts indicate that active system consolidation processes may include LTP-induction in synapses implicated in prior learning (Buzsaki, 1989, Diekelmann and Born, 2010, Chauvette et al., 2012). This could, amongst others, be a prerequisite for LTP-enhancement by DCS.

Above, I have outlined multiple additional accounts which could accommodate both sleep independent enhancement of new learning and sleep dependent enhancement of consolidation. These include potential brain-region, synaptic-activity and modulatory-state dependent differences of DCS effects. While it is presently difficult to judge which of these accounts is most likely, I believe that further research will help to address this. Specifically, further experimentation elucidating the main locations of DCS action and the influence of prior synaptic activity on the direction of the DCS effects could help

integrating and interpreting our current results. It may e.g. be interesting to execute established protocols for LTP and LTD induction with and without concurrent DCS application and examine the effect on long-term plasticity processes.

5 Summary

Synaptic plasticity, specifically long-term potentiation (LTP) and long-term depression (LTD), are well-established correlates of memory processes. The manipulation of NMDA receptors, which are critically involved in these forms of synaptic plasticity, offer a promising and thus frequently used means to gain new insight into the mechanisms of memory. Here, I investigated if the application of the NMDA receptor coagonist d-cycloserine (DCS) during consolidation affected subsequent learning in a sleep deprivation setting. To this end I administered DCS (vs. placebo) shortly after an initial word pair association task and measured the effect on subsequent learning of a similar task after DCS had reached negligible plasma levels. Assessing the indirect DCS effect on subsequent learning, allows to infer the effect of DCS on consolidation of the initial task. I hypothesised that enhanced or reduced subsequent learning performance would result from enhanced or reduced proactive interference from the initial memory task. In the current study subsequent learning was enhanced by DCS, suggesting decreased proactive interference. Such decreased interference could be brought about if DCS enhanced LTD.

A related, methodologically identical study found similarly enhanced new learning under normal sleep settings. Together, this suggests that the LTD-enhancing effect of DCS is independent of the behavioural state (sleep vs wake). By contrast, an earlier study suggests that recall performance of the initial memory task is enhanced by DCS sleep dependently. Taken together these studies show the interesting pattern of sleep dependent enhancement of consolidation and sleep independent enhancement of subsequent learning. A possible explanation is, that DCS enhanced LTD, supporting homeostatic synaptic downregulation and thereby diminishing proactive interference with the subsequent task, independent of the behavioural state. This is in line with accounts proposing sleep independent synaptic homeostatic effects. In contrast, DCS-induced enhancement of LTP may rely on sleep dependent processes, such as sleep dependent synaptic reactivations during active system consolidation. This is in line with previous studies and accounts suggesting that NMDA receptor activation has the potential to induce LTP and LTD, respectively. Indeed, the direction of plasticity depends on a number of factors, such as the previous activity of the respective synapses,

subtypes of the targeted receptors, the modulatory state, patterns and frequencies of activation as well as cell-type and brain region. Further studies selectively addressing these different physiological background conditions in relation to pharmacological NMDA receptor activation would help putting the current results into context.

6 Zusammenfassung

Synaptische Plastizität, insbesondere Langzeitpotenzierung (LTP) und Langzeitdepression (LTD) stellen etablierte Korrelate für Gedächtnisprozesse dar. Die Manipulation von NMDA Rezeptoren, welche maßgeblich an diesen Plastizitätsprozessen beteiligt sind, stellt eine vielversprechende und deswegen viel genutzte Möglichkeit dar, um neue Erkenntnisse über Gedächtnisprozesse zu erlangen. Hier wurde erforscht, ob die Applikation des NMDA Rezeptor Koagonisten D-Cycloserin (DCS) während der Konsolidierungsphase unter Schlafentzug einen Einfluss auf nachfolgendes Lernen hat. Zu diesem Zweck, wurde DCS (vs Placebo) kurz nach Durchführung einer assoziativen Wortpaar-Gedächtnisaufgabe verabreicht. Daraufhin wurde der Effect auf nachfolgendes Lernen einer ähnlichen Aufgabe, nach Erreichen von vernachlässigbaren Plasmakonzentrationen, gemessen. Die Messung des Effektes von DCS auf nachfolgendes Lernen, erlaubt es uns Rückschlüsse auf die DCS Effekte während der vorherigen Konsolidierunsphase zu ziehen. Ich ging von der Annahme aus, dass verstärktes oder reduziertes nachfolgendes Lernen auf verstärkte oder reduzierte proaktive Interferenz durch die initiale Lernaufgabe zurückzuführen ist. Hier zeigte sich ein durch DCS signifikant erhöhtes nachfolgendes Lernen unter Schlafentzug. Dies weist auf eine verminderte proaktive Interferenz hin. Eine Verstärkung von LTD durch DCS könnte eine solche verminderte Interferenz verursachen.
Eine unter physiologischen Schlafbedingungen durchgeführte und ansonsten methodisch identische Studie zeigte ebenfalls eine Erhöhung des nachfolgenden Lernens durch DCS. Zusammengefasst deutet dies auf einen schlafunabhängigen LTD verstärkende Effekt hin. Eine frühere Studie zeigte eine schlafabhängige Verbesserung des nachfolgenden Abrufs der zuvor gelernten Gedächtnisaufgabe, nach Applikation in der Konsolidierunsphase. Zusammengenommen, zeigen diese Studien das spannende Muster einer schlafabhängigen Verstärkung der Konsolidierung des zuvor gelernten in Kombination mit einer schlafunabhängigen Verstärkung des nachfolgenden Lernens. Eine mögliche Erklärung ist, dass DCS schlafunabhängig LTD verstärkt und somit homeostatische Prozesse der synaptischen Herabregulation gestärkt hat. Dies könnte proaktive Interferenz vermindert haben. Diese Erklärung passt zu Ansätzen welche schlafunabhängige homeostatische Effekte annehmen.

Im Kontrast hierzu könnte eine LTP verstärkende Wirkung von DCS ausschließlich im Rahmen von schlafabhängigen Prozessen auftreten. Diese könnte z.B. von synaptischen Reaktivierungen während der aktiven Systemconsolidierung abhängen. Passend hierzu zeigen verschiedene Studien und Ansätze das Potential von NMDA Rezeptoren, LTP als auch LTD zu induzieren, auf. In der Tat, hängt die Richtung der synaptischen Platizität von diversen Faktoren ab. Hierzu gehören z.B. die vorherige synaptische Aktivität, Rezeptorsubtypen, Neuromodulatoren, Aktivitätsmuster und -frequenzen, Zellarten und Gehirnregionen.
Weiterer Studien, welche selektiv diese Einflussfaktoren in Relation zu pharmakologischer NMDA Rezeptoraktivierung erproben, könnten helfen die dargelegten Ergebnisse im Gesamtkontext klarer zu interpretieren.

7 List of publications

1. Alizadeh Asfestani, M., Braganza E., Schwidetzky, J., Santiago, J., Soekadar, S., Born, J. & Feld, G. 2018. Overnight memory consolidation facilitates rather than interferes with new learning of similar materials – a study probing NMDA receptors. *Neuropsychopharmacology*, 43, 2292 – 2298.

8 Literature / Reference List

ABRAHAM, W. C. & ROBINS, A. 2005. Memory retention - the synaptic stability versus plasticity dilemma. *Trends in Neurosciences,* 28**,** 73-78.

ALIZADEH ASFESTANI, M., BRAGANZA, E., SCHWIDETZKY, J., SANTIAGO, J., SOEKADAR, S., BORN, J. & FELD, G. B. 2018. Overnight memory consolidation facilitates rather than interferes with new learning of similar materials—a study probing NMDA receptors. *Neuropsychopharmacology,* 43**,** 2292-2298.

ALVAREZ, P. & SQUIRE, L. R. 1994. Memory Consolidation and the Medial Temporal-Lobe - a Simple Network Model. *Proceedings of the National Academy of Sciences of the United States of America,* 91**,** 7041-7045.

ANS, B. & ROUSSET, S. 1997. Avoiding catastrophic forgetting by coupling two reverberating neural networks. *Comptes Rendus De L Academie Des Sciences Serie III-Sciences De La Vie-Life Sciences,* 320**,** 989-997.

ANTONENKO, D., DIEKELMANN, S., OLSEN, C., BORN, J. & MOLLE, M. 2013. Napping to renew learning capacity: enhanced encoding after stimulation of sleep slow oscillations. *European Journal of Neuroscience,* 37**,** 1142-1151.

ASCHENBRENNER, S., TUCHA, O. & LANGE, K. W. 2000. *Regensburger Wortflüssigkeits-Test: RWT,* Verlag für Psychologie Hogrefe.

BADDELEY, A. 1992. Working memory. *Science,* 255**,** 556-559.

BADING, H., GINTY, D. D. & GREENBERG, M. E. 1993. Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. *Science,* 260**,** 181-6.

BAILEY, C. H. & CHEN, M. 1989. Time course of structural changes at identified sensory neuron synapses during long-term sensitization in Aplysia. *Journal of Neuroscience,* 9**,** 1774-80.

BARTLETT, F. C. 1932. *Remembering; a study in experimental and social psychology,* Cambridge, The University Press.

BATTAGLIA, F. P., BENCHENANE, K., SIROTA, A., PENNARTZ, C. M. & WIENER, S. I. 2011. The hippocampus: hub of brain network communication for memory. *Trends in Cognitive Sciences,* 15**,** 310-8.

BAZHENOV, M., TIMOFEEV, I., STERIADE, M. & SEJNOWSKI, T. J. 2002. Model of thalamocortical slow-wave sleep oscillations and transitions to activated states. *Journal of Neuroscience,* 22**,** 8691-8704.

BEN MAMOU, C., GAMACHE, K. & NADER, K. 2006. NMDA receptors are critical for unleashing consolidated auditory fear memories. *Nature Neuroscience,* 9**,** 1237-1239.

BERGERON, R., MEYER, T. M., COYLE, J. T. & GREENE, R. W. 1998. Modulation of N-methyl-D-aspartate receptor function by glycine transport. *Proceedings of the National Academy of Sciences of the United States of America,* 95**,** 15730-15734.

BILLARD, J. M. & ROUAUD, E. 2007. Deficit of NMDA receptor activation in CA1 hippocampal area of aged rats is rescued by D-cycloserine. *European Journal of Neuroscience,* 25**,** 2260-8.

BLISS, T. V. P. & COLLINGRIDGE, G. L. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature,* 361**,** 31-39.

BLISS, T. V. P. & LOMO, T. 1973. Long-Lasting Potentiation of Synaptic Transmission in Dentate Area of Anesthetized Rabbit Following Stimulation of Perforant Path. *Journal of Physiology-London,* 232**,** 331-356.
BORN, J. & FELD, G. B. 2012. Sleep to upscale, sleep to downscale: balancing homeostasis and plasticity. *Neuron,* 75**,** 933-5.
BORN, J. & WILHELM, I. 2012. System consolidation of memory during sleep. *Psychological Research-Psychologische Forschung,* 76**,** 192-203.
BOSSHARDT, S., SCHMIDT, C. F., JAERMANN, T., DEGONDA, N., BOESIGER, P., NITSCH, R. M., HOCK, C. & HENKE, K. 2005. Effects of memory consolidation on human hippocampal activity during retrieval. *Cortex,* 41**,** 486-498.
BUZSAKI, G. 1989. Two-stage model of memory trace formation: A role for "noisy" brain states. *Neuroscience,* 31**,** 551-570.
BUZSAKI, G. 1998. Memory consolidation during sleep: a neurophysiological perspective. *Journal of Sleep Research,* 7**,** 17-23.
CARPENTER, G. A. & GROSSBERG, S. 1988. The Art of Adaptive Pattern-Recognition by a Self-Organizing Neural Network. *Computer,* 21**,** 77-88.
CHAUVETTE, S., SEIGNEUR, J. & TIMOFEEV, I. 2012. Sleep Oscillations in the Thalamocortical System Induce Long-Term Neuronal Plasticity. *Neuron,* 75**,** 1105-1113.
COAN, E. J., IRVING, A. J. & COLLINGRIDGE, G. L. 1989. Low-frequency activation of the NMDA receptor system can prevent the induction of LTP. *Neuroscience Letters,* 105**,** 205-210.
COHEN, N. J., EICHENBAUM, H. & DEACEDO, B. S. 1985. Different Memory Systems Underlying Acquisition of Procedural and Declarative Knowledge. *Annals of the New York Academy of Sciences,* 444**,** 54-71.
COHEN, N. J. & SQUIRE, L. R. 1980. Preserved Learning and Retention of Pattern-Analyzing Skill in Amnesia - Dissociation of Knowing How and Knowing That. *Science,* 210**,** 207-210.
COLLINGRIDGE, G. L. & BLISS, T. V. P. 1987. Nmda Receptors - Their Role in Long-Term Potentiation. *Trends in Neurosciences,* 10**,** 288-293.
COLLINGRIDGE, G. L., KEHL, S. J. & MCLENNAN, H. 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *Journal of Physiology-London,* 334**,** 33-46.
CORKIN, S. 1968. Acquisition of Motor Skill after Bilateral Medial Temporal-Lobe Excision. *Neuropsychologia,* 6**,** 255-&.
COYLE, J. T. & TSAI, G. 2004. The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia. *Psychopharmacology,* 174**,** 32-38.
DEBIEC, J., LEDOUX, J. E. & NADER, K. 2002. Cellular and systems reconsolidation in the hippocampus. *Neuron,* 36**,** 527-538.
DIEKELMANN, S. & BORN, J. 2010. The memory function of sleep. *Nature Reviews Neuroscience,* 11**,** 114-26.
DIEKELMANN, S., BUCHEL, C., BORN, J. & RASCH, B. 2011. Labile or stable: opposing consequences for memory when reactivated during waking and sleep. *Nature Neuroscience,* 14**,** 381-6.
DINGES, D. F., PACK, F., WILLIAMS, K., GILLEN, K. A., POWELL, J. W., OTT, G. E., APTOWICZ, C. & PACK, A. I. 1997. Cumulative sleepiness, mood

disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. *Sleep,* 20**,** 267-277.
DUDAI, Y. 2004. The neurobiology of consolidations, or, how stable is the engram? *Annual Review of Psychology,* 55**,** 51-86.
DUDAI, Y. 2012. The Restless Engram: Consolidations Never End. *In:* HYMAN, S. E. (ed.) *Annual Review of Neuroscience, Vol 35.* Palo Alto: Annual Reviews.
EBBINGHAUS, H. 1885. *Über das Gedächtnis. Untersuchungen zur experimentellen Psychologie,* Leipzig, Duncker & Humblot.
ELLENBOGEN, J. M., HU, P. T., PAYNE, J. D., TITONE, D. & WALKER, M. P. 2007. Human relational memory requires time and sleep. *Proceedings of the National Academy of Sciences of the United States of America,* 104**,** 7723-8.
FELD, G. B., LANGE, T., GAIS, S. & BORN, J. 2013. Sleep-Dependent Declarative Memory Consolidation-Unaffected after Blocking NMDA or AMPA Receptors but Enhanced by NMDA Coagonist D-Cycloserine. *Neuropsychopharmacology,* 38**,** 2688-2697.
FELD, G. B., WEIS, P. P. & BORN, J. 2016. The Limited Capacity of Sleep-Dependent Memory Consolidation. *Frontiers in Psychology,* 7**,** 12.
FISCHER, S., DROSOPOULOS, S., TSEN, J. & BORN, J. 2006. Implicit learning -- explicit knowing: a role for sleep in memory system interaction. *Journal of Cognitive Neuroscience,* 18**,** 311-9.
FLOOD, J. F., MORLEY, J. E. & LANTHORN, T. H. 1992. Effect on memory processing by D-cycloserine, an agonist of the NMDA/glycine receptor. *European Journal of Pharmacology,* 221**,** 249-254.
FRANKLAND, P. W. & BONTEMPI, B. 2005. The organization of recent and remote memories. *Nature Reviews Neuroscience,* 6**,** 119-130.
GAIS, S., ALBOUY, G., BOLY, M., DANG-VU, T. T., DARSAUD, A., DESSEILLES, M., RAUCHS, G., SCHABUS, M., STERPENICH, V., VANDEWALLE, G., MAQUET, P. & PEIGNEUX, P. 2007. Sleep transforms the cerebral trace of declarative memories. *Proceedings of the National Academy of Sciences of the United States of America,* 104**,** 18778-83.
GAIS, S., RASCH, B., WAGNER, U. & BORN, J. 2008. Visual-procedural memory consolidation during sleep blocked by glutamatergic receptor antagonists. *Journal of Neuroscience,* 28**,** 5513-5518.
GILBOA, A., WINOCUR, G., GRADY, C. L., HEVENOR, S. J. & MOSCOVITCH, M. 2004. Remembering our past: Functional neuroanatomy of recollection of recent and very remote personal events. *Cerebral Cortex,* 14**,** 1214-1225.
GRAF, P. & SCHACTER, D. L. 1985. Implicit and Explicit Memory for New Associations in Normal and Amnesic Subjects. *Journal of Experimental Psychology-Learning Memory and Cognition,* 11**,** 501-518.
GRAF, P., SQUIRE, L. R. & MANDLER, G. 1984. The information that amnesic patients do not forget. *Journal of Experimental Psychology-Learning Memory and Cognition,* 10**,** 164-78.
HARDT, O., NADER, K. & NADEL, L. 2013. Decay happens: the role of active forgetting in memory. *Trends in Cognitive Sciences,* 17**,** 111-20.
HASSELMO, M. E. 1999. Neuromodulation: acetylcholine and memory consolidation. *Trends in Cognitive Sciences,* 3**,** 351-359.

HEBB, D. O., MARTINEZ, J. L. & GLICKMAN, S. E. 1949. The Organization of Behavior - a Neuropsychological Theory - Hebb,Do. *Contemporary Psychology,* 39**,** 1018-1020.

HENGEN, K. B., PACHECO, A. T., MCGREGOR, J. N., VAN HOOSER, S. D. & TURRIGIANO, G. G. 2016. Neuronal Firing Rate Homeostasis Is Inhibited by Sleep and Promoted by Wake. *Cell,* 165**,** 180-191.

HINZ, A., DAIG, I., PETROWSKI, K. & BRAHLER, E. 2012. Mood in the German Population: Norms of the Multidimensional Mood Questionnaire MDBF. *Psychotherapie Psychosomatik Medizinische Psychologie,* 62**,** 52-57.

HODDES, E., ZARCONE, V., SMYTHE, H., PHILLIPS, R. & DEMENT, W. C. 1973. Quantification of sleepiness: a new approach. *Psychophysiology,* 10**,** 431-6.

HUANG, Y. Y., COLINO, A., SELIG, D. K. & MALENKA, R. C. 1992. The Influence of Prior Synaptic Activity on the Induction of Long-Term Potentiation. *Science,* 255**,** 730-733.

INOSTROZA, M. & BORN, J. 2013. Sleep for preserving and transforming episodic memory. *Annual Review of Neuroscience,* 36**,** 79-102.

JANKE, W. & DEBUS, G. 1978. *Die Eigenschaftswörterliste: EWL; eine mehrdimensionale Methode zur Beschreibung von Aspekten des Befindens,* Verlag für Psychologie Hogrefe.

JENKINS, J. G. & DALLENBACH, K. M. 1924. Obliviscence during Sleep and Waking. *The American Journal of Psychology,* 35**,** 605-612.

JOHNSON, J. W. & ASCHER, P. 1987. Glycine Potentiates the Nmda Response in Cultured Mouse-Brain Neurons. *Nature,* 325**,** 529-531.

KALES, A. & RECHTSCHAFFEN, A. 1968. *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects,* Los Angeles Brain Information Service. Brain Information Institute, University of California, Los Angeles.

KANDEL, E. R. 2001. Neuroscience - The molecular biology of memory storage: A dialogue between genes and synapses. *Science,* 294**,** 1030-1038.

KLECKNER, N. W. & DINGLEDINE, R. 1988. Requirement for Glycine in Activation of Nmda-Receptors Expressed in Xenopus Oocytes. *Science,* 241**,** 835-837.

KOCHLAMAZASHVILI, G., BUKALO, O., SENKOV, O., SALMEN, B., GERARDY-SCHAHN, R., ENGEL, A. K., SCHACHNER, M. & DITYATEV, A. 2012. Restoration of synaptic plasticity and learning in young and aged NCAM-deficient mice by enhancing neurotransmission mediated by GluN2A-containing NMDA receptors. *Journal of Neuroscience,* 32**,** 2263-75.

KUHL, B. A., DUDUKOVIC, N. M., KAHN, I. & WAGNER, A. D. 2007. Decreased demands on cognitive control reveal the neural processing benefits of forgetting. *Nature Neuroscience,* 10**,** 908-914.

KUTSUWADA, T., KASHIWABUCHI, N., MORI, H., SAKIMURA, K., KUSHIYA, E., ARAKI, K., MEGURO, H., MASAKI, H., KUMANISHI, T., ARAKAWA, M. & ET AL. 1992. Molecular diversity of the NMDA receptor channel. *Nature,* 358**,** 36-41.

LAROCHE, S., JAY, T. M. & THIERRY, A. M. 1990. Long-term potentiation in the prefrontal cortex following stimulation of the hippocampal CA1/subicular region. *Neuroscience Letters,* 114**,** 184-190.

LECHNER, H. A., SQUIRE, L. R. & BYRNE, J. H. 1999. 100 years of consolidation - Remembering Muller and Pilzecker. *Learning & Memory,* 6**,** 77-87.

LEE, H. K., KAMEYAMA, K., HUGANIR, R. L. & BEAR, M. F. 1998. NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. *Neuron,* 21**,** 1151-1162.

LEE, J. L. C., MILTON, A. L. & EVERITT, B. J. 2006. Reconsolidation and extinction of conditioned fear: Inhibition and potentiation. *Journal of Neuroscience,* 26**,** 10051-10056.

LEWIS, P. A. & DURRANT, S. J. 2011. Overlapping memory replay during sleep builds cognitive schemata. *Trends in Cognitive Sciences,* 15**,** 343-351.

LIU, L., WONG, T. P., POZZA, M. F., LINGENHOEHL, K., WANG, Y., SHENG, M., AUBERSON, Y. P. & WANG, Y. T. 2004. Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science,* 304**,** 1021-4.

LYNCH, M. A. 2004. Long-term potentiation and memory. *Physiological Reviews,* 84**,** 87-136.

MACLEOD, C. M. 1999. The item and list methods of directed forgetting: Test differences and the role of demand characteristics. *Psychonomic Bulletin & Review,* 6**,** 123-129.

MALAMUT, B. L., SAUNDERS, R. C. & MISHKIN, M. 1984. Monkeys with Combined Amygdalo-Hippocampal Lesions Succeed in Object Discrimination-Learning Despite 24-Hour Intertrial Intervals. *Behavioral Neuroscience,* 98**,** 759-769.

MALENKA, R. C. & NICOLL, R. A. 1999. Long-term potentiation--a decade of progress? *Science,* 285**,** 1870-4.

MARR, D. 1971. Simple memory: a theory for archicortex. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 262**,** 23-81.

MARSHALL, L., HELGADOTTIR, H., MOLLE, M. & BORN, J. 2006. Boosting slow oscillations during sleep potentiates memory. *Nature,* 444**,** 610-613.

MARSHALL, U. & BORN, J. 2007. The contribution of sleep to hippocampus-dependent memory consolidation. *Trends in Cognitive Sciences,* 11**,** 442-450.

MAVIEL, T., DURKIN, T. P., MENZAGHI, F. & BONTEMPI, B. 2004. Sites of neocortical reorganization critical for remote spatial memory. *Science,* 305**,** 96-99.

MCCLELLAND, J. L., MCNAUGHTON, B. L. & OREILLY, R. C. 1995. Why There Are Complementary Learning Systems in the Hippocampus and Neocortex: Insights From the Successes and Failures of Connectionist Model sof Learning and Memory. *Psychological Review,* 102**,** 419-457.

MCGAUGH, J. L. 1966. Time-Dependent Processes in Memory Storage. *Science,* 153**,** 1351-&.

MCGAUGH, J. L. 1972. The search for the memory trace. *Annals of the New York Academy of Sciences,* 193**,** 112-&.

MILNER, B., CORKIN, S. & TEUBER, H. L. 1968. Further Analysis of Hippocampal Amnesic Syndrome - 14-Year Follow-up Study of Hm. *Neuropsychologia,* 6**,** 215-&.

MISANIN, J. R., MILLER, R. R. & LEWIS, D. J. 1968. Retrograde Amnesia Produced by Electroconvulsive Shock after Reactivation of a Consolidated Memory Trace. *Science,* 160**,** 554-+.

MONAHAN, J. B., HANDELMANN, G. E., HOOD, W. F. & CORDI, A. A. 1989. D-cycloserine, a positive modulator of the N-methyl-D-aspartate receptor,

enhances performance of learning tasks in rats. *Pharmacology Biochemistry and Behavior,* 34**,** 649-653.
MORRIS, R. G. M. 2006. Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. *European Journal of Neuroscience,* 23**,** 2829-2846.
MOSCOVITCH, M., NADEL, L., WINOCUR, G., GILBOA, A. & ROSENBAUM, R. S. 2006. The cognitive neuroscience of remote episodic, semantic and spatial memory. *Current Opinion in Neurobiology,* 16**,** 179-190.
NADEL, L. & HARDT, O. 2011. Update on Memory Systems and Processes. *Neuropsychopharmacology,* 36**,** 251-273.
NADEL, L. & MOSCOVITCH, M. 1997. Memory consolidation, retrograde amnesia and the hippocampal complex. *Current Opinion in Neurobiology,* 7**,** 217-227.
O'NEILL, J., PLEYDELL-BOUVERIE, B., DUPRET, D. & CSICSVARI, J. 2010. Play it again: reactivation of waking experience and memory. *Trends in Neurosciences,* 33**,** 220-229.
OLNEY, J. W. 2003. Excitotoxicity, apoptosis and neuropsychiatric disorders. *Current Opinion in Pharmacology,* 3**,** 101-109.
ONUR, O. A., SCHLAEPFER, T. E., KUKOLJA, J., BAUER, A., JEUNG, H., PATIN, A., OTTE, D.-M., SHAH, N. J., MAIER, W., KENDRICK, K. M., FINK, G. R. & HURLEMANN, R. 2010. The N-Methyl-D-Aspartate Receptor Co-agonist D-Cycloserine Facilitates Declarative Learning and Hippocampal Activity in Humans. *Biological Psychiatry,* 67**,** 1205-1211.
PARK, P., VOLIANSKIS, A., SANDERSON, T. M., BORTOLOTTO, Z. A., JANE, D. E., ZHUO, M., KAANG, B. K. & COLLINGRIDGE, G. L. 2014. NMDA receptor-dependent long-term potentiation comprises a family of temporally overlapping forms of synaptic plasticity that are induced by different patterns of stimulation. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 369**,** 13.
PENFIELD, W. & MILNER, B. 1958. Memory deficit produced by bilateral lesions in the hippocampal zone. *AMA Archives of neurology and psychiatry,* 79**,** 475-97.
PETROFF, O. A. C. 2002. GABA and glutamate in the human brain. *Neuroscientist,* 8**,** 562-573.
POLSTER, M. R., NADEL, L. & SCHACTER, D. L. 1991. Cognitive Neuroscience Analyses of Memory - a Historical-Perspective. *Journal of Cognitive Neuroscience,* 3**,** 95-116.
POO, M. M., PIGNATELLI, M., RYAN, T. J., TONEGAWA, S., BONHOEFFER, T., MARTIN, K. C., RUDENKO, A., TSAI, L. H., TSIEN, R. W., FISHELL, G., MULLINS, C., GONCALVES, J. T., SHTRAHMAN, M., JOHNSTON, S. T., GAGE, F. H., DAN, Y., LONG, J., BUZSAKI, G. & STEVENS, C. 2016. What is memory? The present state of the engram. *BMC Biology,* 14**,** 40.
PRZYBYSLAWSKI, J. & SARA, S. J. 1997. Reconsolidation of memory after its reactivation. *Behavioural Brain Research,* 84**,** 241-246.
RASCH, B. & BORN, J. 2007. Maintaining memories by reactivation. *Current Opinion in Neurobiology,* 17**,** 698-703.
RASCH, B., BUCHEL, C., GAIS, S. & BORN, J. 2007. Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science,* 315**,** 1426-9.
RIBOT, T. & SMITH, W. H. 1882. *Diseases of memory,* New York, D. Appleton and company.

ROSENZWEIG, E. S., BARNES, C. A. & MCNAUGHTON, B. L. 2002. Making room for new memories. *Nature Neuroscience,* 5**,** 6-8.
RYLE 1949. *The Concept of Mind,* Chicago, University of Chicago Press.
SACHSER, R. M., SANTANA, F., CRESTANI, A. P., LUNARDI, P., PEDRAZA, L. K., QUILLFELDT, J. A., HARDT, O. & ALVARES, L. D. 2016. Forgetting of long-term memory requires activation of NMDA receptors, L-type voltage-dependent Ca2+ channels, and calcineurin. *Scientific Reports,* 6**,** 9.
SALETIN, J. M., GOLDSTEIN, A. N. & WALKER, M. P. 2011. The Role of Sleep in Directed Forgetting and Remembering of Human Memories. *Cerebral Cortex,* 21**,** 2534-2541.
SARA, S. J. 2000. Retrieval and reconsolidation: Toward a neurobiology of remembering. *Learning & Memory,* 7**,** 73-84.
SCHACTER, D. L., EICH, J. E. & TULVING, E. 1978. Richard Semon's theory of memory. *Journal of Verbal Learning and Verbal Behavior,* 17**,** 721-743.
SCOVILLE, W. B. & MILNER, B. 1957. Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery, and Psychiatry,* 20**,** 11-21.
SEMON, R. W. & SIMON, L. 1921. *The mneme,* London, New York,, G. Allen & Unwin ltd.; The Macmillan company.
SHEININ, A., SHAVIT, S. & BENVENISTE, M. 2001. Subunit specificity and mechanism of action of NMDA partial agonist D-cycloserine. *Neuropharmacology,* 41**,** 151-8.
SHIFFRIN, R. M. & ATKINSON, R. C. 1969. Storage and retrieval processes in long-term memory. *Psychological Review,* 76**,** 179-&.
SIAPAS, A. G. & WILSON, M. A. 1998. Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron,* 21**,** 1123-8.
SQUIRE, L. R., STARK, C. E. L. & CLARK, R. E. 2004. The medial temporal lobe. *Annual Review of Neuroscience,* 27**,** 279-306.
SQUIRE, L. R. & WIXTED, J. T. 2011. The cognitive neuroscience of human memory since H.M. *Annual Review of Neuroscience,* 34**,** 259-88.
SQUIRE, L. R., WIXTED, J. T. & CLARK, R. E. 2007. Recognition memory and the medial temporal lobe: a new perspective. *Nature Reviews Neuroscience,* 8**,** 872-83.
SQUIRE, L. R. & ZOLA-MORGAN, S. 1988. Memory: brain systems and behavior. *Trends in Neurosciences,* 11**,** 170-5.
SQUIRE, L. R. & ZOLA-MORGAN, S. 1991. The Medial Temporal-Lobe Memory System. *Science,* 253**,** 1380-1386.
STERNBERG, R. J. & DAVIDSON, J. E. 1995. *The nature of insight,* Cambridge, Mass., MIT Press.
TAKASHIMA, A., NIEUWENHUIS, I. L. C., JENSEN, O., TALAMINI, L. M., RIJPKEMA, M. & FERNANDEZ, G. 2009. Shift from Hippocampal to Neocortical Centered Retrieval Network with Consolidation. *Journal of Neuroscience,* 29**,** 10087-10093.
THOMPSON, L. T., MOSKAL, J. R. & DISTERHOFT, J. F. 1992. Hippocampus-dependent learning facilitated by a monoclonal antibody or o-cycloserine. *Nature,* 359**,** 638-641.

TONONI, G. & CIRELLI, C. 2003. Sleep and synaptic homeostasis: a hypothesis. *Brain Research Bulletin,* 62**,** 143-150.
TONONI, G. & CIRELLI, C. 2014. Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. *Neuron,* 81**,** 12-34.
TSE, D., LANGSTON, R. F., KAKEYAMA, M., BETHUS, I., SPOONER, P. A., WOOD, E. R., WITTER, M. P. & MORRIS, R. G. M. 2007. Schemas and memory consolidation. *Science,* 316**,** 76-82.
TSIEN, J. Z. 2000. Linking Hebb's coincidence-detection to memory formation. *Current Opinion in Neurobiology,* 10**,** 266-273.
TULVING, E. 1972. Episodic and semantic memory. *Organization of memory,* 1**,** 381-403.
VALBUENA, S. & LERMA, J. 2016. Non-canonical Signaling, the Hidden Life of Ligand-Gated Ion Channels. *Neuron,* 92**,** 316-329.
VAN DER WERF, Y. D., ALTENA, E., SCHOONHEIM, M. M., SANZ-ARIGITA, E. J., VIS, J. C., DE RIJKE, W. & VAN SOMEREN, E. J. W. 2009. Sleep benefits subsequent hippocampal functioning. *Nature Neuroscience,* 12**,** 122-123.
VIARD, A., LEBRETON, K., CHETELAT, G., DESGRANGES, B., LANDEAU, B., YOUNG, A., DE LA SAYETTE, V., EUSTACHE, F. & PIOLINO, P. 2010. Patterns of Hippocampal-Neocortical Interactions in the Retrieval of Episodic Autobiographical Memories Across the Entire Life-Span of Aged Adults. *Hippocampus,* 20**,** 153-165.
VILLARREAL, D. M., DO, V., HADDAD, E. & DERRICK, B. E. 2002. NMDA receptor antagonists sustain LTP and spatial memory: active processes mediate LTP decay. *Nature Neuroscience,* 5**,** 48-52.
W. B. SCOVILLE 1954. The Limbic Lobe in Man. *Journal of Neurosurgery,* 11**,** 64-66.
WAGNER, U., GAIS, S., HAIDER, H., VERLEGER, R. & BORN, J. 2004. Sleep inspires insight. *Nature,* 427**,** 352-355.
WATSON, J. D. & CRICK, F. H. C. 1953. Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. *Nature,* 171**,** 737-738.
WIGSTROM, H., GUSTAFSSON, B. & HUANG, Y. Y. 1986. Mode of action of excitatory amino acid receptor antagonists on hippocampal long-lasting potentiation. *Neuroscience,* 17**,** 1105-1115.
WILHELM, I., DIEKELMANN, S., MOLZOW, I., AYOUB, A., MOLLE, M. & BORN, J. 2011. Sleep selectively enhances memory expected to be of future relevance. *Journal of Neuroscience,* 31**,** 1563-9.
WILSON, M. A. & MCNAUGHTON, B. L. 1994. REACTIVATION OF HIPPOCAMPAL ENSEMBLE MEMORIES DURING SLEEP. *Science,* 265**,** 676-679.
WINOCUR, G., MOSCOVITCH, M. & BONTEMPI, B. 2010. Memory formation and long-term retention in humans and animals: Convergence towards a transformation account of hippocampal-neocortical interactions. *Neuropsychologia,* 48**,** 2339-2356.
WIXTED, J. T. 2004. The psychology and neuroscience of forgetting. *Annual Review of Psychology,* 55**,** 235-269.
YASHIRO, K. & PHILPOT, B. D. 2008. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. *Neuropharmacology,* 55**,** 1081-94.

ZHU, M., NIX, D. E., ADAM, R. D., CHILDS, J. M. & PELOQUIN, C. A. 2001. Pharmacokinetics of cycloserine under fasting conditions and with high-fat meal, orange juice, and antacids. *Pharmacotherapy,* 21**,** 891-897.

ZOLA-MORGAN, S., SQUIRE, L. R. & AMARAL, D. G. 1986. Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. *Journal of Neuroscience,* 6**,** 2950-67.

ZOLA-MORGAN, S., SQUIRE, L. R. & RAMUS, S. J. 1994. Severity of Memory Impairment in Monkeys as a Function of Locus and Extent of Damage within the Medial Temporal-Lobe Memory System. *Hippocampus,* 4**,** 483-495.

ZOLA-MORGAN, S. M. & SQUIRE, L. R. 1990. The Primate Hippocampal Formation: Evidence for a Time-Limited Role in Memory Storage. *Science,* 250**,** 288-290.

9 „Erklärung zum Eigenanteil“

Die Konzeption und spätere wissenschaftliche Betreuung der Studie erfolgte durch Herrn Dr. Gordon Feld, Frau Marjan Alizadeh Asfestani, jeweils wissenschaftliche Mitarbeiter am Institut für Medizinische Psychologie und Verhaltensneurobiologie, und Herrn Prof. Dr. Jan Born, Leiter des Instituts für Medizinische Psychologie und Verhaltensneurobiologie, Tübingen.
Die Versuche wurden, nach Einarbeitung durch Marjan Alizadeh Asfestani, von mir eigenständig koordiniert und durchgeführt. Anteilig wurden Retentionsintervalle sowie Kontroll-Tests durch Michael Radloff im Rahmen seiner Anstellung als wissenschaftlicher Mitarbeiter am Institut für Medizinische Psychologie und Verhaltensneurobiologie Tübingen durchgeführt. Alle Daten zu den Wortpaar assoziations Aufgaben (Zielvariablen dieser Arbeit) wurden von mir persönlich erhoben. Die statistische Auswertung erfolgte in Zusammenarbeit mit Marjan Alizadeh Asfestani.
Ich versichere hiermit, das Manuskript selbstständig verfasst und keine weiteren als die angegebenen Quellen verwendet zu haben.
Die Daten der vorliegenden Arbeit wurden in einem von Marjan Alizadeh Asfestani verfassten Articel am 02.07.2018 in der Fachzeitschrift „Neuropsychopharmacology“ (Band 43) unter meiner Ko-Autorenschaft veröffentlicht.

Tübingen, den 27.11.2019

10 Appendix 1, Blood Values

Analyt	Einheit	Referenzbereich		
(Diff)-Blutbild: EDTA-Blut				
Leukozyten	1/ul	3800	–	10300
Normoblasten	%	0	–	1
Normoblasten abs	Tausd/ul	0	–	0.1
Erythrozyten	Mio/ul	4.2	–	6.2
Haematokrit	%	42	–	52
Hb	g/dl	14	–	18
MCH	pg	27	–	34
MCHC	g/dl	32	–	36
MCV	fl	80	–	93
Ery. Verteilungsbreite	%			15
Thrombozyten	Tausd/ul	140	–	392
Verteilungsbreite der Thrombozyten	fl	10	–	16
Mittleres Plättchenvolumen	fl	9	–	12
Gerinnung: Citrat-Blut				
Quick	%	70	–	120
INR				
PTT	sec			40
Elektrolyte, Substrate, Enzyme: Li-Heparin-Plasma				
Natrium	mmol/l	136	–	148
Kalium	mmol/l	3.5	–	4.8
Calcium	mmol/l	2.1	–	2.6
Chlorid	mmol/l	96	–	110
Bilirubin, gesamt	mg/dl			1.1
C-reaktives Protein	mg/dl			0.5
GOT/AST	U/l			50
GPT/ALT	U/l			50
Alkalische Phosphatase	U/l	40	–	130
Laktatdehydrogenase	U/l			250
Gamma-Glutamyl-Transferase	U/l			60
Laktat, Glukose: Na-F-Plasma				
Glukose venös	mg/dl	70	–	110

11 Appendix 2, Study Protocol (exemplary: participant DFW01)

Versuchsablauf

	Anmerkung
Versuchsperson	DFW01
Session	1
Zimmer (eintragen, gleich für beide Sessions)	
PAL Abend 1	Liste E2
PAL Abend 2	Liste D2
PAL Morgen 1	Liste E1
PAL Morgen 2	Liste A2
Nummern Lernen	Version 2
Filme (tag)	Liste 2
Filme (Nacht)	Liste 1

- Essen vorbereitet [2 Scheiben Brot, Käse, Wurst, Butter, Tomate, Tee (keine Koffein)]?
- Versuchsablaufsplan und -material ausdrucken und in Klemmbrett heften
- PAL und 3 x EWL-K dazulegen und beschriften
- Bett beziehen und Zimmer lüften
- Verdunklung runterlassen
- Computer starten
- Lautsprecher an?

Ereignis	**Anmerkung**	**Dauer**	**Uhrzeit geplant**	**Uhrzeit tatsächlich**
Proband erwacht			07:00	
Proband trifft ein	Handy ausschalten und im Vorraum lassen.	2 min	20:30	
Fragebögen I		3 min	20:32	
PAL Abend Lernen (80 Wort-paare, 4s, 1s ISI)	1. Liste E2 2. Liste D2	7 min	20:36	
PAL Abend sofortiger Abruf Kriterium-Check *Pro Liste 60 % (24 Wortpaare) erinnert? Wenn nein, dann diese Liste nochmal lernen und abrufen.*	1. Liste E2 2. Liste D2 Info: 1. Wort gilt, 3. Nur Substantive	53 min	20:43	

PAL	**Kriterium**	**Lern 1**	**Abruf 1**	**Lern 2**	**Abruf 2**	**Lern 3**	**Abruf 3**	**Lern 4**	**Abruf 4**	**Lern 5**	**Abruf 5**
??	>= 24 Wortpaare	✓		o		o		o		o	
??	>= 24 Wortpaare	✓		o		o		o		o	

Anweisung PVT: *Im folgenden Test erscheint in der Mitte des Bildschirms eine Art Stoppuhr, die beginnt sehr schnell Zahlen hochzuzählen. Deine Aufgabe ist es, so schnell wie möglich die Leertaste zu drücken, sobald du die Zahl siehst. Die gestoppte Zeit bleibt dann kurz stehen und entspricht deiner Reaktionszeit. Die Aufgabe wird 5min dauern und beginnt sofort, wenn ich Enter drücke. Nach Ende der Aufgabe komme ich wieder herein und du bekommst die nächste Aufgabe. Hast du verstanden, was du als nächstes machen sollst?*	Mündliche Instruktion. PVT5 starten Speicherort: XXX	1 min	21:36	
PVT		5 min	21:37	
Fragebögen II		10 min	21:42	
Warten bis 22:30				

Ereignis	**Anmerkung**	**Dauer**	**Uhrzeit geplant**	**Uhrzeit tatsächlich**
Das Präparat wird oral verabreicht.	Filme(Nacht): Liste 1		**22:30**	
Fragebögen III		15 min	**7:00**	
Zeit zum Duschen				
Standardisiertes Frühstück	Mensa Mitarbeiterfrühstück (kein Koffein)			
Umzug ins CIN				
Pause im Labor (Filme nacht : Liste2)				

Ereignis	Anmerkung	Dauer	Uhrzeit geplant	Uhrzeit tatsächlich
Abrufsitzung beginnt	**20 Stunden nach Präparat** (doppelte Halbwertszeit), eintragen	0 min	(18:30) _____	
Instruktion vor den Gedächtnistests		0 min	18:30	
PAL Morgen Lernen *80 ortpaare, 4s, 1s ISI*	1. Liste E1 2. Liste A2	7 min	18:31	
PAL Morgen sofortiger Abruf *3 x Lernen und Abruf*	1. Liste E1 2. Liste A2	53 min	18:38	

PAL	Lern 1	Abruf 1	Lern 2	Abruf 2	Lern 3	Abruf 3
E1	✓		✓		✓	
A2	✓		✓		✓	

Instruktion vor dem Abrufen		0 min	19:31	
PAL Abend verzögerter Abruf	1. Liste E1 2. Liste A2	20 min	19:31	

PAL	Abruf 6
E1	
A2	

Nummern Lernen	Version 2	5 min	19:51	
Nummern Abrufen	Version 2	5 min	19:56	
WFT	je 2 Minuten für Buchstabe und Kategorie	6 min	20:01	
Autobiographisches Interview		15 min	20:07	
PVT		6 min	20:22	
Fragebögen IV	Handy zurückgeben!	5 min	20:28	

Nach der zweiten Experimentalsitzung: Nachbefragungsbogen 2		2 min	20:33	

12 Appendix 3, Fragebogen 1

Kennung:
Probandenblatt

Gesundheit heute?

Medikamente/Drogen heute?

Wann zum letzten Mal Kaffee, Cola, Red Bull (oder ähnliches) oder Tee getrunken?

Heute besonderen Stress gehabt?

Hatten Sie in letzter Zeit besonderen Stress (z.B. Prüfungen)? Wenn ja, wann?

Werden Sie in nächster Zukunft besonderen Stress haben? Wenn ja, wann?

Zu welcher Uhrzeit gingen Sie letzte Nacht schlafen?

Wann sind Sie heute aufgestanden?

Wie viele Stunden schliefen Sie letzte Nacht?

Haben Sie heute tagsüber geschlafen?

Wenn ja, wann und wie viel?

Besonderheiten:

13 Appendix 4

"Night time Protocol"

Night time		
Time	Activity	Details
10:30 PM - 11:00 PM	Watch movie	Movie 1
11:00 PM- 11:30 PM	**Play snood**	
11:30PM - 12:00	Snack	(2-4 slices of bread, *cheese & salami , fruit tee, tomato, butter)
12:00AM - 01:08AM	Watch movie	Continue Movie 1 and Movie 2
01:08AM - 01:30AM	**Play snood**	
01:30AM - 02:15AM	Watch movie	Movie 3
02:15AM - 02:45AM	Break(walk outside)	
02:45AM - 03:15AM	Snack	(fruit tee & Biscuit)
03:15AM - 04:09AM	Watch movie	Movie 4
04:09AM - 04:30AM	**play snood**	
04:30AM - 04:45AM	Break(walk outside)	
04:45AM-05:15AM	Snack	(fruit tee & Bread, jam & apple)
05:15 AM -06:00AM	**play snood**	
06:00AM-06:15AM	Break(walk outside)	
06:15AM-06:30	Snack	(fruit tee & Biscuit)
06:30AM-07:00 AM	**play snood**	

*The combination of cheese and salami participant can get: (Please pay attention what they get in first session they should have the same combination also for the second session as well)

3 cheese/9 salami **or** 1 cheese & 6 salami **or** 2 cheese & 3 salami

ListA	Dauer
1) Fremde Welten	(49 min)
2) Giganten	(49 min)
3) Unendliche Weiten	(49 min)
4) Der Mond	(49 min)
total	196 min

ListB	Dauer
1)Die Sonne	(49 min)
2)Atmosphären	(49 min)
3) Lebenszeichen	(49 min)
4) Der Todesstern	(49 min)
total	196min

14 Appendix 5

“Day time Protocol”

Day time		
Time	Activity	Details
7:00 - 7:30 AM/7:45 AM	take a shower/ answer the questionnaire	
7:30/7:45 AM - 8:30 AM	Breakfast at Casino/ move to CIN Building	Tee (not caffeine), bread, *cheese & Salami, Jam or honey.
8:30 AM - 10:00 AM	Watch Animal documentary	movie 1 / movie 2
10:00 AM - 10:30 AM	BREAK	walk out side
10:30 AM - 12:00 AM	Watch Animal Documentary	movie 3 / movie 4
12:00 AM - 1:00 PM	Lunch	drink : water
1:00 PM - 2:30 PM	Watch Animal Documentary	movie 5/ movie 6
2:30 PM - 3:00 PM	BREAK	walk outside & snack (Tee & Biscuit & apple)
3:00 PM - 4:30 PM	Watch Animal Documentary	movie 7 / movie 8
4:30 PM - 4:45 PM	BREAK	walk out side
4:45 PM - 6:15 PM	watch Animal documentary & snack	movie 9 / movie 10/ movie 11 - snack (2 slices of bread,*cheese & Salami , fruit tee, tomato, butter)
6:15 PM - 6:30 PM	BREAK	walk outside, move to the medicine sleep lab, prepare for the test

***The combination of cheese and salami participant can get: (Please pay attention what they get in first session they should have the same combination also for the second session as well)

3 cheese/9 salami **or** 1 cheese & 6 salami **or** 2 cheese & 3 salami

Liste 1	Dauer [min]	Titel
1) Planet Erde 1-1	43.5	Von Pol zu Pol
2) Säugetiere 1-1	43	Ein erfolgreiches Modell
3) Vögel 1-2	43	Die Meister der Lüfte
4) Planet Erde 1-3	44	Wasserwelten
5) Säugetiere 1-3	43	Pflanzenfresser
6) Vögel 1-4	43	Die Fleischfresser
7) Planet Erde 2-3	44	Höhlenwelten
8) Säugetiere 2-2	43	Fleischfresser
9) Vögel 2-2	43	Signale und Gesänge
10) Planet Erde 3-3	43.5	Eiswelten
11) Säugetiere 4-1	43.5	Aufsteiger
Vögel 3-1	43	Das anspruchsvolle Ei
Planet Erde 4-1	43.5	Waldwelten
	563	

Liste 2	Dauer [min]	Titel
1) Planet Erde 1-2	43.5	Bergwelten
2)Säugetiere 1-2	43	Insektenjäger
3) Vögel 1-1	43	Fliegen oder nicht Fliegen
4) Planet Erde 2-1	44	Wüstenwelten
5) Säugetiere 2-1	43	Nagetiere
6) Vögel 1-3	43	Der unersätliche Appetit
7) Planet Erde 3-1	43.5	Meereswelten
8) Säugetiere 3-1	43	Rückkehr ins Wasser
9) Vögel 2-1	43	Die Fischfresser
10)Planet Erde 3-4	43.5	Graswelten
11) Säugetiere 4-2	43.5	Nahrung fürs Gehirn
Vögel 2-3	43	Partnerwahl
Planet Erde 4-3	43.5	Dschungelwelten
	562 . 5	