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The effects of sleep-associated brain rhythms and their manipulation by weak electric stimulation on memory consolidation in the rat

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ZUSAMMENFASSUNG

Die positive Auswirkung von Schlaf auf die Gedächtniskonsolidierung konnte im Menschen in vielen Studien nachgewiesen werden, allerdings gibt es vergleichsweise nur wenige Erkenntnisse zu diesem Thema in der Ratte, eine der prominentesten Modellspezies in der neurowissenschaftlichen Forschung. Es konnte gezeigt werden, dass Tiefschlaf (engl. slow-wave sleep, SWS), gekennzeichnet durch das Auftreten von langsamen Oszillationen (engl. slow oscillations, SO), von besonderer Bedeutung für die Konsolidierung von hippokampusabhängigen Gedächtnisinhalten ist. In einer früheren Studie führte die Anwendung von transkranialer Gleichstromstimulation während SWS, welche in der Frequenz endogener SO oszillierte (so-tDCS), zu verbesserter Gedächtniskonsolidierung in einer hippokampusabhängigen Aufgabe bei menschlichen Probanden und legte somit eine kausale Rolle der SO für die schlafabhängige Gedächtniskonsolidierung nahe. Die Ziele der vorliegenden Arbeit waren nun, diese Ergebnisse in einem Rattenmodell zu replizieren und zu erweitern, und somit die weitere Erforschung der Mechanismen der schlafassoziierten Gehirnoszillationen und deren Rolle bei der Gedächtniskonsolidierung zu ermöglichen. Es wurde angenommen, dass intakte Konsolidierung in hippokampusabhängigen Aufgaben Schlaf mit einen hohen Anteil SO und Schlafspindeln während des Retentionsintervalls erfordert, sowie dass so-tDCS die endogene SO- und Spindel-Aktivität erhöht und somit die Gedächtniskonsolidierung verbessert.

Um diese Hypothesen zu überprüfen, wurden Ratten in 3 separaten Studien hippokampusabhängigen Lernaufgaben unterzogen; während dem darauffolgenden Schlaf wurden EEG-Messungen vorgenommen. Wie zuvor angenommen, deuten die Ergebnisse auf eine wichtige Rolle von ungestörtem Schlaf mit hohem Anteil an SO und erhöhter Spindel-Aktivität für erfolgreiche Gedächtniskonsolidierung in einer räumlichen Lernaufgabe mit einmaligem Lerndurchgang innerhalb des hier getesteten 2h-Intervalls hin (Studie 1). Basierend auf diesen Ergebnissen wurde der Effekt von so-tDCS während SWS in der gleichen Verhaltensaufgabe untersucht, diesmal mit einem langen Retentionsintervalls von 24h (Studie 2). Hypothesenkonform erwies sich die Gedächtnisleistung nur nach so-tDCS als intakt, nicht jedoch infolge einer Scheinstimulation in einer Bedingung ohne so-tDCS, und die Ergebnisse der EEG Analyse wiesen auf eine Erhöhung der endogenen SO-Aktivität im Anschluss an die Stimulation hin. Um im weiteren Langzeiteffekte von multiplen so-tDCS Anwendungen zu untersuchen, wurde eine dritte Studie unter Verwendung einer hippokampusabhängigen Lernaufgabe durchgeführt, welche mehrere Lerndurchgänge über aufeinanderfolgende Tage erfordert (Radiallabyrinth). Eine beschleunigte Verringerung von

Referenzgedächtnisfehlern wies auf eine positive Wirkung von so-tDCS auf hippokampusabhängige Gedächtniskonsolidierung hin. Zusätzlich deutet eine verbesserte Arbeitsgedächtnisleistung für die mit Futterbelohnung versehenen Labyrintharme während der ersten Trainingstage auf durch so-tDCS verbesserte präfrontale Exekutivfunktionen an. Im Gegensatz allerdings zu Studie 2 erhöhte so-tDCS direkt nach der Stimulation nur die Leistung im oberen Deltabereich und reduzierte zusätzlich die im Thetabereich innerhalb der letzten Trainingstage während der akuten Stimulation. Diese Ergebnisse lassen sich möglicherweise auf Resonanzeffekte bzw. auf einen Einfluss auf Langzeitplastizität innerhalb kortikaler Netzwerke zurückführen. Die Unterschiede zwischen Studie 2 und 3 hängen möglicherweise mit aufgabenimmanenten Merkmalen (z.B. satte vs. futterdeprivierte Tiere) sowie mit geringfügigen Unterschieden in den Stimulationprotokollen (z.B. trapezförmige vs. sinusförmige Wellenform von so-tDCS) zusammen.

Zusammenfassend konnte gezeigt werden, dass SO eine wichtige und möglicherweise sogar kausale Rolle für die schlafabhängige Gedächtniskonsolidierung in der Ratte spielen. Außerdem erwies sich oszillatorische tDCS als sehr wertvolles Werkzeug für die weitere Untersuchung der funktionellen Bedeutung endogener kortikaler Netzwerkaktivität. Allerdings sind weitere Studien notwendig um sowohl auf elektrophysiologischer als auch auf molekularer Ebene die zugrundeliegenden Mechanismen der schlafabhängigen Gedächtniskonsolidierung, der damit assoziierten Gehirnoszillationen sowie die Wirkungsweise von so-tDCS genauer aufzuklären.

ABSTRACT

The positive impact of sleep on memory consolidation has been shown for human subjects in numerous studies, but there is still sparse knowledge on this topic in rats, one of the most prominent model species in neuroscience research. Slow-wave sleep (SWS), hallmarked by the occurrence of sleep slow oscillations (SO), were shown of specific importance for the consolidation of hippocampus-dependent memories. Previously, the application of transcranial direct current stimulation, oscillating at the frequency of endogenous SO (so-tDCS), during SWS enhanced memory consolidation for a hippocampus dependent task in humans and therefore suggested a causal role of SOs for sleep dependent memory consolidation. The aims of this work were now to replicate and extend these findings to a rat model, enabling further research on the mechanisms of sleep-associated brain oscillations and its role for memory consolidation. It was hypothesized that intact consolidation in hippocampus-dependent tasks requires sleep containing a high amount of SO activity and sleep spindles within the retention interval, and that so-tDCS boosts endogenous SO and spindle activity and thus improves memory consolidation.

To test these hypotheses, in three separate studies rats were subjected to hippocampus-dependent learning tasks and EEG was measured during post-learning sleep. As hypothesized, results indicate an important role of undisturbed sleep filled with a high amount of SO and increased spindle activity within a 2h retention interval for successful memory consolidation in a one-trial learning spatial task (study 1). Based on these findings, the effect of so-tDCS during early SWS in the very same behavioral task was assessed using a long retention interval of 24 hours (study 2). In support of the hypotheses, memory performance was intact following so-tDCS only, but not in a sham-stimulation condition lacking so-tDCS, and EEG results indicate a post-stimulation enhancement of endogenous SO activity. To further investigate long-term effects of multiple so-tDCS sessions, a third study was conducted in a hippocampus-dependent learning task (radial maze) requiring learning sessions over multiple subsequent days. Here, a transient accelerated decline in reference memory errors indicated again a positive impact of so-tDCS on hippocampus-dependent memory consolidation. Additionally, superior performance in working memory for baited locations within the first days of training hints towards improved prefrontal executive functions by so-tDCS. However, in contrast to study 2, so-tDCS enhanced EEG power only in the upper delta range immediately following stimulation and additionally reduced theta power within the last days of training during acute so-tDCS. These results indicate putatively resonance effects and

impact of so-tDCS on cortical network plasticity, respectively. Differences between study 2 and 3 may be related to task-inherent features (e.g., fed vs. food-deprived animals) as well as to slight differences in stimulation protocols (e.g. trapezoidal vs. sinusoidal wave shape of so-tDCS).

In conclusion, it could be shown that SO play an important and probably even causal role for sleep dependent memory consolidation in rats. Furthermore, oscillatory tDCS was proven to be a highly valuable tool to further investigate the function of endogenous cortical network activity. However, further studies are needed to precisely elucidate the underlying mechanisms of sleep-dependent memory consolidation, associated brain oscillations and the mechanisms of so-tDCS, on an electrophysiological as well as on a molecular level.

1 INTRODUCTION

1.1 MEMORY

Memory can be described under different viewpoints and thereby classified according to taxonomies stressing different aspects, without however being mutually exclusive. Below, three taxonomies are given, describing memories in a time dimension (time course of memory storage), by the processes involved (stages of memory formation) and by the nature of the memory trace (memory systems).

1.1.1 Time course of memory storage

Memories can be divided into different classes by the persistence of the memory trace. The most common (and probably most well-known outside the scientific community) taxonomy is the division between short-term- and long-term memory. Sensory memory (also called ultra-short-term memory or sensory register) can hold a high amount of information for milliseconds to a few seconds (Crowder & Cowan, 2003). Short-term-memory, within modern theoretic frameworks usually called working memory (WM)[1], holds and manipulates goal-relevant representations for seconds to minutes and is highly vulnerable to interference (Baddeley, 2003). Several subregions of the prefrontal cortex (PFC) play a major role in working memory, but also parietal cortex regions were shown to be critically involved (Goldman-Rakic, 1995; Bledowski, Rahm, & Rowe, 2009). On the other hand, long-term memory (LTM) can hold representations from hours to years. Several factors like rehearsal, depth of processing and relevance of the stored information can influence whether a specific content gains access to long-term storage (Baddeley, 1997). Which brain regions are involved in LTM depends critically on the type of memory, but see Figure 1 and the following section 1.1.3 on memory systems for details. Beside this relatively rough division, some authors further divide memory on a time domain into more than two categories, mainly based on the involvement of specialized brain regions and/or processes. Kesner & Hunsaker (2010) divide episodic memory (see below under ‘Memory systems’ for a definition) into short-term (duration of seconds), intermediate (duration of minutes to hours) and long-term or remote memory (days to years). A similar taxonomy is used by Frankland & Bontempi (2005)

[1] Older theories using the term ‘short-term memory’ regarded this form of memory as a unitary store, while modern frameworks acknowledge the short-term store as a collection of subsystems - subsumed under the term ‘working memory’- serving essential cognitive tasks like learning, reasoning and comprehending (Baddeley, 1997).

dividing long-term memory into a recent and a remote form. A detailed explanation of the latter classification system is given in the following section.

1.1.2 Stages of memory formation

Three fundamental processes can be identified necessary for a proper functioning of long-term memory: Encoding (acquisition of information), consolidation (storage and strengthening of the memory trace) and retrieval (recall of stored information). A fourth stage, reconsolidation, has sometimes been additionally defined (Tronson & Taylor, 2007). During encoding, a labile memory trace is formed, which can be subjected to consolidation, i.e. strengthening. If consolidation was successful, the memory trace can be retrieved at a later time point. For consolidation, neuronal plasticity processes have to take place, which may involve for instance the strengthening and/or weakening of synaptic contacts, and changes of dendritic spines (Bailey, Bartsch, & Kandel, 1996; Dudai, 2002).

Two-stage models of memory assume that memory consolidation is a gradual process: a memory is encoded into a temporary store and is transferred during the course of consolidation to a long-term storage site, leading to a further division of long-term memory by its degree of consolidation, a process referred to as systems consolidation. Within two-stage models, "recent memory" refers to memories still dependent on the short-term store, while "remote memories" are fully consolidated residing exclusively in the long-term store (Frankland & Bontempi, 2005).

1.1.3 Long-term memory systems

Memory can be further divided into memory systems, which encode information of differing quality and can work independently from each other. The generally accepted division of memory systems differentiates between declarative and non-declarative memory (Squire, 2004; see Figure 1). Declarative memory - sometimes also called explicit memory[2] - refers to the memory for facts (= semantic memory) and events (= episodic memory), while non-declarative - or implicit - memory refers to different forms of memories like skills, habits and conditioned responses. For declarative memory, the medial temporal lobe (e.g. hippocampus, rhinal cortices) is of critical importance, as shown by lesion studies in animals as well as in patients suffering lesions in this region (Scoville & Milner, 1957). Non-declarative memories

[2] The terms 'explicit/implicit memory' originally referred to different types of memory resulting from different learning modes (i.e., the subject is actively attending to the stimulus and deduces rules facilitating learning or not), but did not state the existence of different underlying memory systems in contrast to the postulation of Squire using the terms 'declarative/non-declarative'. However, in the literature these terms are sometimes used interchangeably.

rely on several brain regions, e.g. while priming depends mostly on neocortical areas, procedural memories like skills and habits are processed mainly by the striatum (Squire, 2004). It is to note that these classical divisions between memory systems and associated brain structures have been challenged by recent findings indicating that memory is more flexible and systems can interact much more than has been assumed based on relatively simplistic theoretical models (Henke, 2010). However, the depicted model is still widely acknowledged as a valid theoretical background for investigations on memory.

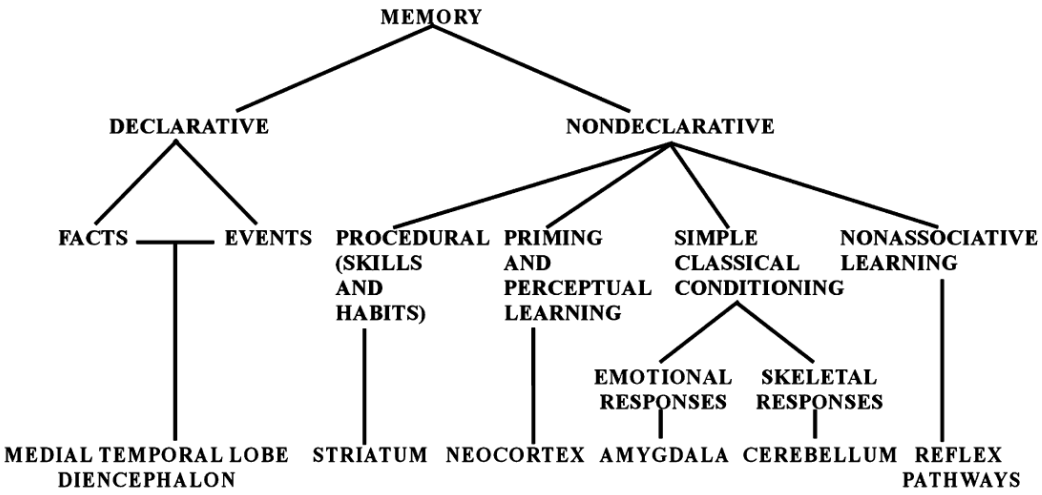

Figure 1.1. Taxonomy of mammalian long-term memory (from Squire, 2004).

1.1.4 Assessment of memory functions in rodents

Although the above described division of memory systems is in general assumed to be valid for all mammalian species, the terms declarative/explicit and non-declarative/implicit stem from research in human subjects and refer to the inherent quality of declarative memories to be easily verbally reportable - while facts and episodes can be told to someone else, it is difficult or even impossible to verbalize procedural or perceptual memories. This quality is reflected by tasks measuring declarative memories in human subjects, which mostly rely on verbalization e.g. in form of reports of prior learned wordlists or a verbal response in recognition tasks. Obviously, in experimental animals this approach is not feasible, so that "declarative" memory tasks in rodents can use only indirect behavioral measures to infer on hippocampus-dependent memory. Examples for these indirect measures are a decline of error rates of responses required to reach a certain goal in the course of memory acquisition (e.g. find food in a maze) or patterns of exploratory behavior (e.g. increased exploration of a novel object). Special care in the conduction of experiments, in the analysis and interpretation of these indirect measures is necessary, since other factors beside a pure memory component

(e.g. motivation, locomotory drive, stress-level) can influence these measures. To investigate hippocampus-dependent memory in rodents, commonly used tasks require remembering spatial or contextual information of the environment, since the dependence of this type of information is well known to rely on intact hippocampal function (Jarrard, 1993). In the following, the memory tasks used in this work are described in detail.

1.1.4.1 The object-place recognition task

The object-place recognition task (OPR) makes use of the innate preference of rodents for novelty in their environment. Therefore, it has the advantages that it relies on natural, spontaneous behavior of the animal and does not involve stressful treatments like prior food deprivation or the application of anxiety or pain inducing procedures to motivate learning. Additionally, it can be conducted using only a single learning trial (= one-trial task), and a repeated measurement of the same animal is feasible by using different objects at different positions in different sessions, which enables a powerful within-design of experiments (i.e., an animal can serve as its own control). The task was developed by Ennaceur, Neave, & Aggleton (1997), based upon a similar task, the novel object recognition task (NOR; Ennaceur & Delacour, 1988). The OPR task – but not the NOR task – depends critically on intact hippocampal function (Bussey, Duck, Muir, & Aggleton, 2000; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; Oliveira, Hawk, Abel, & Havekes, 2010) and was validated for rats as well as for mice (Dere, Huston, & De Souza Silva, 2007).

The general procedure is as follows: The animal is placed in an open field (= an open-top box), which is familiar to it from prior habituation sessions. In the first trial, the open field contains two identical objects, which the animal has never encountered before and which do not have any ethological meaning. The animal is free to explore these objects for a certain amount of time. This part of the task is called Sample trial. Subsequently, the animal is placed somewhere outside the open field, usually in its home cage or a waiting box, and after a retention interval it is put back into the open field. Now, the same two objects are presented again, but one of these objects has been moved to another place. This part of the task is called the Test trial. Due to the innate novelty preference of rodents, the animal will explore the displaced object more intensely than the stationary one. But, most importantly, this intensified exploration of the displaced object will only occur if the animal remembers the spatial configuration of the objects from the Sample trial. A schematic drawing of the procedure is given in Figure 1.2. To measure if the animal shows intact memory, a preference index (P-Index) is computed, calculated by the ratio between the time the animal spent exploring the displaced object and the time the animal explored both objects:

$$P\text{-}Index = \frac{exploration\ of\ displaced\ object}{exploration\ of\ displaced\ object + exploration\ of\ stationary\ object}$$

Thus, if exploration of both objects is equal, the P-Index would be 0.5 (chance level). A P-Index > 0.5 indicates a preference for the displaced, an index < 0.5 a preference for the stationary object. It is to note that the P-Index task infers recognition memory from relative measures of exploratory activity. Since recognition is by definition more or less binary (either something is recognized or not) a P-Index of e.g. 0.9 does not indicate 'better' recognition memory than a P-Index of e.g. 0.75. Moreover, in addition to the memory component, the P-Index of an individual animal reflects further behavioral qualities, for instance an individual tendency to explore more intensely novel objects in its environment. However, by using a within-subjects design, a partial cancellation of non-mnemonic qualities can be expected. In summary, the critical comparisons in this task are the groups' P-Index against chance level of 0.5 and within subjects' comparisons.

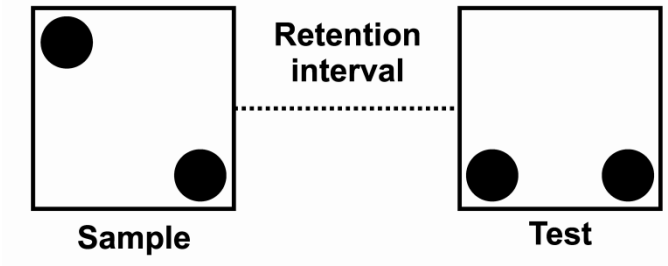

Figure 1.2. Schematic drawing of the Object-Place Recognition task. Two objects are presented in an open field during a Sample trial, and after a retention interval the same objects are presented again in a Test trial. Now, one of the objects is displaced to another corner. If an animal remembers the position of the objects from the Sample trial, due to rodents' innate preference for novelty, it explores the displaced object longer than the stationary one.

1.1.4.2 The radial maze task

The radial maze task uses food or water baits to motivate behaviour, and therefore relies on the motivation of the animal to forage, which is usually reached by prior food or water deprivation. Originally developed by Olton & Samuelson (1976) to test spatial working memory, it was later adapted by Jarrard to additionally assess reference memory (Jarrard, 1983; Jarrard, 1995). Spatial working memory as well as reference memory depend critically on intact hippocampal function (Jarrard, 1995; Bouffard & Jarrard, 1988), however, the working memory component is additionally mediated by prefrontal cortical regions (Ragozzino, Adams, & Kesner, 1998).

The apparatus consists of a central platform equipped usually with 8 arms radiating from it. At the end of each arm, a food well is inserted, where baits not visible from the

central platform can be placed. Several versions of the apparatus are in use: with or without enclosure walls around the arms, being either opaque or transparent, and with or without doors separating the central platform from the arms. A schematic depiction of the apparatus is given in Figure 1.3. The general procedure of the reference-memory version of the task is as follows: Initially, the rat is habituated to the apparatus, usually in the presence of baits scattered everywhere within the maze to associate the apparatus with food reward. During the following learning trials, only food wells of certain arms contain bait.

The animal is placed onto the central platform and has the possibility to explore the maze for a defined amount of time or until it has found all the baits. Every entry into an arm which never contains bait is counted as a reference memory error, and every re-entry into an arm already visited within the same trial is counted as a working memory error. Sometimes working memory errors are further divided into working memory errors made for baited arms (where the food was already consumed) and into working memory errors for never baited arms. In other words, reference memory holds information which has to be kept in mind over different trials ("which arms contain bait?"), working memory holds information which is only relevant for the very same trial ("where have I been already within this trial?"). Most commonly, the task is conducted over 10-15 days, with 1-5 trials per day, always baiting the same arms.

Orientation within the maze is commonly enabled by the use of extra- and/or intra maze cues. While extra-maze cues favor the usage of allocentric navigation and therefore the development of hippocampus-dependent spatial memory, intra-maze cues favor the use of associative learning strategies, which can be accomplished without intact hippocampal functioning (for review see Hodges, 1996).

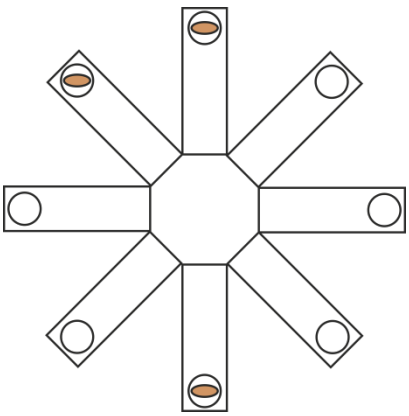

Figure 1.3. Schematic depiction of the radial maze apparatus. Empty circles at the end of the arms represent food wells, and brown ellipses represent food baits.

1.2 SLEEP

1.2.1 Functional and behavioral characterization

Sleep in mammals can be behaviorally characterized by the following criteria: assumption of a species-specific typical body posture, maintenance of behavioral quiescence, an elevated arousal threshold and state reversibility (Campbell & Tobler, 1984). Regular re-occurring periods of sleep or sleep-like behavior can be found in all vertebrates, and also in invertebrates like *C elegans* (roundworm) and *drosophila melanogaster* (fruit fly), although specific characteristics like sleep duration and electrophysiological parameters differ widely between the species (Allada & Siegel, 2008). The preservation of sleep throughout evolution suggests that sleep subserves important and probably even vital functions, especially since inactivity associated with reduced sensory responsiveness is disadvantageous for protection against predators and other vital functions like eating and mating behavior. Indeed, chronic disturbances of sleep or chronic sleep deprivation are risk factors for several diseases (Faraut, Boudjeltia, Vanhamme, & Kerkhofs, 2012) and prolonged total sleep deprivation leads to metabolic, hormonal, immunological and cerebral dysfunctions, and ultimately to death in rats (Everson, 1995). The acknowledgement of sleep as something more than just an “inactive state” of the organism is relatively new, and many of its putative functions are still in need of further investigation. In the light of the impact of sleep loss on so many different systems, the question “why do we sleep?” can at present probably not be answered definitively. However, accumulating findings from humans as well as from other animals indicate that one important function of sleep is the formation of memory (Stickgold, 2005; Diekelmann & Born, 2010).

1.2.2 Electrophysiological characterization

Sleep in mammals is usually subdivided into different stages, and at least two stages - rapid-eye movement (REM) and non-REM (NREM) sleep - could be found in all mammals studied so far (Tobler, 1995; Allada & Siegel, 2008). While NREM sleep can be broadly characterized on the level of electroencephalography (EEG) by the occurrence of synchronized, high voltage slow-wave activity with low muscle tone, during REM sleep a desynchronized, low voltage EEG activity similar to the wake state is seen, accompanied by muscle atonia and rapid eye movements. In humans, NREM sleep is conventionally further divided into 3-4 successively occurring stages. Precise scoring manuals define strict criteria for sleep scoring by EEG, EMG and EOG parameters (Rechtschaffen & Kales, 1968; Iber, Ancoli-Israel, Chesson & Quan, 2007).

In rats, several scoring systems have been developed, and sleep scoring does not follow as strict and unified criteria as in humans. While some authors divide NREM sleep into a light and deep sleep stage (Gottesmann, 1992; Neckelmann, Olsen, Fagerland, & Ursin, 1994), others do assign only one stage for NREM sleep (Borbely, Tobler, & Hanagasioglu, 1984; Fogel, Smith, & Beninger, 2009). With the exception of the staging system proposed by Gottesmann (1992), REM sleep is usually not further subdivided in the rat. Beside the lack of standardized scoring rules, the characteristics of sleep stages are more or less similar to the ones described above for humans: NREM (or SWS) is defined by slow EEG activity, occurrence of spindles and low muscle tone, REM sleep by low-voltage faster activity dominated by theta rhythm (5-10 Hz) and absent muscle tone. Additionally, many authors assign a third sleep stage, not presented by human scalp EEG, the so-called preREM or transitory stage (Gottesmann, 1992; Eschenko, Mölle, Born, & Sara, 2006; Schiffelholz & Aldenhoff, 2002). This short-lasting stage occurs at the transition from NREM to REM sleep (and sometimes from REM sleep to wake or to NREM sleep) and is characterized by high amplitude spindles of a duration up to ~3.5 s superimposed on first signs of theta activity (Gottesmann, 1996).

Sleep architecture is a further feature of sleep derived best from electrophysiological measures. The regularly occurring alternation between NREM and REM sleep allows for dividing a sleep period into sleep cycles. A sleep cycle is the alternation between NREM and REM sleep, with light NREM followed by deep NREM followed by REM sleep. The stability and length of a sleep cycle differs between humans and rats: Healthy humans show typically 3-5 of these cycles per night, each lasting about 90 minutes (see Figure 1.4A). In contrast, sleep cycles of rats last on average only 10-13 minutes, but high variability can be seen, and a cycle can be much shorter depending on its definition criteria (Trachsel, Tobler, Achermann, & Borbely, 1991). Another difference between sleep in humans and rats is the timing of the inactive phase - rats are nocturnal animals. Furthermore, rats' sleep is much more fragmented and not monophasic as in humans: short wake episodes can be seen during the inactive period, and short sleep episodes occur during the active period of the night (Tobler, 1995). Figure 1.4 depicts a comparison of typical 8-h hypnograms in humans and a 1-h hypnogram in rats.

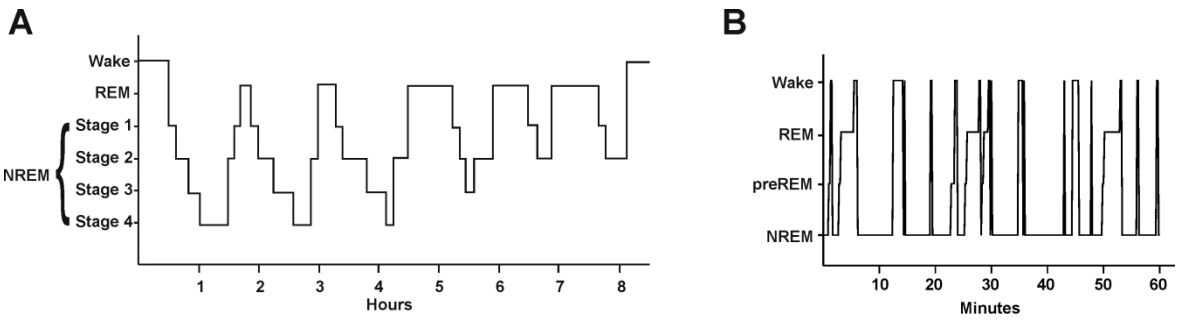

Figure 1.4. Hypnograms. **A**, Typical hypnogram of an 8-hour night sleep in a healthy human subject (modified from Diekelmann & Born, 2010). **B**, Typical hypnogram of one hour sleep in a rat during the early inactive phase. Note the short and variable sleep cycle length and the intermittent short lasting awakenings (data taken from study 1).

1.2.3 Neurochemical control mechanisms

Several brain regions and cell groups within the central nervous system, mainly located in brainstem and diencephalon, show characteristic activity patterns during the different vigilance states of the organism and interact with each other to promote stability of and transition between these states. Key regions are the ventrolateral preoptic area - a cell group rostral to hypothalamus - which shows high activity during sleep and neuromodulatory cell groups in basal forebrain, pons and hypothalamus. Shortly, cholinergic, orexinergic and monoaminergic neurons are highly active during wakefulness and decrease their firing rate during NREM sleep. In REM sleep, orexinergic and monoaminergic neurons are almost silent, while cholinergic neurons show wake-like activity patterns, suggesting a participation in cortical activation (for detailed reviews see Saper, Fuller, Pedersen, Lu, & Scammell, 2010; Swick, 2005). It is assumed that these sleep and wakefulness promoting regions act together like bistable sleep-wake and NREM-REM switches through complex reciprocal excitation and inhibition patterns (Saper et al., 2010). Additionally, adenosine, which accumulates throughout phases of wakefulness due to enhanced energy consumption of the brain, can act as a homeostatic regulated sleep promoting modulator by interacting with these regions (Dunwiddie & Masino, 2001).

1.2.4 Brain rhythms of the sleeping brain

Oscillations of neurons and neuronal networks, which can be measured using EEG in healthy humans or local field potentials (LFP) in animals, do not merely reflect epiphenomena of neuronal activity but appear to be of functional relevance (Buzsaki & Draguhn, 2004). Oscillating neuronal assemblies play an important role for effective information processing, e.g. through binding together neuronal groups in distant cortical regions and by using phase-dependent encoding of external stimuli (Buzsaki & Draguhn, 2004). In the following sections

1.2.4.1-1.2.4.4, the most important brain oscillations during sleep detectable at the EEG level and their putative generating mechanisms are shortly described: sleep spindles, delta activity and slow oscillations during NREM sleep, and theta activity, which is the most prominent rhythm during REM sleep in rats. It is to note, however, that under natural conditions, many different oscillations are present at the same time and interact with each other (including faster oscillations like gamma not described in detail here).

1.2.4.1 Sleep spindles

Sleep spindles are waxing and waning waves between roughly 7 to 15 Hz, although their exact frequency range depends critically on the species studied and the cortical region (Steriade, 2003). In the rat, three different types of spindles have been described, differing in duration, amplitude and frequency: anterior spindles during slow wave sleep and anterior spindles during preREM sleep, both probably comparable in their thalamic origin with spindles in humans or cats, and posterior spindles putatively originating from some other subcortical region (Gandolfo, Glin, & Gottesmann, 1985; Sitnikova & Luijtelaar, 2003). Regarding spindle frequency in the rat, divergent findings were reported, ranging from relatively fast and narrow bands between 12-16 Hz (Fogel et al., 2009) to broad ranges of 5-15 Hz (Terrier & Gottesmann, 1978). If these discrepancies are related to recording conditions, analysis techniques and/or putative strain differences remains to be determined.

Spindles are generated mainly by the reticular nucleus of the thalamus (RE). Rhythmic spike bursts of the RE induce inhibitory postsynaptic potentials (IPSPs) in thalamocortical (TC) neurons, which in turn promote burst firing and are transferred to the cortex where rhythmic EPSPs are induced (Steriade, 2003). Additionally, spindle generation by the RE is facilitated by monoaminergic brainstem and corticothalamic afferents (Fuentealba & Steriade, 2005). During sleep spindles, TC neurons are inhibited, thus preventing external stimuli to be transferred to the neocortex (Fuentealba & Steriade, 2005). However, spindles trigger synaptic plasticity in thalamus and neocortex, which may contribute to sleep-dependent memory consolidation (Steriade & Timofeev, 2003). An example trace of spindle activity is given in Figure 1.5A.

1.2.4.2 Delta activity

Delta waves (1-4 Hz) originate from two different sources, a thalamic and a neocortical one. The thalamic component derives from intrinsic, clock like oscillations of TC neurons, which occur only after these neurons have become sufficiently hyperpolarized (< -65 to -70 mV). It is assumed that a progressive hyperpolarisation of the TC neurons during deepening of

NREM sleep is caused by a decrease in firing rates of afferent corticothalamic, midbrain and mesopontine cholinergic neurons. Another component of delta wave generation is cortically driven and can still be seen after thalamectomy (Steriade, 2003).

1.2.4.3 Slow oscillations

The existence of this slow rhythm (~ 1Hz) of cortical origin was first reported just about 20 years ago (Steriade, Nunez, & Amzica, 1993a). As depicted in Figure 1.5B, on the single-cell level slow oscillations consist of alternating phases of de- and hyperpolarizations, also termed UP- and DOWN states, which are reflected also in EEG and cortical LFP recordings (Achermann & Borbely, 1997; Steriade et al., 1993a). That slow oscillations (SOs) are indeed generated within the neocortex could be shown by their survival after thalamectomy (Steriade, Nunez, & Amzica, 1993b), their absence in the thalamus of decorticated animals (Timofeev & Steriade, 1996) and by their occurrence in cortical slices in vitro (Sanchez-Vives & McCormick, 2000). However, in the intact animal SOs are reflected in several subcortical structures as the basal ganglia and hippocampus (Magill, Bolam, & Bevan, 2000; Wolansky, Clement, Peters, Palczak, & Dickson, 2006). Importantly, SOs can group other sleep oscillations like delta waves and spindles through interaction with thalamic nuclei, but also faster rhythms like beta and gamma are coupled to the UP-state of the SO (Sirota & Buzsaki, 2005; Steriade, 2006). It has been suggested that the delta waves of SWS, especially their hyperpolarizing phase, probably represent a faster and less potent equivalent of the SO (Buzsaki, 2006). As spindle activity, SOs have been linked to plasticity processes in thalamus and neocortex (Steriade, 2006; Steriade & Timofeev, 2003).

1.2.4.4 Theta activity

Theta activity (~ 5-9 Hz) is generated in hippocampus and entorhinal cortex and is present mainly during active exploration and REM sleep (Sirota & Buzsaki, 2005). In the rat, the prominent theta rhythm seen in epidural recordings is most probably a reflection of these subcortical oscillators, although there are indications that other brain regions including the neocortex are capable of generating their own theta fields (Sirota & Buzsaki, 2005; Kahana, Seelig, & Madsen, 2001; Young & McNaughton, 2009). Hippocampal theta depends strongly on cholinergic and GABA-ergic inputs from medial septum/diagonal band of broca, and has been implicated in phase-dependent synaptic plasticity and encoding of place information during wakefulness (Kahana et al., 2001). An example trace of theta activity in rats is shown in Figure 1.5C.

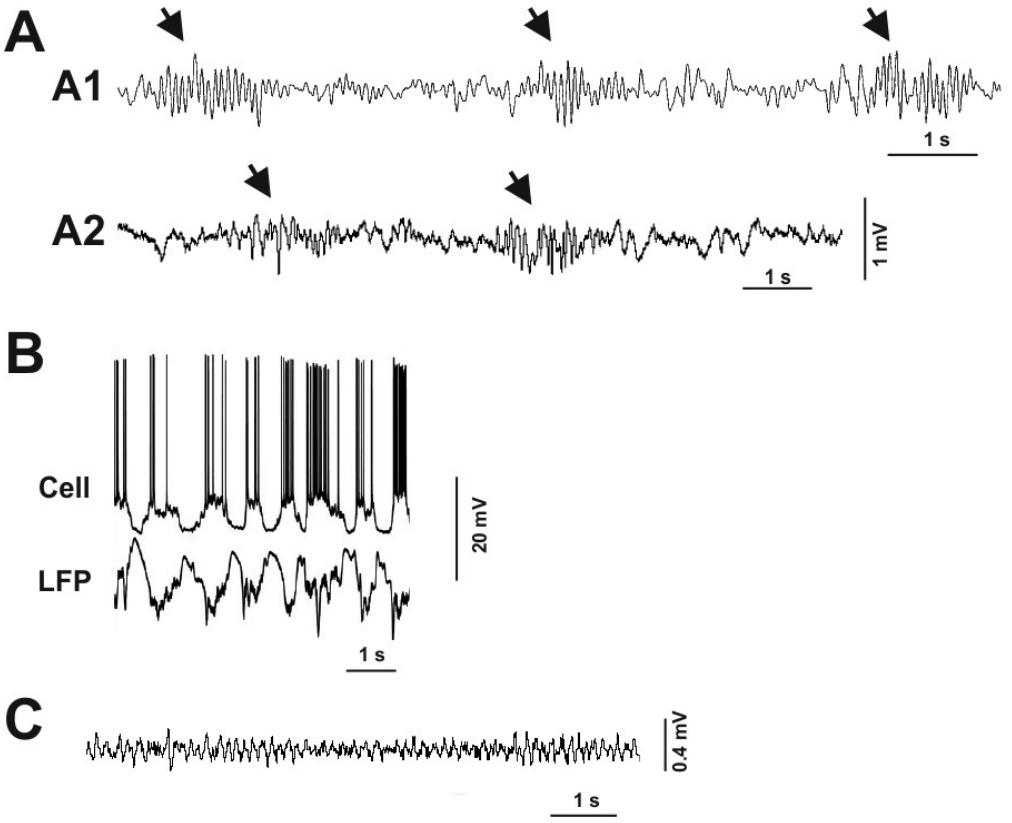

Figure 1.5. Examples of major brain oscillations during sleep. A, Example trace of human scalp EEG (A1; modified from Contreras, Destexhe, Sejnowski, & Steriade (1997) and a rat epidural EEG (A2; from study 1) during early SWS. Spindle events are marked by arrowheads. **B,** Slow oscillations (SO) recorded simultaneously intracellular (Cell) and extracellular (LFP) in the cortex of a sleeping cat. During UP states, the cell is highly active; during DOWN states no action potentials are generated. Note the reflection of UP- and DOWN states in the LFP recording below. (modified from Mukovski, Chauvette, Timofeev, & Volgushev, 2007). **C**, Theta activity during REM sleep in an epidural recording of a rat (from study 2).

1.3 SLEEP AND MEMORY

1.3.1 Memory consolidation during sleep and neurophysiological mechanisms

That sleep promotes the consolidation of memory has been shown in numerous studies on several memory systems, in humans as well as in rodents (Diekelmann & Born, 2010; Stickgold, 2005). It is assumed that two types of consolidation are favored by sleep, namely cellular consolidation (sometimes also referred to as 'synaptic consolidation', see Diekelmann & Born, 2010), which refers to plasticity processes at the cellular level induced by learning; and systems consolidation, which refers to the transfer of the memory trace from a temporary store to a long-term storage site (Frankland & Bontempi, 2005; McClelland, McNaughton, & O'Reilly, 1995; Marr, 1971). For declarative memories, the widely accepted model for system

consolidation proposes that the hippocampus serves as the temporary store, while the neocortex is the site for long-term storage: Memories are encoded in parallel into hippocampus and neocortical areas, and subsequent reactivations of the hippocampal-neocortical network ('replay' of the memory trace) lead to a gradual strengthening of cortico-cortical connections, ultimately leading to independence of the memory trace from the hippocampus. This process is assumed to take place preferentially and most effectively 'offline', when no new to be encoded stimuli are present, i.e. during sleep (Diekelmann & Born, 2010; Frankland & Bontempi, 2005). Indeed, such replay of neuronal activity patterns during sleep mirroring those patterns which occurred during prior learning have been found in several brain regions and species (Frankland & Bontempi, 2005). One example is the coordinated replay seen in the CA1 region of the hippocampus in rats, where neurons, which code for place, fire during subsequent sleep in the same temporal order as during prior waking exploration of the environment (Lee & Wilson, 2002). Interestingly, these replay events in rodents take place preferentially during hippocampal sharp-wave ripples (SPWs), high-frequency bursts which occur abundantly during SWS (Wilson & McNaughton, 1994; Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009). Suppression of these ripples in rats impairs heavily the consolidation of spatial memory (Girardeau, Benchenane, Wiener, Buzsaki, & Zugaro, 2009), hinting towards a causal role of replay for memory consolidation. Animals experiments have shown SPWs again to occur in tight temporal correlation with neocortical sleep spindles (Siapas & Wilson, 1998; Mölle, Eschenko, Gais, Sara, & Born, 2009), a rhythm which was also implicated to trigger plasticity processes in neocortex (Rosanova & Ulrich, 2005; Sejnowski & Destexhe, 2000; Timofeev et al., 2002). Several studies in humans as well as in rodents have shown increased spindling activity after learning, as well as a correlation between spindle activity and later retrieval success (Eschenko et al., 2006; Schabus et al., 2004; Fogel & Smith, 2011). As mentioned before, spindles and SPWs again are coordinated by the neocortical SO, which makes this rhythm a candidate key player in the process of memory consolidation (Isomura et al., 2006; Mölle, Yeshenko, Marshall, Sara, & Born, 2006). Indeed, SO activity was shown to be enhanced after learning, especially in brain regions involved in pre-sleep learning (Huber, Ghilardi, Massimini, & Tononi, 2004; Mölle et al., 2009; Mölle, Marshall, Gais, & Born, 2004), and as to be explained in greater detail in the following sections, boosting SOs by acoustic stimulation (Ngo, Martinetz, Born, & Molle, 2013) or transcranial direct current stimulation (Marshall, Helgadottir, Mölle, & Born, 2006) lead to enhanced memory consolidation for a declarative task. A schematic depiction of systems consolidation and involved neuronal oscillations is given in Figure 1.6.

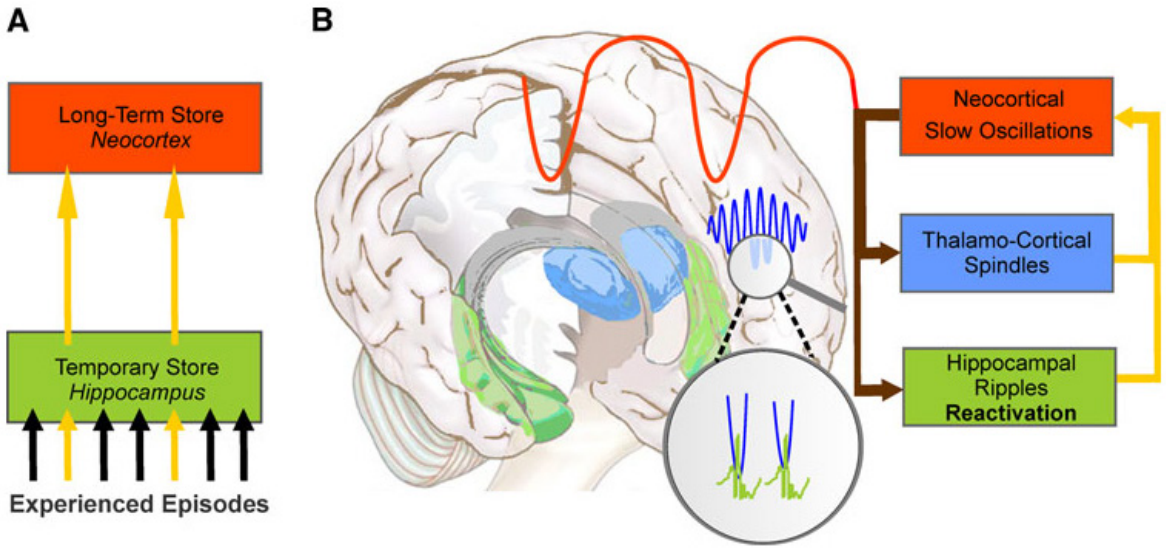

Figure 1.6. Active system consolidation during sleep. A, Episodes (black and yellow arrows) are encoded during wakefulness temporarily into hippocampus. Only some of them (yellow arrows) are reactivated during subsequent sleep and redistributed into neocortex for long-term storage. **B**, The depolarizing UP-state of the neocortical SOs (red) groups thalamo-cortical spindles (blue) and hippocampal SPWRs (green), which are nested within the troughs of spindles (from Born & Wilhelm, 2012).

Other hypotheses focus on the role of different sleep stages for memory consolidation: It has been suggested, that SWS and REM sleep are of differential importance for different memory systems and aspects of memory: As described in the preceding paragraph, SWS has been associated with the consolidation of hippocampus-dependent (declarative) memories in general (Diekelmann & Born, 2010), whereas REM sleep favors the consolidation of procedural memory and emotional aspects of declarative memories (Nishida, Pearsall, Buckner, & Walker, 2009; Wagner, Gais, & Born, 2001; Plihal & Born, 1997). On the other hand, sequential hypotheses of sleep-dependent memory consolidation assume that the succession of SWS and REM sleep is necessary for successful memory consolidation to take place (Ambrosini & Giuditta, 2001), which lead to the proposal that systems consolidation takes place mainly during SWS, while synaptic plasticity is mainly confined to the following REM sleep episodes (Diekelmann & Born, 2010). Indeed, an up-regulation of plasticity-related immediate early genes (IEGs) was found in an experience-dependent manner during REM sleep in neocortex, but not during preceding SWS (Ribeiro et al., 2007). A putative mechanism could be that the induction of IEG up-regulation is primed by spindle activity and the related Ca^{+}-influx into neurons during prior SWS (Diekelmann & Born, 2010), and congruent with this assumption IEG levels were correlated to SWS-spindle activity in the study by Ribeiro et al. (2007). An additional factor which may favor synaptic plasticity

processes during REM sleep could be the elevated cholinergic tone during this sleep stage, which is known to facilitate IEG expression and long-term potentiation (LTP) (Diekelmann & Born, 2010; Teber, Kohling, Speckmann, Barnekow, & Kremerskothen, 2004; Lopes et al., 2008).

SWA has also been attributed a main role in synaptic homeostasis: The synaptic homeostasis hypothesis assumes that sleep serves mainly a homeostatically regulated downscaling of synaptic weights (Tononi & Cirelli, 2006), and in this way contribute to memory consolidation during sleep. In contrast to the aforementioned model, within this framework, synaptic plasticity processes are assumed to take place mainly during SWS: Synapses are potentiated during wakefulness, and depotentiated ("downscaled") during SWS, especially during SO, thereby increasing the signal-to-noise ratio, which benefits learning and memory. Evidence for these assumptions stem e.g. from increased and decreased levels of LTP-related proteins and genes during wakefulness and sleep, respectively (Cirelli, Gutierrez, & Tononi, 2004; Vyazovskiy, Cirelli, Pfister-Genskow, Faraguna, & Tononi, 2008), and the phenomenon of locally increased SWA in brain regions highly demanded during prior wakefulness (Huber et al., 2004). The synaptic homeostasis hypothesis has however recently been criticized as being oversimplifying in its view on synaptic plasticity, as no clear statement is made about the precise plasticity mechanisms involved: e.g., the sleep-dependent up- and downregulation of plasticity related substances like BDNF or Arc, regarded as supporting the hypothesis, can have strengthening as well as weakening effects on synaptic plasticity (Frank, 2012).

1.3.2 Rodent models in research on sleep and memory

Early studies on the memory function of sleep in rodent models mainly concentrated on REM sleep and the effects of sleep deprivation. With the exception of work done on the sequential hypothesis of sleep, investigations on the specific functions of SWS in the rodent remained sparse until recently. As an illustrative example, a database search on Pubmed using the keywords "SWS", "learning" or "memory", and "rat" yields just 35 results, contrasting a hit list of 268 articles by replacing "SWS" with "REM".

First evidence for an increase in REM sleep duration in rats after massed learning of food locations in a maze was found already in the early 1970s (Lucero, 1970). Following investigations could replicate and extend this finding (Pearlman, 1979). Most importantly, many studies found specific time windows during post-learning sleep termed REM-windows, during which selective deprivation of REM sleep lead to memory impairments (Smith & Rose, 1996; Smith, Conway, & Rose, 1998). It was postulated that the exact timing of these

REM-windows depend heavily on learning-intensity (Smith & Rose, 1996). A role of SWS for learning and memory in the rodent model was firstly postulated by the sequential hypothesis (Giuditta, 1985), and studies conducted within this framework could show that successful learning in an active-avoidance task is not only correlated with specific REM windows, but also with an increment in SWS after learning (Ambrosini, Langella, Gironi Carnevale, & Giuditta, 1992). Some later findings confirmed an influence of learning on several parameters of SWS in rodents, including on its duration (Hellman & Abel, 2007; Magloire & Cattarelli, 2009) and on spindle activity (Eschenko et al., 2006; Fogel et al., 2009). Studies investigating the impact of post-learning sleep deprivation come to the conclusion that sleep deprivation has especially detrimental effect on hippocampus-dependent memory, but not on memories which does not rely critically on hippocampal function, and that this effect is most pronounced if sleep deprivation is applied immediately after learning during a time of day when naturally SWS power is highest (Graves, Heller, Pack, & Abel, 2003; Guan, Peng, & Fang, 2004). These findings on the importance of SWS in the rodent compares well with similar findings in human subjects.

The value of rodent models for research on sleep-dependent memory function is manifold. Firstly, as in other fields in neuroscience, rodent models allow the application of invasive techniques like selective brain lesions, pharmacological interventions and intracranial electrophysiology. Secondly, in comparison with other animals, the techniques to apply the aforementioned interventions as well as behavioral paradigms are well developed. Furthermore, progress in genetical engineering provides more and more genetically modified animals giving the opportunity to selectively study the impact of specific genes and proteins on sleep and memory. Thirdly, beside the already mentioned differences in sleep cycle length, the characteristics of sleep and its relevance for learning (e.g. high SWA during the beginning of the inactive period, learning induced increase in spindle activity, dependence of hippocampus-dependent memory on undisturbed early sleep) are comparable with humans.

1.4 OSCILLATORY TRANSCRANIAL ELECTRIC STIMULATION

Different techniques are in use to influence neuronal activity by electric fields, varying in their mode of action, their degree of invasiveness and the setting within they are applied. Prominent examples used in a clinical setting are electro-convulsive therapy to treat severe depression and deep brain stimulation to alleviate symptoms of Parkinson disease, two techniques considered highly invasive (Kellner et al., 2012; Fasano, Daniele, & Albanese, 2012). A technique to interfere with neuronal activity noninvasively is transcranial magnetic stimulation (TMS), mainly used in the context of neuroscientific research, which induces

action potentials in the affected brain regions (Pascual-Leone et al., 1998). In contrast, weak forms of transcranial electric current stimulation (tES) - a term used to characterize a family of non-invasive stimulation techniques - does not induce action potentials, but is presumed to modulate the membrane potentials of neurons and interfere with brain rhythms as in case of oscillatory stimulation by application of weak currents (Paulus, 2011; Herrmann, Rach, Neuling, & Struber, 2013). An overview of different tES types is given in table 1.1.

Table 1.1 Overview of different types of transcranial electric stimulations

Name	*Abbreviation*	*Description*
Transcranial electric stimulation	tES	General term to describe several forms of transcranially applied weak electric stimulation
Transcranial direct current stimulation	tDCS	Weak transcranially applied DC currents
Transcranial alternating current stimulation	tACS	Weak transcranially applied AC currents
Transcranial random noise stimulation	tRNS	Weak transcranially applied currents of randomly fast changing intensity
Oscillatory tDCS	e.g. so-tDCS, theta-tDCS	Weak transcranially applied oscillating DC currents, either cathodal or anodal

1.4.1 tES - Modes of action and applications

Early animal experiments could show that the polarization of the cortex by weak, subthreshold DC fields leads to acute effects on spontaneous neuronal activity: While the application of anodal fields led to an increase in neuronal firing, cathodal fields reduced neuronal activity (Bindman, Lippold, & Redfearn, 1962; Creutzfeld, Fromm, & Kapp, 1962). However, the precise effect these fields exert on specific neurons depends heavily on their orientation towards the DC field and depth within the cortical tissue (Purpura & McMurty, 1965; Kabakov, Muller, Pascual-Leone, Jensen, & Rotenberg, 2012). Most interestingly, depending on strength and duration of the applied current, long-lasting after effects in form of increased or decreased neuronal excitability could be seen for anodal and cathodal polarization, respectively (Bindman et al., 1962). It could be shown that these after-effects of anodal polarization depend on NMDA-receptors, associated intracellular calcium accumulation and/or IEG expression (Islam, Aftabuddin, Moriwaki, Hattori, & Hori, 1995; Islam et al., 1995). Later studies confirmed the importance of glutamatergic neurotransmission for after-effects of anodal and cathodal tDCS and extended these findings,

implicating the involvement of GABA for the effects of anodal, but probably not cathodal tDCS (Stagg et al., 2009) and the neuromodulators acetylcholine, serotonin and dopamine (Stagg & Nitsche, 2011). On the contrary, human studies mainly conducted by the Göttingen group using several pharmacological interventions suggest that the acute (= intrastimulation) effects of both anodal and cathodal tDCS on neuronal excitability indeed rely solely on effects on resting membrane potential but not on modulation of GABAergic or glutamatergic neurons (Stagg & Nitsche, 2011).

The vast majority of studies in basic research on tES has been done on human subjects, concentrating on effects on the motor cortex and on motor learning (Stagg & Nitsche, 2011; Nitsche et al., 2008), but there are also studies investigating the effects of tDCS in humans in other domains, e.g. working memory, time perception, attention and language (Jacobson, Koslowsky, & Lavidor, 2012; Nitsche et al., 2008; Elbert, Rockstroh, Lutzenberger, & Birbaumer, 1981). Another ever growing field consists of clinical research, assessing possible therapeutic use of tDCS in depression, stroke rehabilitation, schizophrenia, epilepsy and other neurological conditions.

To apply tDCS, most commonly big sponge electrodes are used, placed over the brain region of interest, with reference electrodes either placed over the contralateral orbit, extracephalic regions or somewhere over the contralateral skull (Nitsche et al., 2008). Recent modeling studies, however, suggest that small electrodes are superior in producing focal effects and furthermore stress the relevance of careful placement of reference electrodes (Faria, Hallett, & Miranda, 2011; Bikson, Datta, Rahman, & Scaturro, 2010).

Another important aspect of tES is the applied current density (defined as the quotient of current strength and electrode size), as larger current densities were shown to induce stronger effects of tDCS (Nitsche et al., 2008). However, too high current densities can cause adverse effects as skin irritation in humans, and very high intensities (> 14.3 mA/cm²) of cathodal tDCS in rats were shown to induce lesions in brain tissue (Liebetanz et al., 2009; Nitsche et al., 2008). Commonly, in studies on human subjects current densities below 0.03 mA/cm² are used (Nitsche et al., 2008), but even much higher values of up to 0.52 mA/cm² were successfully applied without any reports of adverse side effects (e.g. Marshall et al., 2006; Eggert et al., 2013).

1.4.2 Oscillatory weak electric currents - a method to probe brain rhythms

In contrast to tDCS, stimulation techniques using oscillatory forms of stimulation (e.g. tACS and oscillatory tDCS) are presumed to be effective by interacting with ongoing oscillations of the brain (Paulus, 2011). Neuronal activity creates electric gradients within the extracellular

space. As depicted in Figure 1.7, these fields can potentially influence the membrane potential of a neighboring neuron, a principle which is called ephaptic coupling (Jefferys, 1995). While it is unlikely that activity or subthreshold oscillations of a single neuron affect the excitability of other neurons to a greater extent, a group of neurons, which have similar orientation in space and oscillate in synchrony produce much larger fields and therefore exert much stronger effect on neighboring neurons as well as on itself (Weiss & Faber, 2010; Buzsaki, Anastassiou, & Koch, 2012). It was proposed that these fields, which occur during endogenous brain oscillations, may play a functional role in synchronizing neuronal networks (Weiss & Faber, 2010). Experimental findings conducted in slice preparations indeed could show that externally applied fields, which lie within the range of the very weak endogenous fields produced by network oscillations, are effective in entraining neuronal activity and that neuronal networks are more sensitive to these fields than single neurons (Deans, Powell, & Jefferys, 2007; Francis, Gluckman, & Schiff, 2003; Anastassiou, Perin, Markram, & Koch, 2011).

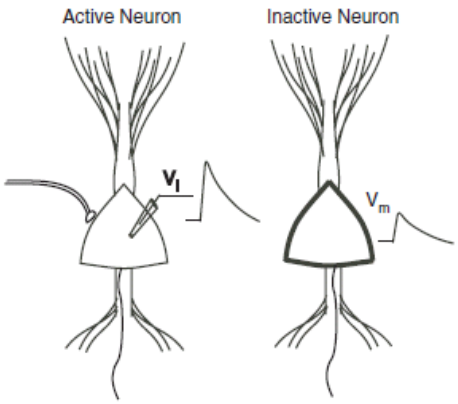

Figure 1.7. Ephaptic coupling between two neurons. An EPSP in the soma of the left neuron causes a depolarization of the inactive right neuron (modified from Weiss and Faber, 2010).

Several studies in human subjects could show the efficacy of oscillatory tES on endogenous brain oscillations as well as on behavior (Zaehle, Rach, & Herrmann, 2010; Kirov, Weiss, Siebner, Born, & Marshall, 2009; Marshall, Kirov, Brade, Mölle, & Born, 2011). The application of slow oscillatory tDCS (so-tDCS) during early SWS in human subjects, oscillating at the endogenous frequency of SOs, could transiently enhance endogenous SO and spindle activity and improved the consolidation of declarative, but not non-declarative memory (Marshall et al., 2006). This study is of special relevance, as it could show for the first time a causal relationship between SO activity, sleep spindles and memory consolidation, and, additionally, hints toward a functional role of extracellular fields for memory consolidation during sleep. It was assumed that so-tDCS leads to an acute

entrainment of endogenous SO activity, as deduced from the coherent SOs detected immediately after stimulation ended. Due to artifactual EEG a direct measurement was precluded during acute so-tDCS. Work done by Fröhlich & McCormick (2010) in ferret brain slices, which exhibit spontaneous SO-like activity patterns, strengthened this assumption: As depicted in figure 1.8, the application of a weak sinusoidal field at SO frequency entrained the endogenous oscillation. Similar entrainment was found in rats in vivo by Ozen et al. (2010), who could show acute entrainment of neuronal activity to sinusoidal tES at 1.25 Hz. Interestingly, the magnitude of entrainment depended heavily not only on stimulation intensity, but also on the behavioral state of the animal, as stimulation was most effective if the animal was in SWS.

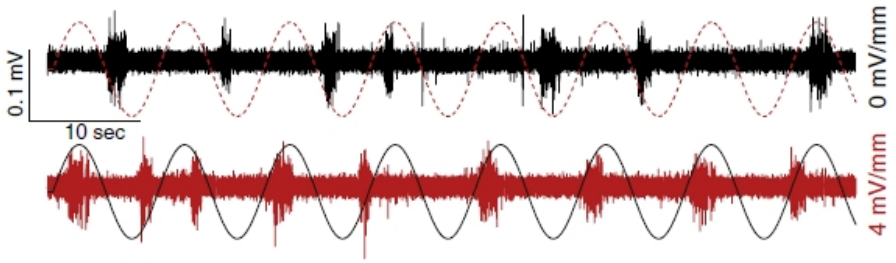

Figure 1.8. Multi-unit activity of a ferret brain slice showing spontaneous UP- and DOWN states (upper trace, black). The dashed sinusoidal line in the upper trace represents a 'virtual extension' of the sinusoidal field, however, no field is applied here. During the application of a weak sinusoidal field, multi-unit activity becomes entrained to the external field - represented by the black sinusoidal line - over time (lower trace, red; taken from Fröhlich and McCormick, 2010).

1.4.3 Animal models of tES

Beside the aforementioned study on freely moving rats by Ozen et al. (2010), very few chronic animal studies have investigated the effects of tES. Studies in rats by Islam et al. were conducted in awake and unrestrained animals, however, stimulation electrodes were firstly in contact with the dura mater, and therefore possibly not comparable to transcranial stimulation, and secondly and most importantly, electrodes made of silver were used, a material known to be toxic for tissue and therefore not well suited for chronic experiments (Merrill, Bikson, & Jefferys, 2005). Another tDCS model in rats, developed by the group around Liebetanz (e.g. Liebetanz et al., 2009) and transferred with minor variations also to mice (Cambiaghi et al., 2010), uses relatively big electrodes (~ 3-4 mm²), in this way disabling focality on defined brain areas. Additionally, in this model very large counterelectrodes on the chest of the animal are used, fixed by a jacket the animal has to wear continuously, may hindering the animal to show natural behavior, thus making this model less suitable for behavioral studies. Recently, a rabbit model for tDCS has been developed (Marquez-Ruiz et al., 2012). However, the tDCS

setup here requires the animal to be restrained in a holding frame, reducing the possibility to conduct behavioral studies to very simple conditioning paradigms. In summary, most tDCS models established in animals so far either require restraint before application of the current, use electrode montages not suited for long-term behavioral experiments or lacking focality by polarizing wide areas of the brain.

1.5 OBJECTIVES AND HYPOTHESES

Following aims should be reached by this work: In a first step, a behavioral rat model should be established, enabling the investigation of the role of sleep for memory consolidation on both a behavioral and an electrophysiological level in a hippocampus-dependent task providing good comparability to tasks applied in human subjects. This model should be further used to characterize brain oscillations participating in sleep-dependent memory consolidation in the rat. In a second step, a rat model of tDCS should be developed, enabling the focal application of weak electric current transcranially in awake, unrestrained and naturally behaving animals. This technique should be used to investigate the effects of so-tDCS on behavior and sleep-associated brain oscillations in the behavioral model established in step one. In a third step, the effects of multiple so-tDCS sessions should be investigated, using another learning task which requires learning over several days, thereby investigating more closely long-term memory processes.

Study 1: Sleep-dependency of memory consolidation in a hippocampus-dependent task in rats

As stated in the introduction, in the past most studies investigating the contribution of sleep to memory processes in rats have focused on REM sleep and less work has been done on the relevance of SWS. More importantly, compared to human studies, most mnemonic tests for long-term memory assessment in rodents, e.g., maze learning and conditioning experiments, employ relatively extensive training sessions and stressful procedures; and in part massive sleep deprivation protocols were involved (Fishbein & Gutwein, 1977; Rabat, Bouyer, George, Le, & Mayo, 2006; Smith et al., 1998; Youngblood, Zhou, Smagin, Ryan, & Harris, 1997). Also, in animal research a vast body of literature deals with post-learning modifications in brain electric activity ranging from the EEG/LFP to the cellular level (reviewed in Girardeau & Zugaro, 2011; Smith, 2011). In light of this increasingly detailed assessment of underlying neurophysiological processes, essentially from animal research and acknowledgement of the highly task-specific nature of sleep-dependent mnemonic processes

in animals and humans (Diekelmann, Wilhelm, & Born, 2009), a convergence of animal and human research is becoming a more pressing task.

To establish an animal model more closely comparable to human experiments on hippocampus-dependent episodic encoding in the declarative memory system, a behavioral task should be used fulfilling following criteria: hippocampus-dependency of the task, no involvement of stressful procedures like prior food-deprivation, feasibility for one-trial learning, possibility to conduct in a within design A task matching all these criteria is the object-place recognition task (Ennaceur et al., 1997; Mumby et al., 2002; Oliveira et al., 2010), which was therefore chosen as a behavioral model for study 1. Firstly, it was investigated if the consolidation of the task depends on sleep at all (spontaneous sleep vs. spontaneous wakefulness), in a second step it should be investigated which sleep-associated brain oscillations may play a role by using polysomnographic recording techniques during the retention interval. Additionally, to exclude any purely circadian influences, two sleep deprivation conditions were incorporated. It was hypothesized that:

- Only rats which predominantly slept during the retention interval are able to solve the task
- Test performance depends not critically on the time of day, but on sleep
- Characteristic electrophysiological parameters of SWS (SO, spindles) during the retention interval spent asleep after learning are enhanced in comparison to a baseline sleep episode
- REM sleep is not changed by this type of hippocampus-dependent task low in emotional content

Study 2: Effects of so-tDCS in rats on memory consolidation

Based upon the results of study 1, where enhanced SO activity during the retention interval could be shown, it was asked if boosting SO activity using so-tDCS during SWS would lead to a further enhancement in memory consolidation as shown before in humans (Marshall et al., 2006). To answer this question, and due to a lack of in vivo methods for focal tDCS in rodents, a method to apply so-tDCS in the rat had to be developed, which exerts focal field effects on brain regions comparable to the study by Marshall et al (2006), namely fontal/prefrontal areas. In pilot studies, the retention interval in the OPR task needed to be adjusted to ensure chance performance in the non-stimulation condition. Additionally, pilot studies examined any putative unspecific effects of so-tDCS on locomotion and emotionality. Since study 3 aimed at extending the results of this study using similar stimulation techniques, joint hypotheses are given at the end of the following section.

Study 3: Effects of repeated so-tDCS in rats on memory consolidation in a multiple trial learning paradigm

Based on the results of study 1 and 2, supporting the importance of SWS and its associated SO activity on hippocampus-dependent memory consolidation in rats, study 3 was conducted to investigate the effect of so-tDCS in a multi-trial task (radial maze) incorporating a learning phase of several days. For this task, studies by other groups already showed dependence of performance on intact sleep and on hippocampal ripple activity (Smith et al., 1998; Ramadan, Eschenko, & Sara, 2009; Girardeau et al., 2009). As explained in detail in the introduction, SO group ripple activity and boosting SO may lead to enhanced hippocampal-neocortical communication and thereby enhances memory consolidation. Additionally, this task is well suited to assess remote memory in follow-up studies. Instead of using trapezoidal so-tDCS as in Experiment 2 and in Marshall et al. (2006), here a sinusoidal stimulation signal was used, facilitating analysis of EEG signals during stimulation. Following hypotheses on the effects of so-tDCS applied during SWS should be tested:

- So-tDCS during SWS leads to an enhancement in memory consolidation as measured by (i) above chance performance in the Test trial of the OPR task (study 2) and (ii) faster decrease across days in reference memory errors in the radial maze task (study 3) compared to corresponding sham-stimulation conditions without so-tDCS.
- So-tDCS has no effects on unspecific behavior or working memory, as measured by (i) open field behavior in the pilot study of open field behavior in study 2 and (ii) locomotion and (iii) working memory in study 3.
- So-tDCS leads to a post-stimulation increase in endogenous SO and spindle activity (study 2 and 3), as well as to an enhancement in spindle activity during acute so-tDCS (study 3).

2 STUDY 1: SLEEP DEPENDANCY OF MEMORY CONSOLIDATION IN A HIPPOCAMPUS-DEPENDENT TASK IN RATS

Published as: Binder, S., Baier, P.C., Mölle, M., Inostroza, M., Born, J., Marshall, L. (2012). Sleep enhances memory consolidation in the hippocampus-dependent object-place recognition task in rats. *Neurobiology of Learning and Memory*, 97(2):213-9.

2.1 Methods

2.1.1 Experiment 1

2.1.1.1 Animals

Twenty-two male Long Evans rats (Janvier, Le Genest-Saint-Isle, France), 9-10 weeks old at the beginning of the experiments, were used. Animals were housed individually in Standard type IV Macrolon cages with ad libitum access to food and water under a 12h/12h light-dark cycle (lights-on 06.00 A.M.). Ten animals already took part in a pilot study to find optimal exploration times for the Sample trial of the OPR task, and underwent one to six trials in the same set up, but with different objects. All experimental procedures were performed in accordance with the European animal protection laws and policies (directive 86/609, 1986, European Community) and were approved by the Schleswig-Holstein state authority.

2.1.1.2 Handling

Before starting behavioral testing animals were handled daily for 10 min on seven consecutive days. Handling refers to a procedure to accustom the animals to human contact and to procedures which will be conducted in the following experiment. The rationale behind it is to reduce stress reactions during behavioral testing, which may influence the outcome. Handling procedures applied here consisted in taking the animal out of its home cage; keep it on the lap of the experimenter while touching it for ~ 9 min. Then, the animal was carried on the arm of the experimenter across the room, and finally put back in its home cage.

2.1.1.3 Apparatus and objects

OPR testing took place in a quadratic dark grey open field made of PVC (OF; 80 x 80 cm W, 40 cm H), dimly lit with 12 lux. A camera (model DFK1BU03, The Imaging Source, Germany) was mounted above the OF. The arm and foot of the camera as well as surrounding

furniture and posters affixed to the walls represented potential extra maze cues to facilitate spatial orientation.

Objects were glass bottles of different shape, texture and size (height 17-26 cm, bottom diameter 6-9 cm), each type filled with sand of a different color. They had sufficient weight to ensure the rats could not displace them. Objects and OF were cleaned thoroughly between trials with 60% ethanol solution.

2.1.1.4 General procedure and design

On three consecutive days prior to the first OPR session animals were habituated to the empty OF for five minutes per day. Habituation trials took place during lights-on between 11.00 A.M. and 05.00 P.M.

Each session consisted of a Sample trial, followed by a 2-hour retention interval, and a Test trial. During the Sample trial two identical objects were positioned in two far corners of the OF (Figure 2.1). The rat was put in the center of the OF and explorative behavior in reference to the objects was measured. Touching the object with vibrissae, nose and/or forelegs was counted as 'object exploration', merely close proximity to the object or contact to it while passing were not counted. After 60 s of exploration time across both objects or after reaching the cut-off criterion of 10 min spent in the OF the Sample trial was terminated and the animal was brought back to a transient housing room using a transportation box where it spent the 2-hour retention interval in its home cage. In the Test trial, the open field contained the same objects as before, but one object was now displaced to another corner. Test trial duration was 2 min.

Six to 13 hours prior to each testing session animals were brought from their initial housing room to a transient housing room next to the experimental room to reduce possible stress effects due to transportation and, in case of early morning tests, to prevent exposure to light during the dark phase. Animals were already habituated to this room during the open-field habituation sessions. Each rat was tested on two conditions according to a within-subject crossover design, a Morning session at the beginning of the inactive phase (between 06.00 – 07.00 A.M.), and an Evening session at the beginning of the active phase (between 06.00 – 07.00 P.M.). Order of sessions was balanced. Sessions were separated by five to six days, and different objects were used in each session. Positions of objects in Sample and Test trials and type of object were counterbalanced between the two retention interval conditions. Figure 2.1 shows a schematic depiction of the experimental procedure.

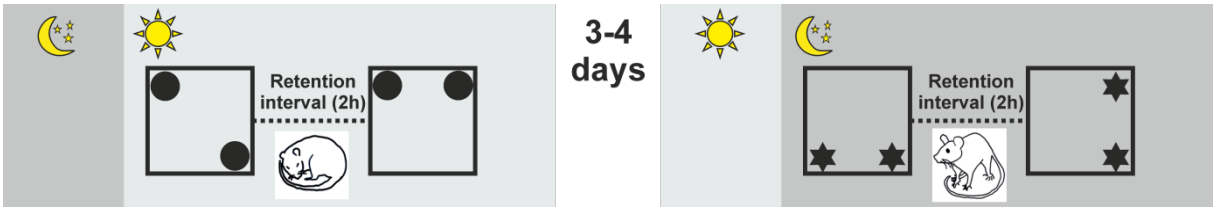

Figure 2.1. Schematic depiction of the experimental procedure. Animals are presumed to spent the retention interval mostly asleep in the Morning retention interval and mostly awake in the Evening retention interval due to their natural sleep-wake cycle. Note that order of Morning and Evening sessions as well as positions and types of objects were balanced.

2.1.1.5 Data reduction and statistical analyses

Scoring of explorative behavior was conducted semi-manually using tracking software (ANY-maze, Stoelting Europe, Ireland) by an experienced observer according to the above mentioned criteria. P-Index for object exploration within the Test trials was calculated as the quotient of exploration time of the displaced object and total exploration time. Because the preference for the displaced object tends to fade with elapsed time (Dix & Aggleton, 1999) the P-Index was computed separately for the first minute and for the total Test trial duration of 2 min.

Student's one-sample t-tests investigated whether the P-Index differed from chance level. Additionally, t-tests for dependent samples were used to compare the P-Index of Morning and Evening condition for the first and the total 2 min of the Test trial.

Total exploration time across both objects for each Sample and Test trial were compared between conditions using Student's t-test. Additionally, for the Sample trials Morning and Evening trial durations were compared.

2.1.2 Experiment 2

2.1.2.1 Animals

Twenty-two male Long Evans rats (Janvier, France), 9-10 weeks old by start of the experiments, were used. These animals were not identical with the ones used for experiment 1. Prior to start of the long-time recordings, they were housed in Standard type IV Macrolon cages with ad libitum access to food and water under a 12h/12h light-dark cycle (lights on 06.00 A.M.), first in groups of four, after surgery individually to prevent damage to the electrode montage. Seven days after surgery animals were moved to the recording boxes (see section 2.2.3) within the testing room and were housed there until the end of the experiments. All experimental procedures were performed in accordance with the European animal

protection laws and policies (directive 86/609, 1986, European Community) and were approved by the Schleswig-Holstein state authority.

2.1.2.2 Handling

Starting seven days prior to surgery animals were handled for 10 minutes daily. Handling procedure was in principle identical to experiment 1, except that animals were placed into the recording boxes (described under section 2.1.3.4) for 5 min afterwards to accustom to these boxes.

2.1.2.3 Surgery

Animals were anesthetized with ketamine (i.p., 75 mg/kg initial dose, 35 mg/kg supplements for maintenance) and xylazine (i.p., 5 mg/kg). Four screw-electrodes (Plastics One, USA) were used for EEG-recordings, two frontal (AP: - 2,6 mm, L: +/- 1,5 mm; according brain coordinates described by Paxinos & Watson, 2007) and two occipital reference electrodes (AP: -10,0 mm; L +/- 1,5 mm). For EMG-recordings, two insulated stainless steel wire electrodes (Plastics One, USA) were implanted bilaterally in the neck muscles. All electrodes were connected to a plastic pedestal (Plastics One, USA) and fixed to the skull with cold polymerizing dental resin (Palapress, Heraeus Kulzer GmbH, Germany). Following surgery, rats were given 5 ml 0.9% NaCl-solution s.c. for fluid substitution. Animals had at least 7 days for recovery from surgery.

2.1.2.4 Sleep recordings

Four recording boxes (35 cm x 35 cm x 46 cm), made of dark-grey PVC and containing plexiglas-windows on two opposite sides for visual contact to the neighboring box were placed in a light-proof chamber within the behavioral testing room with a consistent 12 h light-dark-cycle (lights on at 06.00 A.M.). The electrodes were connected through a swiveling commutator (Plastics One, USA), allowing free movement inside the box, to a Grass Model 15A54 amplifier (Grass Technologies, AstroMed GmbH, Germany) in an adjacent room. EEG and EMG signals were amplified, filtered (EEG: high pass 0.01 Hz, low pass 300 Hz; EMG: high pass 30 Hz, low pass 300 Hz, -6 dB cutoff frequency and at least -12 dB per octave roll-off), subsequently digitized at a sampling rate of 1000 Hz (CED 1401, Cambridge Electronics, UK), recorded using Spike2 software (Cambridge Electronics, UK) and stored on hard disk. The animals could be visually monitored on a PC-monitor in the adjacent room via cameras mounted above the recording boxes.

Days 1 to 3 served to adapt animals to the environment. On day four, a 24 h baseline recording was taken, starting at 05.00 P.M. Twenty four hour recordings were taken throughout the complete experimental period, interrupted only during behavioral testing.

2.1.2.5 General procedure and design

The procedure of object-place recognition testing was the same as in Experiment 1. However, each animal underwent four sessions, with Sample and Test trials separated again always by a 2-h retention interval, according to a within-subject design with the order of conditions balanced across animals. For each animal, two of the sessions started at the beginning of the inactive phase (between 06.00 - 07.00 A.M.), the other two at the beginning of the active phase (between 06.00 - 07.00 P.M.). Animals were deprived of sleep (SD) in the 2-hour retention interval between Sample and Test trial during one of the morning (Morning SD) and evening sessions (Evening SD), whereas during the respective other session, they were not deprived (Morning S, Evening S). Figure 2.2 shows a schematic depiction of the experimental procedure.

To achieve shorter delays between the sessions, two different open-fields were used (the grey one used in Experiment 1 and a white one of the same dimensions). On three consecutive days, following the 24-h baseline recording and prior to the first experimental session animals were habituated to both empty open-fields for ten minutes daily. A pulley-system mounted above the open field served to affix the commutator-end of the recording cable and thus minimize stress due to manipulations at the head-side of the cable before placing the animal in the open field. Habituation trials took place during the inactive phase between 08.00 A.M. and 05.00 P.M and were separated by at least 4 h. The following testing sessions were separated by at least 36 h, using the different open-fields alternately. Sleep deprivation was achieved by "gentle handling": At the first sign of falling asleep (adopting a sleep posture) the experimenter tapped at the box, shuffled the bedding or, if necessary, gently touched the animal.

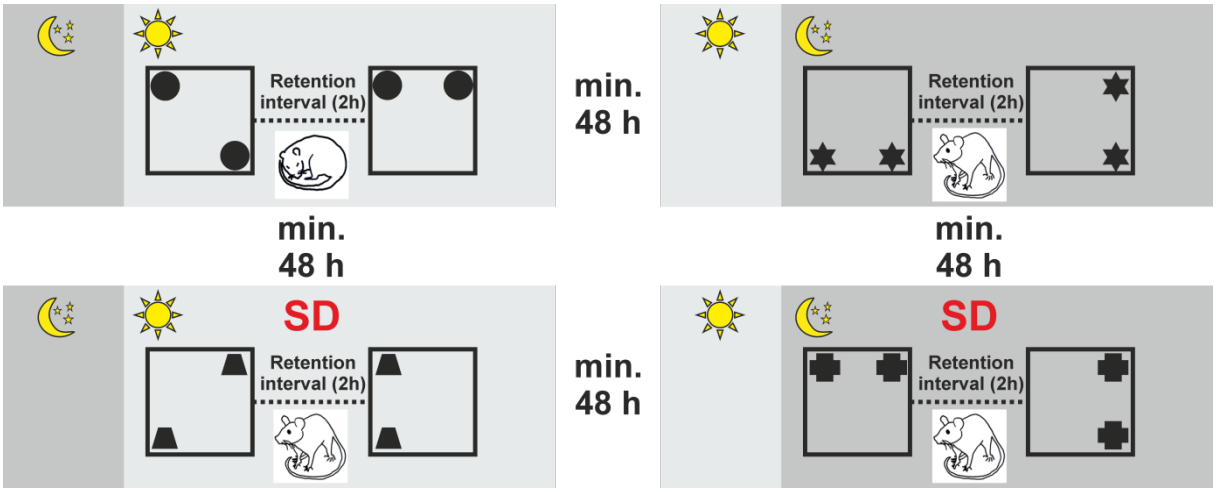

Figure 2.2. Schematic depiction of the experimental procedure. SD: sleep deprivation. Order of sessions as well as positions and types of objects were balanced.

2.1.2.6 Data reduction and statistical analyses

Data analyses of behavioral measures were essentially the same as in Experiment 1. Sleep (EEG and EMG) recordings during the retention intervals between Sample and Test trial as well as a corresponding morning time interval of the baseline recording were scored using 10-s epochs according to similar criteria as in Neckelmann et al. (1994) with the software SleepSign for Animal (Kissei Comtec, Japan). In short, 'waking' was identified by sustained EMG activity and mixed-frequency EEG, 'NREM sleep' by low EMG, high-amplitude low-frequency EEG with a high proportion of delta activity, 'Pre-rapid eye movement sleep' (PreREM; in the literature also referred to as 'transition sleep') by low EMG and high-amplitude EEG spindle activity, and 'REM sleep' by a further reduced EMG-signal and low-amplitude EEG with high theta (5-10 Hz) activity. Regarding sleep architecture, the following measures were computed: total sleep time (TST), duration of the different stages in min (Wake, NREM, REM, PreREM).

Further analysis of the sessions without SD involved 35 Hz low-pass filtering (finite impulse response [FIR] filter, attenuation of stop band: -80 db, transition width: 15.5 Hz) of the EEG signal and two subsequent EEG power spectral analyses for NREM and REM sleep epochs within a frequency range of 0.85-35 Hz (bin size of 0.061 Hz) calculated via Fast Fourier Transformation (FFT). Mean power was determined for the following bands: slow oscillations (SO) 0.85-2.0 Hz, upper delta 2.0-4.0 Hz, theta 5.0-10.0 Hz, and spindle 10.5-13.5 Hz. The spindle band was chosen after identifying the peak frequency of spindle activity in individual rats within a broader (10-15 Hz) frequency range; for a peak-to-peak frequency distribution of spindles see Figure A1 and Table A1 in the Appendix. The SO and upper delta band together represent slow wave activity (SWA). The latter was split to enable a more

detailed analysis and since slow oscillations in the rat lie below 2 Hz (Ozen et al., 2010; Vyazovskiy, Riedner, Cirelli, & Tononi, 2007). Filtering and FFT analysis were done with the software Spike 2 (Cambridge Electronics, UK).

Data from animals showing a disturbed circadian sleep-wake pattern in sessions without sleep deprivation, i.e., a TST in the Morning retention interval lower than the mean TST of all animals for the Evening condition and/or a TST in the Evening retention interval higher than the mean TST for the Morning interval, were excluded. This was the case for 4 animals. Data from 3 further rats were discarded due to technical problems (control of ambient temperature during the session), thus resulting in a final N = 15. FFT analysis were conducted for N = 14, due to artifacts in one recording. Statistical analyses relied on ANOVAs with a repeated measures factor for "Condition" (Morning S, Morning SD, Evening S, Evening SD). For behavioral data, directed Helmert contrasts were conducted, and on FFT data Fisher's LSD tests were used as post-hoc contrasts.

2.2 Results

2.2.1 Experiment 1

The two conditions did not differ regarding total object exploration time across both objects (Morning: 49.24 +/- 3.03 s, Evening: 43.81 +/- 3.32 s, $p = 0.256$) nor trial duration (Morning: 522.43 +/- 23.41 s, Evening: 547.05 +/- 22.44 s, $p = 0.429$) during the Sample trials.

Figure 2.3A reveals that the P-Index measured against chance was significant only in the Morning condition (1st min: $p < 0.01$, total 2 min: $p < 0.05$), but not in the Evening condition (1st min: $p = .43$, total 2 min: $p = .86$). More importantly, results reveal a higher P-index for Morning than Evening sessions (main effect for the factor "Time of Day" ($F(1, 20) = 11.42$, $p < .01$). Neither the factor "Minute" nor the interaction reached significance (Minute: $F(1,20) = 2.09$, $p = .164$, "Time of Day" x "Minute": $F(1,20) = 3.23$, $p = .087$). Altogether, these results indicate that the animals were able to discriminate between displaced and stationary object only in the Morning, but not in the Evening condition. There was no difference in general exploratory behavior between the two conditions (total object exploration: Morning: 14.1 +/- 1.7 s, Evening: 14.57 +/- 1.5 s, $p = 0.818$)

2.2.2 Experiment 2

2.2.2.1 Sample trials

The four conditions did not differ statistically regarding total object exploration (Morning S: 56.67 +/- 2.56 s, Evening S: 54.19 +/- 2.88 s, Morning SD: 55.49 +/- 2.64 s, Evening SD:

52.96 +/- 4.04 s, $F(3, 42) = .31$, $p = 0.806$) nor regarding trial duration (Morning: 417.9 +/- 42.53 s, Evening: 399.66 +/- 46.88 s, Morning SD: 445.3 +/- 44.28 s, Evening SD: 420.99 s +/- 42.53 s, $F(3, 42) = .29$, $p = 0.833$).

2.2.2.2 Test trials

In agreement with the findings of Experiment 1, the P-Index was significantly increased above chance only in the Morning condition with undisturbed sleep (1st min: $p < .001$, total 2 min: $p = 0.001$), but not in the three other conditions (Evening S: 1st min: $p = .193$, total 2 min: $p = 0.344$, Morning SD: 1st min: $p = 0.147$, total 2 min: $p = 0.508$, Evening SD: 1st min: $p = 0.439$, total 2 min: $p = 0.859$; 1st min: $F(3, 42) = 2.92$, $p < 0.05$, total 2 min: $F(3, 42) = 3.22$, $p < 0.05$, Figure 2.4). The significant difference between the Morning condition and the others was confirmed by Helmert contrasts (1st min: $p < 0.05$, total 2 min: $p < 0.05$) indicating that the animals were only able to solve the task after the Morning retention interval with undisturbed sleep. Again, there was no difference regarding general exploratory behavior between the conditions (total object exploration: Morning S: 32.0 +/- 2.66 s, Evening S: 45.13 +/- 4.30 s, Morning SD: 39.96 +/- 5.50 s, Evening SD: 39.07 +/- 6.40 s, $F(3, 36) = 1.697$, $p = 0.185$). Results are depicted in Figure 2.3B.

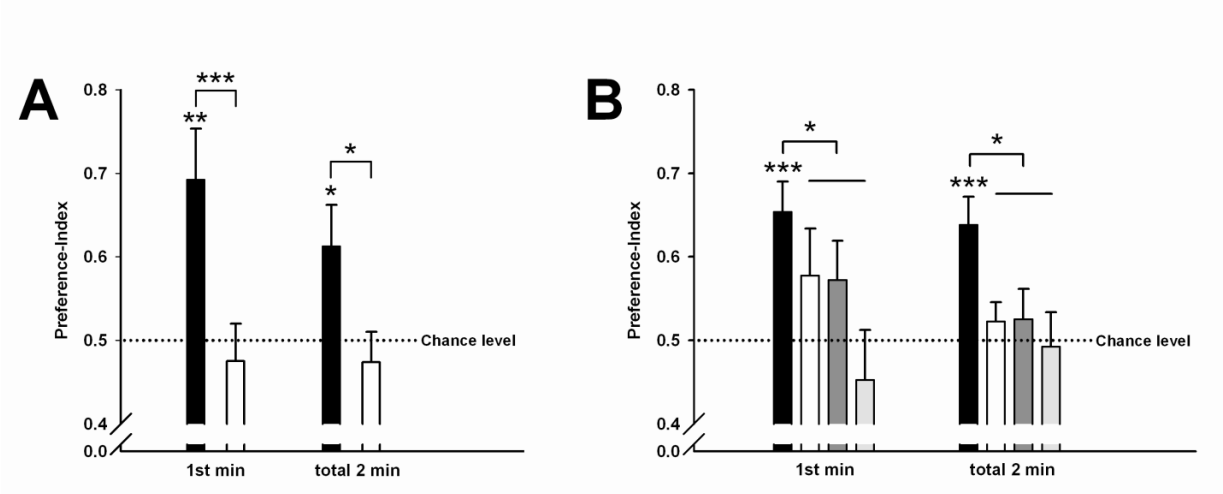

Figure 2.3 Preference-Index (mean +/- SEM) for the displaced object during the Test trials for all conditions, separated for the 1st and the total 2 min of the trial. **A** Experiment 1: An exploration pattern above chance level is only observed in the Morning (black bars), but not in the Evening (white bars) condition. **B** Experiment 2: Place object recognition above chance level is only observed in the Morning S (black bars), but not in the Evening S (white bars) or SD conditions (Morning SD: dark grey bars, Evening SD: light grey bars). Performance in the Morning S condition differs significantly from all other conditions. * $p < 0.05$, ** $p < 0.01$, *** $p \leq 0.001$

2.2.2.3 Sleep Parameters and EEG Power

Sleep architecture for each condition is given in Table 2.1 and Table 2.2. As expected, animals overall slept more during the Morning S than Evening S session and SD effectively hindered rats from falling asleep. Furthermore, the composition of sleep differed between conditions regarding NREM and REM sleep ($F(2, 28) = 14.28$, $p < 0.001$, and $F(2, 28) = 29.59$, $p < 0.001$, respectively), whereas the proportion spent in PreREM sleep did not differ significantly (see Table 2.2 for detailed results). Generally, rats spent in all of these sleep stages more time during the Morning S than Evening S session.

Table 2.1. Sleep architecture in minutes for the retention interval between Sample and Test trials and for corresponding Morning Baseline period.

	Baseline	Morning S	Evening S	Morning SD	Evening SD
Awake	40.31 +/- 5.56	57.51 +/- 4.88*	95.36 +/- 2.75$^{\$}$	116.96 +/- 0.28	108.19 +/- 7.75
NREM	57.26 +/- 4.47	47.97 +/- 3.98*	18.06 +/- 2.20$^{\$}$	0.24 +/- 0.23	0.64 +/- 0.32
REM	15.63 +/- 1.44	8.59 +/- 1.01*§	1.37 +/- 0.45$^{\$}$	0.00 +/- 0.00	0.00 +/- 0.00
PreREM	4.24 +/- 0.41	3.35 +/- 0.35*	1.82 +/- 0.40$^{\$}$	0.00 +/- 0.00	0.00 +/- 0.00
TST	77.13 +/- 5.85	59.91 +/- 4.85*	21.25 +/- 2.67$^{\$}$	0.24 +/- 0.23	0.64 +/- 0.32

Notes: Values are given in mean +/- SEM. TST: total sleep time, REM: rapid eye movement sleep, NREM: NREM sleep, PreREM: pre-rapid eye movement sleep. Duration of all sleep stages and TST differed significantly between Baseline, Morning and Evening conditions ($p < 0.001$). Bonferroni corrected pairwise comparisons revealed significant differences between Morning and Evening condition for Awake, NREM, REM, TST (* $p < 0.001$) and PreREM (* $p = 0.01$) and between Evening and Baseline condition for all stages and TST ($^{\$}$ $p < 0.001$). Morning and Baseline condition differed only in the duration of REM (§ $p = 0.001$), while TST and duration of Awake failed to reach significance ($p = 0.067$ and 0.066, respectively). Sleep architecture of SD sessions were not included in analysis. N = 15.

Table 2.2 Sleep architecture in percentage of TST for the retention interval between Sample and Test trials and for corresponding Morning Baseline period.

	Baseline	Morning S	Evening S
NREM	74.45 +/- 1.22	79.87 +/- 1.28*§	87.71 +/- 2.36$
REM	19.92 +/- 1.25	14.24 +/- 1.06*§	4.74 +/- 1.53§
PreREM	5.62 +/- 0.40	5.89 +/- 0.60	7.55 +/- 1.50

Notes: Values are given in mean +/- SEM. REM: rapid eye movement sleep, NREM: NREM sleep, PreREM: pre-rapid eye movement sleep. Proportion of NREM and REM differed significantly between Baseline, Morning and Evening conditions ($p < 0.001$). Bonferroni corrected pairwise comparisons revealed significant differences between Morning and Evening condition for both NREM and REM (*$p < 0.05$ and $p = 0.001$, respectively). The same prevailed for comparison between Morning and Baseline condition (§ $p < 0.01$) and Evening and Baseline condition (NREM: $ $p < .01$, REM: $ $p < .001$). Sleep architecture of SD sessions were not included in analysis. N = 15.

The EEG power spectra of NREM sleep epochs during the retention interval differed in the Evening, Morning and at the corresponding morning Baseline interval. Figure 2.4 reveals for the NREM sleep epochs that the EEG slow oscillation (0.85- 2.0 Hz) and upper delta (2.0 - 4.0 Hz) bands contain less power during the Evening compared to the Morning and Baseline condition ($F(2, 26) = 9.08$, $p = 0.001$, and $F(2, 26) = 4.81$, $p < 0.05$, respectively, Figure 2.4). In contrast, power in the spindle frequency band (10.5 - 13.5 Hz) was higher in the Evening S compared to the Morning S condition and also higher in the Morning S compared to the respective morning Baseline interval ($F(2, 26) = 23.67$, $p < 0.001$, Figure 2.4). Regarding REM sleep, EEG power of Morning S did not differ from the Baseline condition for SO (power in μV^2/Hz: Morning S:356.29 +/- 12.27, Baseline: 348.6 +/- 13.46, $p = .62$), upper delta (power in μV^2/Hz: Morning S:234.48 +/- 13.3, Baseline: 233.07 +/- 9.92, $p = .87$) and spindle band (power in μV^2/Hz: Morning S:127.96 +/- 7.59, Baseline: 140.58 +/- 7.23, $p = .24$), but there was a trend towards decreased theta power in the Morning condition (power in μV^2/Hz: Morning S:834.7 +/- 48.07, Baseline: 902.38 +/- 43.77, $p = .09$). A comparison to Evening S could not be made, since half of the animals (N = 7) showed no REM sleep at all.

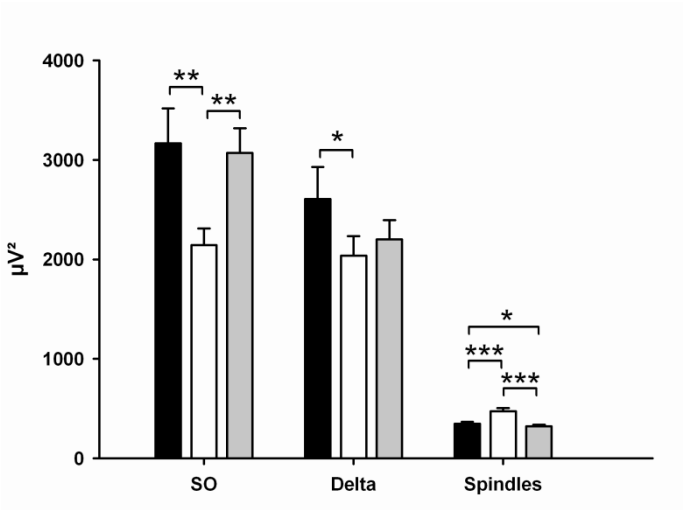

Figure 2.4. Power of selected EEG bands for the NREM epochs during Morning (black bars) and Evening retention intervals (white bars) with undisturbed sleep and during corresponding Morning Baseline taken from 24-h recordings before the experiment proper (grey bars). SO: slow oscillations, 0.85 -2.0 Hz, upper Delta 2.0 - 4.0 Hz, Spindles 10.5 - 13.5 Hz. * $p < 0.05$, ** $p < 0.01$, *** $p \leq 0.001$

3 STUDY 2: EFFECTS OF SO-TDCS IN RATS ON MEMORY CONSOLIDATION AND BRAIN OSCILLATIONS

In preparation as: Binder, S., Berg, K., Gasca, F. Lafon, B., Parra, C.L., Born, J. & Marshall, L.; Transcranial slow oscillation stimulation enhances memory consolidation in rats

3.1 Materials and Methods

3.1.1 Animals

Twelve male Long Evans rats (Janvier, France), 10 to 11 weeks old at time of surgery, were used. Animals were housed individually in Standard type III Macrolon cages with ad libitum access to food and water under a 12h/12h light-dark cycle (lights-on 07.00 A.M.). To prevent possible stress due to transportation, behavioral testing and sleep recordings took place in the housing room. All experimental procedures were performed in accordance with the European animal protection laws and policies (directive 86/609, 1986, European Community) and were approved by the Schleswig-Holstein state authority.

3.1.2 Handling

Before surgery animals were handled daily for 5 min on seven consecutive days, followed by a 5 min exposure to the recording boxes. Handling procedures were similar as in study 1, but additionally to touching the animals' body while keeping it on the lap of the experimenter, starting with day 3 of handling, the heads of the animals were touched repeatedly and pressed gently down to accustom them to the attachment of recording cables later in the experiment.

3.1.3 Surgery

Animals were anesthetized with isoflurane (induction: 3.5 ml/min in 700 ml/min O_2, maintenance: 1.3 - 1.9 ml/min in 700 ml/min O_2). Additionally, 0.6 mg/kg medetomidine (Dorbene, Dr. E. Graeub AG, Switzerland) was given i.p. for intrasurgical pain relief and 0.04 mg/kg atropin (Atropinum Sulfuricum, Eifelfango, Germany) s.c. to prevent breathing problems. This change in anesthesia agents to study 1 was done since surgical procdures required more time due to the placement of more electrodes. For longer surgeries, inhalable agents are to be preferred for safety reasons for the health of the animal. For epidural EEG recording a stainless steel screw-electrode (diameter 1.57 mm, shaft length 2.4 mm, Plastics One, USA) was placed over the left frontal cortex (AP: + 1.6 mm, L: - 0.5 mm) and referenced to an occipital site (AP: -12.0 mm; L +/- 0.0 mm). For bilateral stimulation screw-electrodes of same size as above were drilled halfway through the skull. Anodes for so-tDCS

were positioned over the PFC (AP: + 2.5 mm, L: +/- 2.0 mm) and the return electrodes over the cerebellum (AP: -10.0 mm, L: +/- 2.0 mm). Two additional electrodes were placed at AP - 4.0 mm, L +/- 2.0 mm, and one at AP + 0.3 mm, L: + 2.1 mm). Another anterior electrode (AP: + 6.9, L + 1.1) was used as ground. For EMG recordings, two insulated stainless steel wire electrodes (Plastics One, USA) were implanted bilaterally in the neck muscles. All electrodes were connected to two plastic pedestals (Plastics One, USA), one for recording and one for so-tDCS, covered with adhesive luting dental cement to enable long-term stability on the skull (C & B MetaBond, Parkell Inc, USA) and finally fixed with cold polymerizing dental resin (Palapress, Heraeus Kulzer GmbH, Germany). Following surgery, rats were given 1 mg/kg atipamezol (Alzane, Dr. E. Graeub AG, Switzerland) i.p. to antagonize the effects of medetomidine, 5 mg/kg caprofen (Rimadyl, Pfizer AG, Switzerland) i.p. for pain relief and 5 ml 0.9% NaCl-solution s.c. for fluid substitution. Animals had at least 7 days for recovery from surgery.

3.1.4 Apparatus and objects

Object-place recognition testing took place in the same quadratic dark gray open field as in study 1, dimly lit with 11 lux. Behavior was recorded by a camera (model DFK1BU03, The Imaging Source, Bremen, Germany) mounted above the open-field. The arm and stand of the camera, surrounding furniture and a curtain separating the open field area from the remaining room could serve the animal as extra maze cues to facilitate spatial orientation. Objects were similar as in study 1. Again, objects and open field were cleaned thoroughly between trials with 60% ethanol solution.

3.1.5 Habituation to the open field

On three consecutive days prior to the first object-place recognition session animals were habituated to the empty open-field for five minutes per day. Habituation trials took place during lights-on between 08.00 A.M. and 02.00 P.M. Following habituation, animals' sleep was recorded for two hours to adapt them to the recording conditions.

3.1.6 Sleep recordings

Three dark-grey PVC recording boxes (identical to the ones used in study 1) were placed in the housing room. EEG and EMG recording conditions were also identical to study 1.

3.1.7 Pilot studies 1: Retention intervals

To find optimal retention intervals 11 animals underwent pilot trials using in general the same procedure as described below in section 3.1.9, but with retention intervals of different length. Due to strong dependence of task performance on external factors (e.g. housing conditions, type of extra-maze cues) results of prior studies revealing a P-Index above chance for a 2h retention interval of sleep without so-tDCS (study 1, Binder et al., 2012) were first replicated. Secondly, experiments using a 5-h retention interval were conducted in a within subject design with so-tDCS applied in one of these trials. A third pilot experiment extended the retention interval to 7-8 h, assigning 5 and 6 animals to a stimulation and sham group, respectively. Stimulation parameters were similar as described below (section 3.1.9.2), except only 15 stimulation trains were applied. To prevent interfering with the light-dark cycle, it was refrained from investigating a retention interval between 8-24 h.

3.1.8 Pilot studies 2: Unspecific effects of so-tDCS on behavior

To investigate if a single session of so-tDCS may induce long-lasting unspecific effects on behavior, additional behavioral tests were conducted within the pilot studies of 11 animals. The open-field test was chosen, since it allows assessing simultaneously different behaviors, more precisely it can test for locomotion, emotionality/anxiety and spatial habituation (Karl, Pabst, & von, 2003; Leussis & Bolivar, 2006). Shortly, if a rodent is placed into an open field, it will show behavior resulting from conflicting tendencies to explore the novel environment and from anxiety of open space. With time, the animal habituates to the new environment and an increase in exploration conjoint with a decrease in anxiety related behaviors are expected to occur (Leussis & Bolivar, 2006). Commonly applied measures, which are also to be applied here, are locomotion (measured as distance travelled; a measure for general activity as well as an anxiety related behavior), number and duration of rearings (= standing on hind legs; a measure for exploratory activity), grooming (= licking and scratching of fur and washing of face; a displacement behavior which decreases with increased habituation) and time spent in the center area of the open field (a measure for anxiety and exploration). Since rodents are afraid of brightly lit open space, they spent usually more time in the “safe” areas close to the walls. Therefore, the OF was virtually divided in 9 equally big quadrants and time spent in the wall-near quadrants and in the center quadrant was separately assessed.

To investigate OF behavior in four sessions, the OF used for the OPR task was modified three times. As a baseline trial, the first OF habituation sessions was analyzed (OF1, described in section 3.1.5). For the second OF session (OF2), taking place ~30 h after the first 5 h pilot

OPR test trial, one wall of the grey OF was replaced by a white wall. For the third OF session (OF3), taking place ~30 h after the second 5 h pilot OPR test trial, two adjacent walls of the grey OF were replaced by white walls. For the fourth OF session (OF4), taking place 7-9 days after the second 5 h pilot OPR test trial, two opposite walls of the grey OF were replaced by white walls. For all OF trials, the extra maze cues were changed to prevent orientation on familiar spatial cues. Each rat was tested on all four conditions according to a within-subject design: One baseline test (OF1), a test ~ 30h after so-tDCS (OF2 or OF3), a test ~ 30h after sham-stimulation (OF2 or OF3) and a last remote test (OF4). The latter was conducted to investigate putative remote effects of a single so-tDCS session. See Figure 3.2 for an overview of the timeline for an example animal.

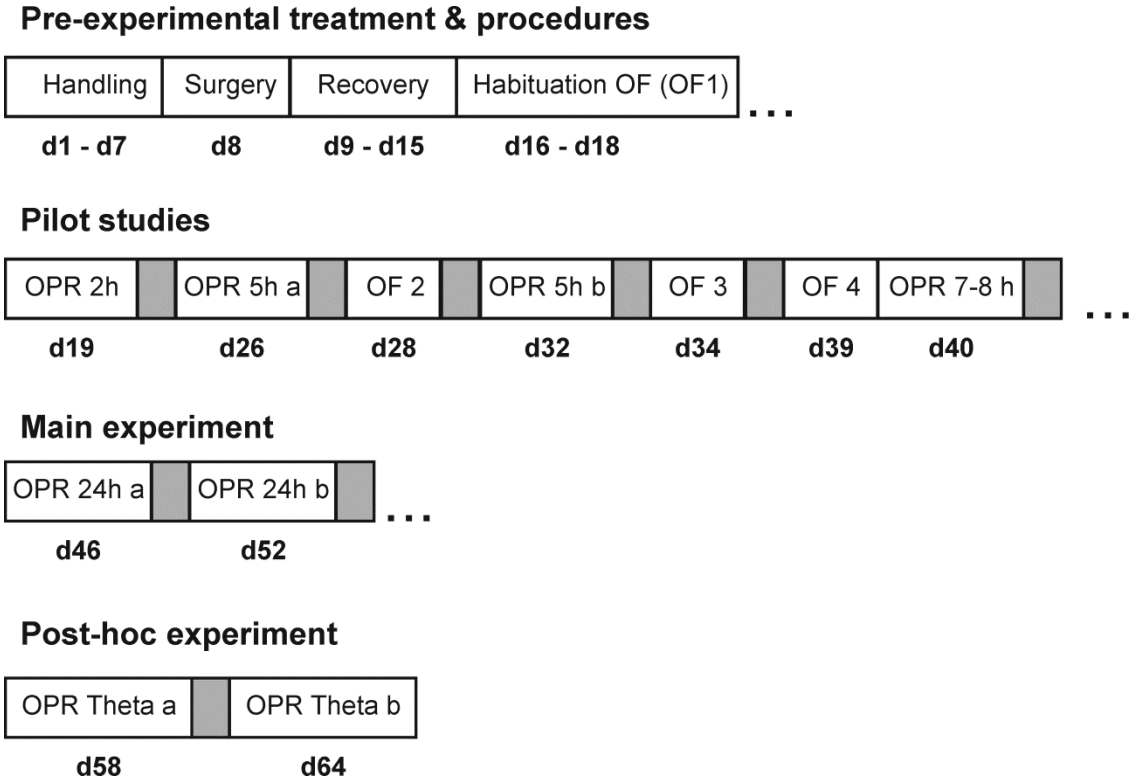

Figure 3.2 Timeline of an example animal in study 2. Grey boxes represent days between experimental procedures. d = day, OPR = object-place recognition task, OF = open field task

3.1.9 Main study

3.1.9.1 General procedure and design

The OPR task was conducted similar as described in study 1 (Binder et al., 2012). Main differences were as follows: The evening prior to object-place recognition session animals were brought into the recording boxes without connecting them to the cables. Behavioral testing started the next day around 08.00 AM. Animals were connected to EEG and so-tDCS after termination of the Sample trial and brought into the recording box, where it spent the

whole retention interval (Pilot studies) or 8 hours (Main study, post-hoc experiment). Animals were subsequently subjected to STIM (so-tDCS) or SHAM (sham-stimulation). After the retention interval, the 2 min Test trial took place. Positions of objects in Sample and Test trials and type of object were counterbalanced between retention interval conditions. Each rat was tested on both conditions according to a within-subject crossover design, a STIM session and a SHAM session. Order of sessions was counter balanced. Sessions were separated by five to six days, and different objects were used in each session.

3.1.9.2 Stimulation parameters

The so-tDCS electrodes were connected through the same swiveling commutator as the EEG and EMG, but through a separate cable to a battery driven constant current stimulator (designed by the Electronics Facility of the University of Luebeck) in the adjacent room. Current intensity of trapezoidal so-tDCS fluctuated between 0 and 9 μA. This maximum current was chosen on the basis of FEM modeling (described below) to reach sufficient fields within the frontal cortex without affecting wide subcortical structures. Stimulation frequency was in the range of slow oscillation (1.33 - 1.5 Hz), with oscillatory currents applied to both hemispheres in phase-synchrony. Duration of each stimulation train was 30 s, separated by a stimulation-free period of at least 30 s.

Stimulation started after the first occurrence of 60 s stable NREM sleep and lasted for 30 s, followed by a 30 s stimulation-free interval. If the animals showed signs of awakening during stimulation (movement and/or increased EMG activity), stimulation was terminated, and if any sleep stage change was observed during the stimulation-free period, the next 60s of stable NREM sleep were awaited before the next stimulation began. Animals received in total 20 stimulations during NREM sleep (prematurely terminated stimulations and those immediately followed by REM or PreREM sleep were repeated). In the SHAM condition, no stimulation was applied.

3.1.9.3 Electric field calculations using the Finite Element Method (FEM)

To estimate the spatial distribution of the magnitude of the electric field for our electrode positioning at different current intensities, a realistic 3D model was calculated on the bases of Gasca, Marshall, Binder, Schlaefer, & Schweikard (2011). Since development of this model is not part of this thesis, only the main features are described to make clear on which bases the selected stimulation current was chosen, its restriction to cortical areas and to support the assumption of focality of the electrode montage. Details of this model are given in Gasca et al., 2011. Briefly, based on an MRI of a living male Wistar rat, scalp, skull, cerebrospinal

fluid (CSF), brain, eyes and airways were segmented and converted to solids. Electric conductivity of the different media was taken from averaged human values used in a similar FE study (Datta et al., 2009). Electrodes were modeled in size and position according to the values given in section 3.1.3 'Surgery'. The resulting induced electric field distributions are shown in Figure 3.2.

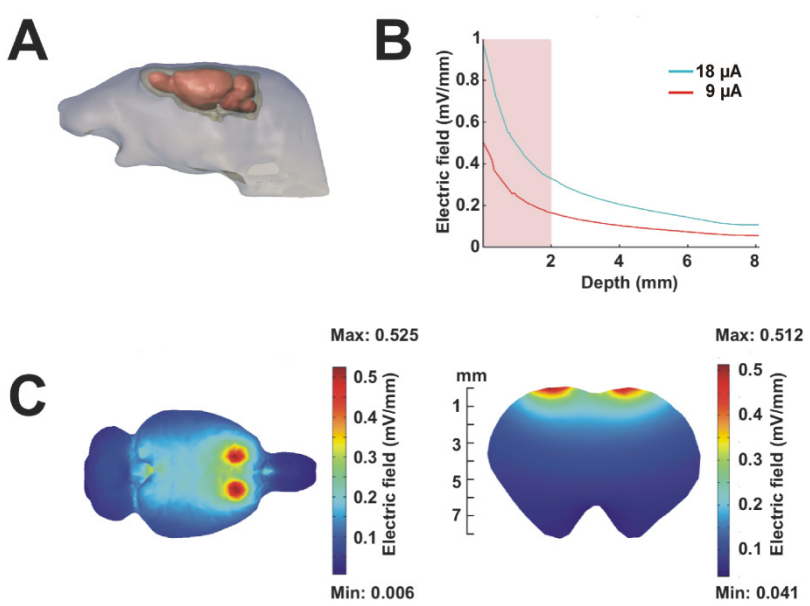

Figure 3.2. Estimated electric field distributions according to the finite element (FE) rat brain model. **A**, Anatomically realistic geometries of the rat head model. **B**, Electric field profile within the brain for two different currents; cortical surface corresponds to 0 mm. Pink shaded area represents cortex. **C,** Electric field distribution (9µA applied current) on the cortical surface (left) and along a coronal slice positioned at the height of the anodes (right). A detailed description of the FE model is in Gasca et al., 2011.

As depicted in Figure 3.2B-C, the estimated electric fields induced by currents of 9µA were most pronounced directly beneath the electrodes and almost exclusively affected cortical tissue. Field strength reached maximum values of ~ 0.5 mV/mm at cortical surface, which is slightly below the fields of ~0.8 mV/mm induced by endogenous SO activity measured on multi-site recordings from PFC in rats (calculations were done on a recording taken from Fujisawa & Buzsaki, 2011). Figure 3.2C shows field strength of different current intensities and its decay within deeper brain tissue. It is to note that an increase to higher currents would lead to higher field effects in subcortical structures, which would be an unwanted effect since it was aimed to affect primarily the neocortex.

3.1.10 Post-hoc experiment: theta-tDCS during REM sleep

In a post-hoc experiment, possible effects of theta-tDCS during REM sleep on OPR performance, EEG power and sleep architecture were investigated. Since so-tDCS during SWS proved to be effective in improving memory consolidation and enhancing SO activity

(see section 3.2 on Results), firstly, the specifity of so-tDCS during SWS to elicit these effects should be investigated. Secondly, prior studies emphasized the importance of REM sleep on memory consolidation in rats. However, results of study 1 did not point towards such a role of REM sleep on memory consolidation in the OPR task.

The procedure was similar to the one in the main experiment: Each animal underwent to additional OPR sessions (STIM and SHAM in a balanced order), using a retention interval of 24 h. This time, however, stimulation parameters and procedure was changed. For application of theta-tDCS, the additional electrodes over the somatosensory cortex were used (see section 3.1.3 on surgery). Stimulation took place after reaching stable REM sleep for the first time, defined as its first uninterrupted occurrence for 15s. In contrast to the main experiment, this time a sinusoidal shape of stimulation was chosen, to facilitate analysis during acute theta-tDCS. Stimulation frequency was 6.85 Hz, as determined by the mean peak amplitude of theta activity during REM sleep in the SHAM condition of the pilot studies. Current intensity fluctuated between 0 and 7 μA, again applied in synchrony in both hemispheres. Stimulations lasted for 28.9 s, and to account for the shorter mean duration of REM episodes, the stimulation-free intervals were 15s; and 20 stimulations were applied in total. Again, in case of a sleep stage change, stimulation was interrupted and repeated with occurrence of next stable REM sleep.

3.1.11 Data reduction and statistical analyses

3.1.11.1 Behavioral data

Scoring of explorative behavior was conducted identical as is study 1. The P-Index was again computed separately for the first Test trial minute and for the entire 2 minutes of the Test trial. Additionally, in the main experiment only, following behavioral control measures were assessed to more safely exclude any pre-existing differences between the conditions (control measures on Sample trials) and unspecific effects of stimulation on behavior during Test trial (control measures on Test trials):

- Amount of time spent in each of the 4 quadrants of the open field during Sample trials: A differential pattern of time spent in pure vicinity of the objects could, beside the active exploration expressed by P-Index, putatively lead to differences in encoding of object position between the conditions.
- Amount of time spent in each of the 4 quadrants of the open field during Test trials: A differential pattern of time spent in pure vicinity of the objects could, beside the active exploration expressed by P-Index, putatively show differences in retention of object position between the conditions.

- Relationship of P-Index during Test trial to time spent in each quadrant during the Test trials: As above, a time spent in pure vicinity of the objects could, beside the active exploration expressed by P-Index, putatively be an expression of retention of object position. To test for this possibility, its relation to P-Index was assessed and compared between the conditions.
- Sample trial duration: Since Sample trial is terminated after reaching 60s of active object exploration, the duration of Sample trials differ between animals. A systematic difference between the conditions in this measure could lead to differences in encoding.
- Relationship of P-Index during Test trial to Sample trial duration: Similar as above, a longer Sample trial may have led to a better encoding and therefore to a better consolidation and retention. However, this measure could only hint towards such a relation, since P-Index is in general a measure for novelty preference and not a direct measure of memory; see also section 2.1 on OPR and section 3.1.11.4 on statistics below.
- Relationship of P-Index in the Test trial to exploration of the to-be-displaced object in the Sample trial: An increased exploration of the to-be-displaced object in the Sample trial could lead to a better encoding of the position of this object and therefore to a better consolidation and retention. Again, this relation should be interpreted with care (see above).
- Relationship of P-Index during Test trial to time spent in each quadrant during Sample trial: Similar as above, an increased time spent in vicinity of the objects during Sample trial could have led to better encoding and therefore retention during Test. Again, this relation should be interpreted with care (see above).

In the pilot study on effects of so-tDCS on unspecific behavior, following measures were used in the four OF-trials: Distance travelled, mean speed, number of rearings, mean duration of rearings, number of groomings, mean duration of grooming, duration of time spent in the center of the OF and duration of time spent in the quadrants close to the wall.

3.1.11.2 Sleep scoring and sleep architecture

Sleep scoring was in principle the same as in study 1. Additionally, stimulation epochs were scored as a separate "stage" (STIM or SHAM), because a reliable assignment to a distinctive sleep stage was not always possible due to a stimulation artefact. For control days without stimulation, sham-stimulation intervals were inserted according to the same rules as for real stimulation, i.e., sham-stimulation started after 60 s of stable NREM sleep, each sham-stimulation lasted 30 s with a 30 s sham-stimulation-free interval, and was repeated if the

animal woke up or changed sleep stage. Regarding sleep architecture, following measures were computed hourly, starting at sleep onset (defined as first occurrence of 60 s stable NREM sleep): total sleep time (TST), duration of the different stages (W, NREM, REM, PreREM) in minutes and as percentage of TST. Furthermore, sleep latency (start of recording to first occurrence of stable NREM), REM latency and duration of the stimulation period (time from start of first stimulation to the end of the last stimulation) were computed. Sleep was scored for the main experiment and the post-hoc experiment on theta-tDCS only, but not for the pilot experiments.

3.1.11.3 EEG analysis

Before power spectral analyses, EEG data were first low pass filtered (FIR filter, 35 Hz, attenuation of stop band: -80 db, transition width: 15.5 Hz) using Spike2 software (Cambridge Electronics, UK). Subsequently, a Hanning window was applied on 16384 blocks (~ 16.4 s) of EEG data before power spectra were calculated using FFT (Spike2, Cambridge Electronics, UK). Generally, data was normalized using the percentage of each bin (bin size 0.06 Hz) with reference to the total spectral power between 0.85 and 35 Hz. This normalization was necessary due to differences in total power between animals. To account for possible violations of the assumption of normal distribution, the normalized data was logarithmized according to the method proposed by Gasser, Bacher, & Mocksn (1982): (log(x/[1-x]), where log refers to the natural logarithm and x represents the relative power in a given frequency band. For statistical analyses, these transformed values were used. Spectral analyses were conducted after onset of the 1st (sham-)stimulation for:

(i) all NREM sleep epochs of the entire 8 h recording (hourly mean)
(ii) all REM sleep epochs of the entire 8 h recording (hourly mean)
(iii) all NREM sleep epochs between the first and the last (sham-)stimulation
(iv)all REM sleep epochs between the first and the last (sham-)stimulation
(v) the first 10s of all stimulation-free intervals.

For (v) intervals containing epochs other than NREM sleep were excluded, and analysis was done using a customized script (written by Fernando Gasca, Institute for Robotics and Cognitive Systems, University of Luebeck) in MATLAB (Mathworks, USA) applying slightly different filtering to remove the artifact immediately following stimulation caused by interplay between the hardware-implemented high-pass filter and so-tDCS: Data blocks were high pass backward filtered (0.5 Hz, 20^{th} order Butterworth) in order to avoid filter-induced artifacts at the beginning of the epochs. Low-pass filtering of 35 Hz was done by a 10^{th} order Butterworth filter. FFT length for (v) was 10.000 blocks (10s).

For the post-hoc experiment involving theta-tDCS during REM sleep, spectral analysis was similar, except that for (v) identical filtering as for analysis (i-iv) was used, as the sinusoidal stimulation did not induce gross post-stimulatory artifacts. For (v) intervals containing epochs other than REM sleep were excluded; a Hanning window was applied on 8192 blocks (~ 8.2 s) of EEG data before power spectra were calculated using FFT. Temporal dynamics of spectral power can be assessed by comparing the short (i-iii) and long (iv-v) time ranges.

Mean spectral power was calculated for the slow oscillation (SO) band (0.85-2.0 Hz), upper delta (2.0-4.0 Hz), theta (5.0-9.0 Hz), and the spindle band (10.5-14.0 Hz). The delta range was divided since slow oscillations in the rat lie below 2 Hz (Ozen et al., 2010; Vyazovskiy et al., 2007) and prior results of study 1 revealed relevance of the SO band for successful memory consolidation (Binder et al., 2012). The spindle band was selected according spindle detection procedure described below which revealed the majority of sleep spindles to lie in the 10.5-14.0 Hz range and according to similar results of study 1; for a peak-to-peak frequency distribution of spindles see Figure A1 and Table A1 in the Appendix. Theta range was adjusted from study 1 since the mean spectrum during REM sleep showed most power within this adjusted range.

Sleep spindles were detected based on the algorithm used by Eschenko et al. (2006). Briefly, data was band pass filtered (10-15 Hz, attenuation of the stop bands: -80 dB, transition width: 0.99 Hz), root-mean square of the resulting signal (rms) and standard deviation (SDev) were computed. Spindles were defined for a rms signal above 1.5 x SDev (mean SDev of the two recordings of an animal) for a duration of at least 0.5 s. Preprocessing of data and spindle detection was done using the in-built script language of Spike 2 software (Cambridge Electronics, UK). Spindle density was calculated across all 1 min intervals of NREM sleep for the same time ranges as used for the FFT analyses.

3.1.11.4 Statistics

For the behavioral analysis Student's one-sample t-tests investigated whether the P-Index differed from chance level. Additionally, ANOVAs for repeated measures were used to compare the P-Index for the first and the entire 2 min of the Test trial. Total exploration time across both objects for each Sample and Test trial, and trial duration for the Sample trials were compared between the STIM and SHAM condition using Student's t-test. ANOVAs for repeated measures followed by Bonferroni corrected pairwise comparisons were used to analyze the amount of time spent in the 4 quadrants of the open field, and Pearsons correlation to investigate the relation of P-Index to time spent in the quadrants. In addition, the P-Index

was correlated to the relative exploration time of the to-be displaced object during Sample trial. It is to note, that correlation with the measure P-Index are to be interpreted with caution, as stated above in section 2.1.1 on the OPR task. Yet, correlations were conducted to obtain a rough estimate for our specific behavioral model of individual performance and relation of exploratory activity between Sample and Test trials. For sleep architecture and EEG power analysis and spindle density over the whole recording period, ANOVAs for repeated measures were used, followed by post-hoc Student's t-tests where appropriate. For the pilot studies on OF behavior, Student's t-tests were conducted to compare the STIM and the SHAM condition ~ 30h after the last OPR test. To investigate a possible effect of time on OF behavior and a putative remote effect of so-tDCS, ANOVAs for repeated measures were conducted on all four OF trials irrespective of condition prior to OF 2 and OF3. Bonferroni corrected pairwise comparisons were conducted if appropriate. A p-value $< .05$ was considered significant. Results are given as means +/- SEM unless indicated otherwise.

3.2 Results

3.2.1 Pilot studies 1: Retention intervals

As expected, a P-Index above chance level was found after a 2h retention interval, but in contrast to study 1 only for the first minute of Test (see Figure 3.3A). Secondly, the experiment was conducted using a 5-h interval between Sample and Test trial in a within subject design (STIM and SHAM). As depicted in Figure 3.3B, P-Index differed significantly from chance level only in the 1st minute of STIM condition, but the mean of the SHAM group was still quite high although a high variability of performance could be seen. In a third pilot experiment the retention interval was extended to 7-8 h, assigning 5 animals to the STIM and 6 animals to the SHAM condition. No statistical analysis was used here due to small group size, but on a descriptive level again a high variability in performance could be seen. Interestingly, the mean in the SHAM condition was still quite high (see Figure 3.3C).

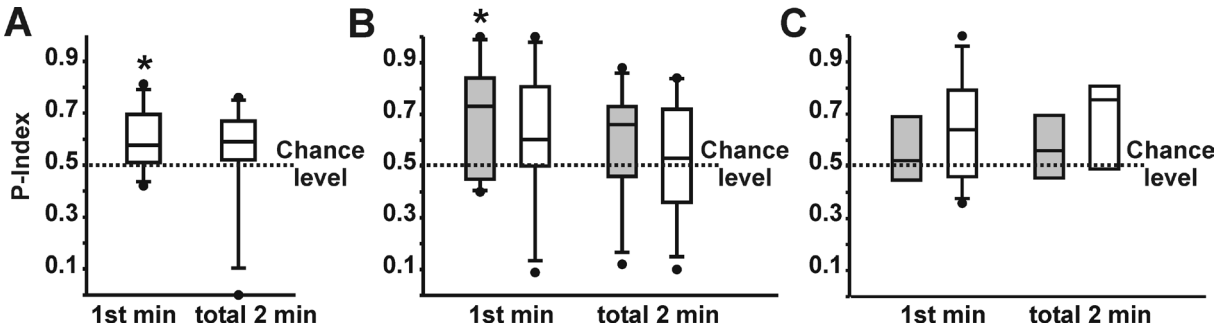

Figure 3.3. Performance during pilot experiments. Whiskers show the 90th and 10th percentile, dots are outliers. **A**, Preference (P)-Index within the first and total two minutes of time within the Test trial after a retention interval of 2h without application of so-tDCS (N = 11). **B**, P-Index after a retention interval of 5h for both STIM and SHAM conditions, within-subject design (N = 11). In the first minute of Test, a preference for the displaced objects differs significantly from chance only in the STIM condition. Note the high spreading of the P-Index. No significant difference between the conditions could be detected. **C**, P-Index as in B for a retention interval of 7-8h, between subject design (N = 5, STIM; N=6, SHAM). No group statistics used due to small number of animals. Missing whiskers indicate 90th and 10th percentiles lying within the interquartile range. Note the high preference index in the SHAM group and the high spreading. Grey boxes represent STIM condition, white boxes SHAM condition. * $p < .05$, t-test against chance level.

3.2.2 Pilot studies 2: Unspecific effects of so-tDCS on behavior

The comparison between different measures of OF behavior ~30 h after the first application of so-tDCS vs. sham-stimulation during the 5h retention interval in the pilot studies (OF2 and OF3) revealed reduced grooming activity after so-tDCS, in both duration (STIM: 44.0 +/- 6.1 s, SHAM: 65.1 +/- 9.8 s; $T(10) = -3.49$, p = .006) and total number of occurrence (STIM: 9.4 +/- 1.4, SHAM: 12.1 +/- 1.5; $T(10) = -2.40$, p = .037). No further measures on locomotion, exploratory behavior and emotionality differed between conditions ($p > .05$).

If all four OF tests were taken into account to investigate the effect of time and remote effects of so-tDCS, increased rearing activity (duration and number of occurrence) and reduced time spent in the center time as well as increased time spent in the wall near quadrants could be seen in the baseline OF test (OF1) in comparison to all further OF trials. Results of pairwise comparisons on these parameters are given in Table 3.1. However, OF2, OF3 and OF4 did not differ in any of the parameters measured. A detailed overview of mean values and *F*-statistics is given in Table 3.2.

Table 3.1. P-values for pairwise comparisons with Baseline open field test (OF1)

	OF2	OF3	OF4
Rearing (count)	.063#	.086#	.127
Rearing (duration; s)	.037*	.457	.235
Center quadrant (time; s)	.070#	.091#	.034*
Wall near quadrants (time; s)	.067#	.098#	.061#

Note: Only behavioral parameters showing at least a trend ($p < .1$) in one comparison are shown. * p < .05, # p < .1,Bonferroni corrected pairwise comparisons.

Table 3.2. Means +/- SEMs and F-statistics of all four open field tests

	OF1	OF2	OF3	OF4	*F*	*p*
Distance travelled (cm)	53.0 +/- 1.7	49.9 +/- 2.8	50.9 +/-3.0	53.6 +/-2.2	.86	.473
Mean speed (m/s)	.087 +/- .003	.083 +/-.005	.085 +/-005	.089 +/-.004	.87	.466
Rearing (count)	137.0 +/- 5.4	115.8 +/-4.9	121.1 +/-5.4	123.1 +/-4.6	6.15	.002*
Rearing (duration; s)	204.9 +/- 9.0	178.4 +/-9.0	190.5 +/-11.7	188.8 +/-8.4	4.07	.015*
Grooming (count)	7.6 +/- 1.3	9.6 +/-1.1	12.1 +/-1.6	9.6 +/-1.1	1.51	.233
Grooming (duration; s)	45.4 +/-8.0	53.0 +/-7-6	49.4 +/-7.2	35.2 +/-4.8	1.20	.327
Center quadrant (time; s)	23.1 +/-2.5	39.9 +/-4.4	35.7 +/-6.0	43.4 5.0	4.13	.014*
Wall near quadrants (time; s)	925.4 +/-11.1	839.1 +/-17.8	855.1 +/-18.5	847.4 +/-17.5	7.22	.004*

Note: Degrees of freedom: $F(3,30)$; Huynh-Feldt corrections were used if necessary. * p < .05, ANOVAs for repeated measures.

3.2.3 Memory performance in the Test trial

Figures 3.4A-B depicts the distribution of P-Index for both conditions and that 9 out of 12 animals revealed an increased P-Index after applying so-tDCS within the 24h retention interval: one performed equally well in both conditions while only two showed the opposite pattern. Most importantly, only these two animals in the STIM condition perform below chance level in the Test trial, while the P-Index is equally distributed above and below chance level in the SHAM condition (Figure 3.4A). Interestingly, one of the animals seemed to prefer the stationary object in both conditions, and this pattern was more pronounced after so-tDCS. Figure 3.4C depicts the means for STIM and SHAM, with t-tests revealing that the preference for the displaced object was only above the level of chance for the STIM group (STIM: $T(11) = 3.09$, $p = .01$ and $T(11) = 2.42$, $p = .034$ for the P-Index during the 1st min and, total 2 min, respectively; SHAM, 1st min: $T(11) = .65$, $p = .53$, total 2 min: $T(11) = .22$, $p = .83$). More

importantly, preference for the displaced object was significantly higher in the STIM than SHAM group (1st min: $T(11) = -2.28$, $p = .043$; total 2 min: $T(11) = -1.77$, $p = .104$). Thus, only animals in the STIM group discriminated between the displaced and stationary objects. Overall exploratory behavior did not differ between the two conditions (total object exploration: STIM: 37.2 +/- 3.3 s, SHAM: 37.1 +/- 3.9 s, $p = .99$).

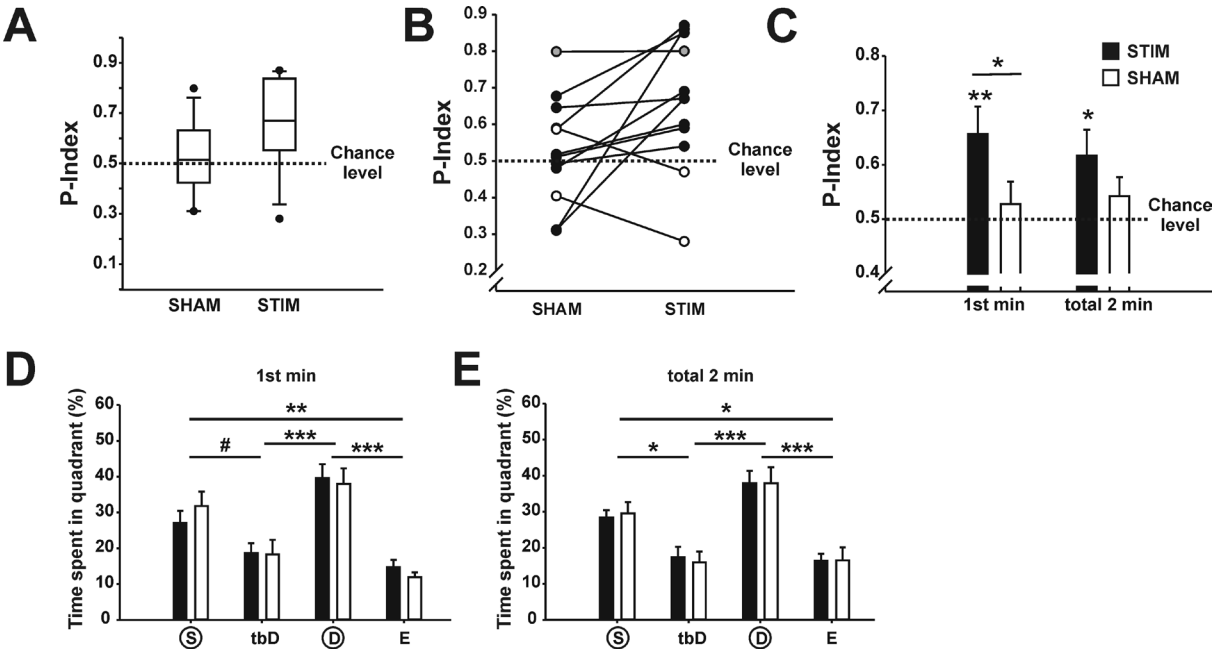

Figure 3.4. Test trial performance. **A-C** Preference-Index. **A**, Boxplots for both conditions. **B,** P-Index of individual animals. Most animals show an increase of the P-Index in the STIM condition (black circles, N = 9), only one shows identical high preference in both conditions (gray circle) and two show a reduced P-Index in the STIM condition (white circles). Note that in the STIM condition only these two animals perform below chance level. **C**, P-Index (mean +/- SEM) for the displaced object during the Test trials for both conditions, separated for the 1st and the total 2 min of the trial. An exploration pattern above chance level is only observed in the STIM condition but not in the SHAM condition. **D-E,** Behavioral control measures. **D**, Amount of time spent in each of the 4 quadrants of the open field during the 1st Test trial minute. 'Stationary' (S) and 'displaced' (D) refer to quadrants where an object is present, 'to-be-displaced' (tbD) and 'empty' (E) do not contain an object. **E**, same as in D, but for the total 2 minute Test trial. There is no difference between the conditions, but animals spent more time in the quadrants where objects are presented. Most importantly, there is no significant difference between the quadrants 'stationary' and 'displaced', indicating this measure not as sensitive to recognition memory as the preference index. One-sample t-tests against chance level and t-tests for dependent samples for comparisons between conditions (C). ANOVA followed by Bonferroni corrected pairwise comparisons (D+E). # $p < .1$, * $p < .05$, ** $p < .01$, *** $p < .001$.

In addition the amount of time spent in the 4 quadrants of the open field during the Test trial was analyzed to investigate if memory performance may be reflected also by this more indirect measure. As depicted in Figure 3.4D-E, there was no difference between the

conditions (1st minute: condition: $F(1,11) = 1.32$, $p = .275$; condition x quadrant: $F(2.06,19.3) = .31$, $p = .713$; total 2 min: condition: $F(1,11) = 1.38$, $p = .266$; condition x quadrant: $F(1.73,19.02) = .067$, $p = .914$), but time spent in each quadrant differed (1st min: $F(3,33) = 21.45$, $p < .001$; total 2 min: $F(3,33) = 19.94$, $p < .001$). In both conditions, animals spent more time in the quadrants containing objects (quadrants 'stationary' and 'displaced') than in quadrants without objects (quadrants 'to-be-displaced' and 'empty'). Most importantly, there was no difference between the two object-containing quadrants, and no interaction effect. Thus, this measure is not as sensitive as P-Index to detect novelty preference. In contrast to open field behavior in pilot study 2, grooming activity in the Test trial was not changed by the application of so-tDCS during the retention interval, neither regarding its duration (STIM: 6.2 +/- 1.1 s, SHAM: 5.1 +/- 1.0 s, $p = .35$) nor the total number of occurrence (STIM: 3.0 +/- 0.6 s, SHAM: 2.7 +/- 0.5 s, $p = .57$).

3.2.4 Exploratory activity during Sample trials and its relation to Test trial performance

An overview of Sample trial duration for single animals is given in Figure 3.5A and B. During the Sample trials, the two conditions did not differ regarding either total object exploration time across both objects (STIM: 60.92 +/-.71 s, SHAM: 60.68 +/- .82 s, $p = .81$) or trial duration (STIM: 249.91 +/- 24.42 s, SHAM: 284.47 +/- 34.28 s, $p = .42$). Exploration times are slightly longer than 60 s due to a discrepancy between time measured online by stopwatch and the offline computer-assisted analysis. Trial duration in the Sample trial does not significantly correlate with exploration of the displaced object reflected by P-Index in the Test trial (Figure 3.5B). As in the Test trial, amount of time spent in the 4 quadrants of the open field did not differ between conditions, and animals spent more time in the object-containing quadrants than in the empty quadrants (condition: $F(1,10) = .09$, $p = .773$; condition x quadrant: $F(3,30) = .15$, $p = .928$; quadrant: $F(3,30) = 18.57$, $p < .001$; Figure 3.5C). Figure 3.5D reveals no systematic relation between time spent in a specific quadrant and P-Index in the Test trial. The relative amount of time the animals spent exploring the to-be-displaced object in the Sample trial and the P-Index in the Test trial was also analyzed, since one could hypothesize that a more intense encoding of the removed object could have led to a better memory performance. As depicted in Figure 3.5E, no significant correlation between these measures could be detected for either condition.

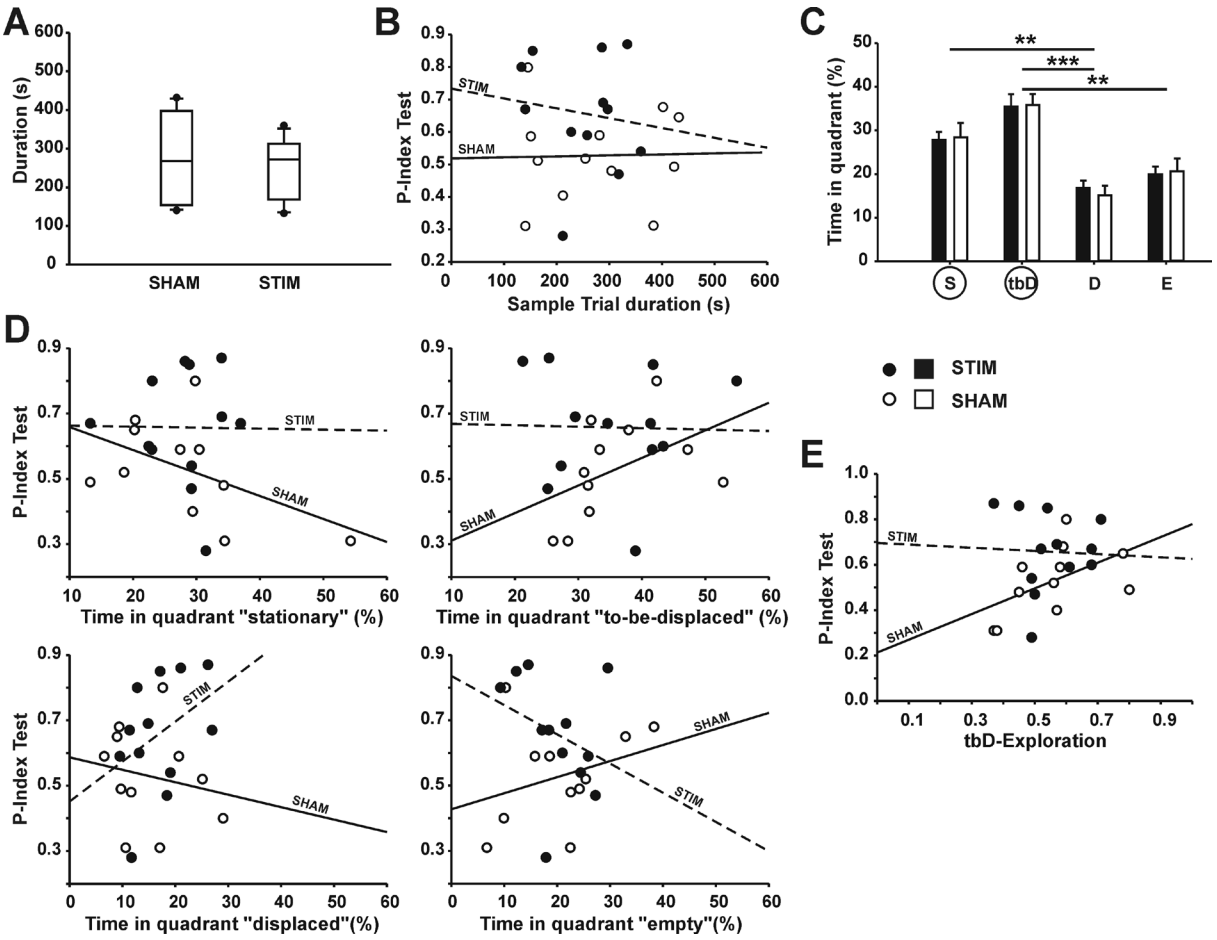

Figure 3.5. Sample trial performance and its relation to Test trial performance. **A**, Boxplot of Sample trial duration, revealing no difference in means (t-test). **B**, Relationship of the P-Index in the 1st Test trial minute to Sample trial duration, with regression lines shown for both conditions. There is no significant correlation for either condition ($p > .1$). **C**, Amount of time spent in each of the 4 quadrants of the open field during the Sample trial. Animals spent more time in the quadrants occupied by objects, but there is no difference between conditions. ANOVA followed by Bonferroni corrected pairwise comparisons. ** $p < .01$, *** $p < .001$. **D**, Relation of P-Index in the 1st Test trial minute to time spent in each quadrant during Sample trial . Animals in the SHAM condition tend to explore the displaced object relatively more intense in the Test trial if they have spent less time in the empty quadrant during Sample ($r = -.54$, p = .068), but no further significant correlations or trends could be seen (all p > .1). Regression lines are shown for both conditions. **E**, Relationship of P- Index in the 1st Test trial minute to relative amount of exploration of the to-be-displaced object in the Sample trial . The relative amount of active exploration of the to-be-displaced object does not appear to influence the P-Index (STIM: $r = -.03$, $p = .93$; SHAM: $r = .52$, $p = .10$). Note that for C-D only 11 animals were analyzed due to a failure in the camera system for one animal.

3.2.5 Brain electric activity during the 8-hour recording period

Measures of sleep architecture are depicted in Figure 3.6A-C and an overview of F-statistics of the ANOVA results is given in Table 3.3. Across the entire recording period no significant differences between the conditions occurred in time spent awake, in NREM and PreREM

sleep, nor in total sleep time (TST; $p > 0.05$). However, animals did spend slightly less time in REM sleep in the STIM condition ($p = .016$), but this effect was not statistically maintained for REM sleep as percentage of TST ($p = .068$). Neither the proportion of NREM and PreREM sleep nor latency to reach stable NREM sleep (STIM: 51.26 +/- 3.77 min, SHAM: 52.47 +/- 4.69 min, $p = .864$) differed between conditions. A reduction in REM sleep latency following stimulation failed to reach significance (STIM: 62.86 +/- 4.14 min, SHAM: 73.22 +/- 4.48 min, $p = .063$). Total duration of the stimulation period was comparable between STIM and SHAM as well (STIM: 55.28 +/- 4.87 min, SHAM: 52.5 +/- 3.70 min, $p = .596$).

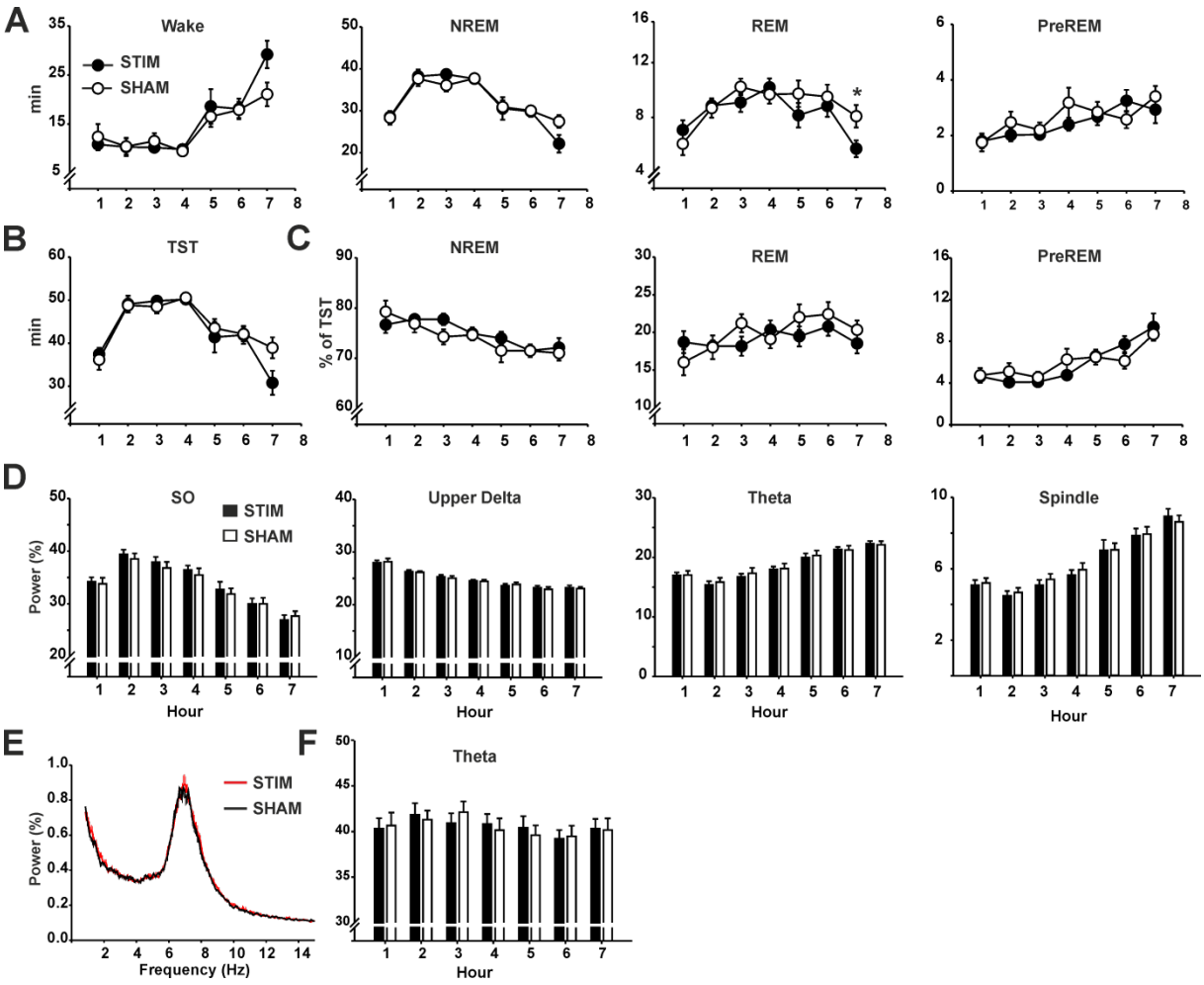

Figure 3.6 Sleep Architecture (mean +/- SEM) and Power analyses across the entire recording period within the retention interval. Sleep staging and EEG power analyses begin after stable NREM sleep was first obtained (on average 52 min after the Test trial). **A**, Time spent in the different sleep stages, expressed as minutes. In the STIM condition, animals spent less time in REM, an effect most likely due to significantly reduced REM duration at the end of the recording period. There were no further differences between the conditions. * $p < .05$, post-hoc t-tests. **B**, Total sleep time (TST) in minutes. **C**, NREM, REM and PreREM sleep in percentage of TST. Neither TST nor the amount of any sleep stage differed between the conditions. Note that REM duration does not differ between the conditions anymore if expressed as percentage of TST. **D**, EEG power for relevant frequency bands during NREM sleep. No group differences were seen. SO: 0.85-2.0 Hz, Upper Delta: 2.0-4.0 Hz, Theta: 5.0-9.0 Hz, Spindle: 10.5-14.0 Hz. **E**, EEG power spectrum of REM sleep **F**, Power in the theta band during REM sleep. No group differences were seen. In figures D-F, power is depicted as percentage of total power between 0.85-35 Hz.

EEG power during NREM sleep across the 8-hour recording period did not differ between conditions for any of the examined frequency bands. Both conditions revealed during NREM sleep to the same extent a decrease in the slower frequencies across the day, while spindle activity increased (Figure 3.6D, Table 3.4). Spindle density per minute did not differ between the conditions for the entire recording period (STIM: 3.23 +/- 0.32, SHAM: 3.02 +/-0.39;

condition: $F(1,11) = 1.31$, $p = .26$; condition x time: $F(6,66) = 2.01$, $p = .068$). Of the detected spindles, 99.4% lay between 10.5-14.0 Hz, with a mean frequency of 12.48 +/- 0.2 Hz, and followed a normal distribution. So-tDCS during SWS also remained without effect on theta activity in REM sleep (Figure 5E-F, condition: $F(1,11) = .08$, $p = .783$; condition x time: $F(6,66) = 1.11$, $p = .367$).

Table 3.3. F-statistics for sleep architecture

	Time (min)		% of TST	
	F	p	F	p
TST				
condition	2.90	.117	-	-
time	17.32	<.001*	-	-
condition x time	1.51	.221	-	-
W				
condition	2.88	.117	-	-
time	14.29	<.001*	-	-
condition x time	1.50	.220	-	-
NREM				
condition	.40	.543	1.17	.302
time	21.90	<.001*	5.61	<.001*
condition x time	1.34	.266	.91	.494
REM				
condition	7.52	.019*	1.96	.189
time	5.46	<.001*	2.11	.064
condition x time	1.40	.229	1.27	.283
PreREM				
condition	.60	.456	.03	.864
time	6.74	<.001*	16.43	<.001*
condition x time	1.61	.197	1.87	.123

Note: Degrees of freedom: 'condition' $F(1,11)$, 'time' and 'condition x time' $F(6,66)$; Huynh-Feldt corrections were used if necessary. * p < .05, ANOVAs for repeated measures.

Table 3.4. F-statistics on EEG power the entire ~ 8 h recording period

	Spectral power	
	F	*p*
SO (0.85-2 Hz)		
condition	.35	.568
time	66.55	<.001*
condition x time	.64	.699
Upper delta (2-4 Hz)		
condition	.06	.808
time	103.16	<.001*
condition x time	.33	.92
Theta (5-10 Hz)		
condition	.28	.608
time	119.09	<.001*
condition x time	.547	.771
Spindle (10.5-14 Hz)		
condition	.40	.543
time	126.0	<.001*
condition x time	.59	.735

Note: Degrees of freedom: 'condition' $F(1,11)$, 'time' and 'condition x time' $F(6,66)$; Huynh-Feldt corrections were used if necessary. * $p < .05$, ANOVAs for repeated measures.

3.2.6 Brain electric activity within the stimulation-free intervals of so-tDCS

Only 30s post-stimulation intervals consisting of NREM sleep only following 'successful stimulations' (= no awakening/EMG activity during stimulation) were considered for analysis on first 10s of the stimulation-free intervals. The number of intervals matching these criteria did not differ between conditions (STIM: 14.8 +/- 0.5, SHAM: 15.4 +/- 0.43; $T(11) = -1.0$, $p = .339$). Figure 3.7 reveals that endogenous SO EEG power (0.8 - 2 Hz) tended to be enhanced in first the 10-s stimulation-free interval immediately following so-tDCS as compared to SHAM ($T(10) = 2.04$, $p = .069$) with 7 of the 11 analysed animals showing this enhancement (Figure 3.7A-C). Binwise comparisons revealed a significant enhancement within the 1.4-1.5 Hz bin (inset in Figure 3.7A). The enhancement within the first 10s of the stimulation-free interval does not merely reflect a general enhancement in SO power within the time period spanning from the first to last stimulation event in STIM as compared to SHAM (SO: $T(11) = .23$, $p = .824$). EEG spectral power within the other relevant frequency bands was also comparable between STIM and SHAM, both within the first 10s of stimulation-free intervals (upper delta: $T(10) = .55$, $p = .595$; theta: $T(10) = -.90$, $p = .391$; $T(10) = .15$, spindle $p = .882$) as well as within the whole time range (upper delta: $T(11) = -.56$, $p = .589$; theta: $T(11) = .69$, $p = .507$; spindle: $T(11) = .08$, $p = .936$; Figure 3.7C). Spindle density was also not affected by so-tDCS within the complete 30-s stimulation-free intervals ($T(11) = -.38$, $p = .708$).

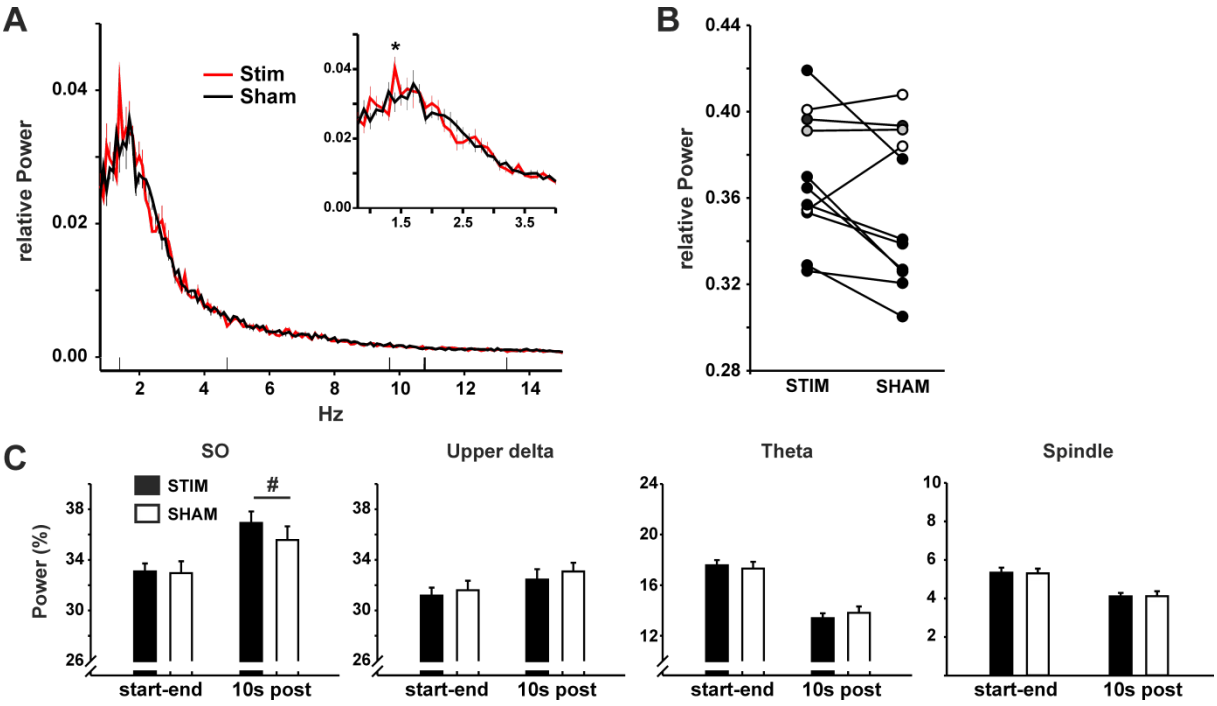

Figure 3.7. EEG spectral measures during the first 10s of the stimulation-free intervals and the entire time period from the first to the last (sham)stimulation epochs. Only intervals containing undisturbed NREM sleep were used for analysis. **A**, Power spectrum (Mean+/- SEM) for all animals and all 10s post-stimulation intervals (N=11). Power is presented as relative power between 0.8-35 Hz. Red curve – STIM condition, black curve – SHAM condition. Vertical bars on the x-axis indicate bins differing significantly between conditions (binwise t-tests). For better visualization of the relevant frequencies, the spectrum is only depicted up to 15 Hz. The inset magnifies the delta frequency range. **B**, SO power between the conditions for individual animals. Most animals show increased power in the STIM condition (black circles, N = 7), while a decrease is only observed in 2 animals (white circles). One animal shows no difference between conditions (grey circle). **C**, Mean spectral power for the entire time period from the first to the last (sham)stimulation epochs ('Start-End') and for the mean of all first 10-s of stimulation free intervals ('10s post'). SO power was slightly increased in the STIM condition in the stimulation-free intervals, whereas differences did not occur in any other band. * $p < 0.05$. # $p < 0.1$.

3.2.7 Post-hoc experiment: theta-tDCS during REM sleep

As revealed by Figure 3.8, P-Index did not differ from chance level neither in the STIM nor in the SHAM condition (STIM: $T(11) = 1.04$, $p = .322$ and $T(11) = .08$, $p = .941$ for the P-Index during the 1st min and, total 2 min, respectively; SHAM, 1st min: $T(11) = 1.13$, $p = .284$, total 2 min: $T(11) = 1.08$, $p = .302$), and both condition did not differ from each other (1st min: $T(11) = -.16$, $p = .873$; total 2 min: $T(11) = -.05$, $p = .487$), indicating a lacking effect of theta-tDCS during REM sleep on memory consolidation.

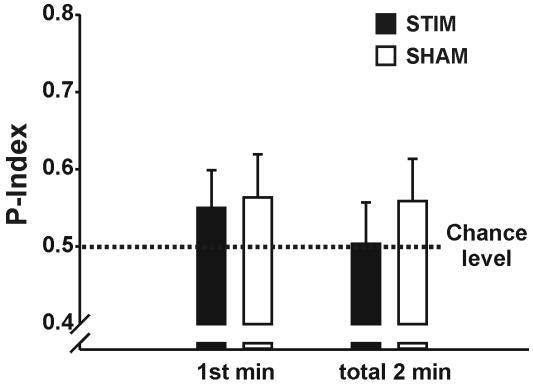

Figure 3.8. P-Index (mean +/- SEM) for the displaced object during the Test trials for both conditions in the post-hoc experiment on theta-tDCS, separated for the 1st and the total 2 min of the trial. Neither the comparison to chance level nor the comparison between conditions was significant ($p > .05$).

Sleep architecture during the whole 8h recording period did not differ between conditions, neither if durations spent in each sleep stage were expressed in min nor in percentage of TST ($p > .05$, for all comparisons between conditions). A detailed overview of F-statistics is given in Table 3.5.

Table 3.5. F-statistics for sleep architecture (post-hoc experiment)

	Time (min)		% of TST	
	F	p	F	p
TST				
condition	1.10	.325	-	-
time	21.81	<.001*	-	-
condition x time	.65	.693	-	-
W				
condition	.39	.546	-	-
time	12.40	<.001*	-	-
condition x time	.65	.691	-	-
NREM				
condition	.54	.479	.02	.902
time	32.57	<.001*	47.68	<.001*
condition x time	.92	.468	.96	.462
REM				
condition	.11	.744	.18	.677
time	23.04	<.001*	44.76	<.001*
condition x time	.41	.843	1.02	.419
PreREM				
condition	2.89	.117	1.55	.239
time	2.34	.062#	5.45	.008*
condition x time	1.21	.311	1.13	.357

Note: Degrees of freedom: 'condition' $F(1,11)$, 'time' and 'condition x time' $F(6,66)$; Huynh-Feldt corrections were used if necessary. * $p < .05$, # $p < .1$, ANOVAs for repeated measures

EEG power across the 8-hour recording period did not differ between conditions for any of the examined frequency bands, neither during NREM nor REM sleep ($p > .05$ for all comparisons between conditions). Changes in power of the frequency bands over time were

similar as in the main experiment (data not shown). A detailed overview of F-statistics is given in Table 3.6. Within the first 10s of stimulation-free intervals, no difference between STIM and SHAM was found in EEG power for theta power ($T(11) = .63$, $p = .544$).

Table 3.6. F-statistics on EEG power the entire ~ 8 h recording period

	NREM sleep		REM sleep	
	F	*p*	*F*	*p*
SO (0.85-2 Hz)				
condition	.01	.927	-	-
time	79.43	< .001*	-	-
condition x time	.54	.708	-	-
Upper delta (2-4 Hz)				
condition	.76	.402	-	-
time	71.40	< .001*	-	-
condition x time	.52	.789	-	-
Theta (5-9 Hz)				
condition	<.01	.961	<.01	.983
time	125.94	< .001*	1.38	.259
condition x time	.37	.883	1.78	.137
Spindle (10.5-14.0 Hz)				
condition	.04	.843	-	-
time	136.59	< .001*	-	-
condition x time	.42	.759	-	-

Note: For REM sleep, only theta power was calculated since SO, upper delta and spindle activity are negligible during this sleep stage (compare Fig. 3.6 E). Degrees of freedom: 'condition' $F(1,11)$, 'time' and 'condition x time' $F(6,66)$; Huynh-Feldt corrections were used if necessary. * $p < .05$, ANOVAs for repeated measures.

3.2.8 Post-mortem verification of electrode placement

After experiments, animals were killed under isoflurane anaesthesia by decaptication, the brain was extracted and skulls were visually examined for correct positioning of stimulation electrodes halfway through the skull. In conclusion, the frontal electrodes used for so-tDCS and counter electrodes were positioned as intended in 7 animals; in 4 animals at least one electrode protruded at least partly the skull. However, the stimulation electrodes over somatosensory cortex which were used for theta-tDCS were correctly positioned in 5 animals only, which occurred most likely due to much thinner bone at this position. An overview of these results is given in Table 3.7.

Table 3.7. Post-mortem inspection of stimulation electrode placement

Animal ID	SO-tDCS electrode LH	SO-tDCS electrode RH	theta-tDCS electrode LH	theta-tDCS electrode RH	Counter electrode LH	Counter electrode RH
2	+	+	-	+	(-)	-
3	+	+	-	(-)	+	+
4	+	+	-	(-)	+	+
5	+	+	-	-	+	+
6	+	+	+	+	+	+
7	+	+	+	+	+	+
8	(-)	+	+	+	+	+
9	-	+	(-)	(-)	+	-
10	-	+	(-)	+	(-)	+
11	+	+	+	-	+	+
12	+	+	+	+	+	+
13	-	+	+	+	+	(-)

Note: LH: left hemisphere, RH: right hemisphere, +: electrode correctly placed halfway through skull, - : electrode fully protruded skull, (-): electrode partly protruded skull

4 STUDY 3: EFFECTS OF REPEATED SO-TDCS IN RATS ON MEMORY CONSOLIDATION IN A MULTIPLE TRIAL LEARNING PARADIGM

In preparation as: Binder, S., Rawohl, J., Born, J. & Marshall, L.; Transcranial slow oscillation stimulation changes learning behavior in the radial maze task

4.1 Methods

4.1.1 Animals

Twenty-two male Long Evans rats (Janvier, Le Genest-Saint-Isle, France), 10 - 11 weeks old at time of surgery, were used. Before surgery, animals were housed individually in Standard type IV Macrolon cages with ad libitum access to food and water under a 12h/12h light-dark cycle (lights-on 07.00 A.M.). All experimental procedures were performed in accordance with the European animal protection laws and policies (directive 86/609, 1986, European Community) and were approved by the Schleswig-Holstein state authority.

4.1.2 Handling

Animals were handled daily for 5 min on seven consecutive days prior surgery. Handling procedure was identical to study 2.

4.1.3 Surgery

Anesthesia procedure and medical treatment was identical to study 2. For epidural EEG recording a stainless steel screw-electrode (diameter 1.57 mm, shaft length 2.4 mm, Plastics One, USA) was placed over the left frontal cortex (AP: + 1.7 mm, L: - 0.5 mm) and referenced to an occipital site (AP: -12.0 mm; L +/- 0.0 mm). For bilateral stimulation screw-electrodes of same size as above were drilled halfway through the skull. Anodes for so-tDCS were positioned over the PFC (AP: + 3.9 mm, L: +/- 2.0 mm) and the return electrodes over the cerebellum (AP: -10.0 mm, L: +/- 2.0 mm). Two holding screws were positioned over the right somatosensory cortex (AP - 4.0 mm, L: + 2.0 mm), another anterior electrode (AP: + 6.9, L + 1.1) was used as ground. For EMG recordings, two insulated stainless steel wire electrodes (Plastics One, USA) were implanted bilaterally in the neck muscles. All electrodes were connected to two plastic pedestals (Plastics One, USA), one for polysomnographic recording and one for so-tDCS, covered with adhesive luting dental cement to enable long-term stability on the skull (C & B MetaBond, Parkell Inc, USA) and finally fixed with cold

polymerizing dental resin (Palapress, Heraeus Kulzer GmbH, Germany). Animals had 7 days for recovery from surgery before moving to the experimental room.

4.1.4 Apparatus and setting

The experimental room was divided by a curtain and light wood walls into three areas: one housing/recording area, one radial maze area and one observation area for the experimenter. The radial maze was made of black PVC with eight arms (L 40 cm, W 9 cm) radiating from a central platform (diameter 24 cm). The arms were enclosed with 17 cm high walls, one of them made of black PVC, the other wall and the end wall made of transparent Plexiglas. At a recess at the end of each arm a glass cup which served as food well was inserted. The central platform was separated from the arms by 30 cm high doors, which could be mechanically operated from the observation area. The whole maze was positioned 50 cm above the ground and a camera was mounted above the apparatus. Surrounding furniture and posters affixed to the walls could serve the animal as extra maze cues to facilitate spatial orientation. Below the end of each arm a cup containing bait food was placed to impede orientation on potential olfactory cues. To prevent the use of intra-maze cues, the maze was rotated daily by 45°.

4.1.5 General procedure and design

Following surgery, animals were housed individually in the recording boxes described in section 2.1.2.4. Food restriction started when animals reached their pre-surgical weight or latest on the 6th post-surgical day. During the course of the whole experiment, rats were kept between 85% - 90% of presurgical bodyweight and weighted daily. On the 6th post-surgical day, they were moved to the housing area in the experimental room where they stayed until the end of the experiment. All procedures described beneath were conducted between 8 AM and 1 PM if not stated otherwise. Animals were randomly assigned to the so-tDCS (STIM) or the control group (SHAM). On the 7th post-surgical day, a 2 h habituation recording was conducted to adapt the animals to the recording conditions. On the 8th post-surgical day, a 2 h baseline recording was conducted, and on day 9 a baseline stimulation recording took place, where the animals which were randomly chosen to receive so-tDCS were stimulated to check for signal quality and proper functioning of the whole setup. One day later, animals were habituated to the radial maze. Therefore, 16 food baits (Choco Krispies, Kellogg GmbH, Germany) were scattered throughout the whole apparatus. The animal was placed on the central platform (doors closed), after ~ 5 s the doors were opened and the animal had the possibility to explore the maze until all baits were eaten or 20 min had elapsed. If after this time the animal did not have consumed any of the bait, the sojourn time was prolonged by 5

min. Afterwards the animal was connected to the recording and so-tDCS cables, placed back in its box and recorded for 2 h. Here, no so-tDCS was applied. On the following day, the experiment proper started. During 12 consecutive days, each animal received 3 trials in the radial maze per day, separated by 3 min the animal spent on the central platform with doors to the arms closed. Every day, the same three arms were baited, see also Figure 4.1. A trial ended when the animal found and consumed all baits or after 3 min. The experimenter placed the animal back onto the central platform while the doors were closed. After 3 trials, animals were connected to the cables and recorded for 2 h. Between two animals the maze was thoroughly cleaned with 60% ethanol solution.

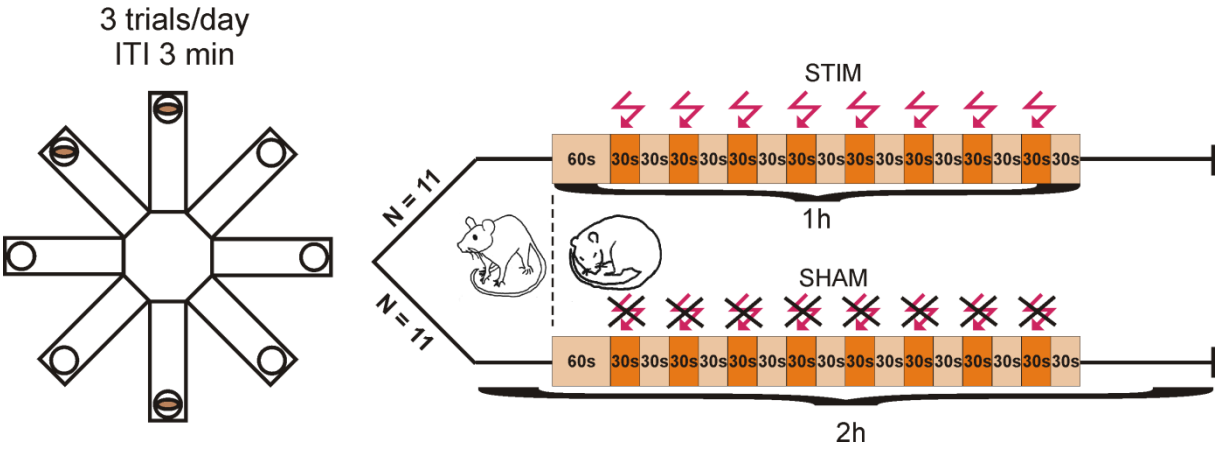

Figure 4.1. Schematic depiction of task procedure and design. ITI = inter-trial interval. For a detailed description of procedure and design see section 4.1.5 above.

4.1.6 Sleep recordings

Six recording boxes were placed in the experimental room. Recording conditions were identical to the ones described in study 1 (section 2.1.3.4).

4.1.7 Stimulation parameters

The so-tDCS electrodes were connected through the same swiveling commutator as the EEG and EMG, but through a separate cable to a battery driven constant current stimulator (designed by the Electronics Facility of the University of Luebeck) in the adjacent room. Current intensity of sinusoidal so-tDCS fluctuated between 0 and 5.6 µA. Stimulation frequency was in the range of slow oscillation (1.5 Hz) and was bilaterally synchronized. Duration of each stimulation train was always 30s, separated by a stimulation free period of at least 30s.

Stimulation started after the first occurrence 60s stable NREM sleep and lasted for 30s, followed by a 30s stimulation free interval. If the animals showed signs of awakening

during stimulation (movement and/or increased EMG activity), stimulation was terminated, and if the animal showed any sleep stage change during the stimulation free period, again 60s of stable NREM sleep was awaited before the next stimulation to take place. Animals received stimulations for one hour, starting with the time of the first stimulation. In the SHAM condition, no stimulation was applied.

4.1.8 Data reduction and statistical analysis

4.1.8.1 Behavioral data

Arm entries, consumption of baits and trial duration were scored online by the experimenter. An arm entry was scored if the animal entered an arm with all four paws. Following measures were computed: i) reference memory errors (entries into arms which never contained a bait), ii) working memory errors (re-entries into arms already visited during the ongoing trial), further divided in iii) working memory errors for baited arms and iv) working memory errors for never baited arms, and v) speed of trial completion (speed-index: ratio of duration of the trial and number of arm entries). The mean values over the 3 consecutive trials/day of all measures were subjected to statistical analysis. Within the habituation trial, number of consumed baits and the duration of the trial were recorded.

4.1.8.2 Sleep architecture

Sleep scoring was conducted according to the same criteria as described for study 1 (see section 2.1.2.6), stimulation epochs were again scored as a separate stage and insertion of sham-stimulation intervals was identical to study 2 (see section 3.1.11.2). Regarding sleep architecture, following measures were computed: total sleep time (TST), duration of the different stages (W, NREM, REM, PreREM) in minutes and as percentage of TST. Furthermore, sleep latency (start of recording to first occurrence of stable NREM), REM latency and the number of stimulations were computed.

4.1.8.3 EEG analysis

Before power spectral analyses, EEG data were first low pass filtered (FIR filter, 35 Hz, attenuation of stop band: -80 db, transition width: 15.5 Hz). Subsequently, a Hanning window was applied on 8192 blocks (~ 8.2 s) of EEG data before power spectra were calculated using FFT. Filtering and FFTs were conducted using Spike2 software (Cambridge Electronics, UK). Generally, data was normalized using the percentage of each bin (bin size 0.12 Hz) with reference to the total spectral power between 0.85 and 35 Hz. To account for possible violations of the assumption of normal distribution, the normalized data was logarithmized according to the method proposed by Gasser et al. (1982): log(x/[1-x]), where log refers to the

natural logarithm and x represents the relative power in a given frequency band. For statistical analyses, these transformed values were used. Analyses were conducted for:

i) all NREM sleep epochs of the baseline and the experimental recordings
ii) all REM sleep epochs of the baseline and the experimental recordings
iii) during (sham)stimulation
iv) the first 10-s stimulation free epochs immediately following stimulation if they consisted of NREM sleep only

The latter analysis was further refined by additionally analyzing just the first and the last 10-s stimulation free interval of the day. This was done due to effects found in a similar study by Marshall et al. (2006) in human subjects, where changes in EEG activity after sotDCS were most pronounced in the first stimulation-free intervals. For all analyses on NREM sleep, mean spectral power was calculated for the slow oscillation (SO) band (0.85-2.03 Hz), the upper delta band (2.03-4.01 Hz), theta band (5.00-9.03 Hz) and the spindle band (10.50-14.04 Hz). For REM sleep, only the theta band was analyzed.

Sleep spindles were detected based on the algorithm used by Eschenko et al. (2006) as described above in section 3.1.11.3. Spindle density was calculated across 1 min intervals of NREM sleep for the same time ranges as for the FFT analyses.

4.1.8.4 Statistics

For behavioral measures, sleep architecture and EEG power analysis ANOVAs for repeated measures were used, followed by post-hoc Student's t-tests where appropriate. A p-value < .05 was considered significant. Results are given as means +/- SEM unless indicated otherwise.

4.2 Results

4.2.1 Behavioral data

Measures of behavioral performance are depicted in figures 4.2 and 4.3. The number of reference memory errors declined in the course of training from day 1 to 12 (time: $F(11, 220) = 47.88$, $p < .001$), without an overall difference between groups (condition: $F(1, 20) = 1.06$, $p = .316$). However, the decline in errors tended to be temporally different for the two groups (condition x time: $F(11, 220) = 1.68$, $p = .08$). Post-hoc tests revealed a baseline difference on day 1, with STIM animals showing significantly more reference memory errors (see Figure 1A). A baseline correction was conducted thereupon, with data now revealing a significant interaction (condition x time: $F(11, 220) = 2.58$, $p = .009$): Performance of the STIM group on days 2-4 was enhanced as indicated by post-hoc tests (see Figure 1B). Overall both groups

revealed as above a significant decline in errors over time (time: $F(11, 220) = 39.92$, $p < .001$; condition: $F(1, 20) = 2.03$, $p = .17$).

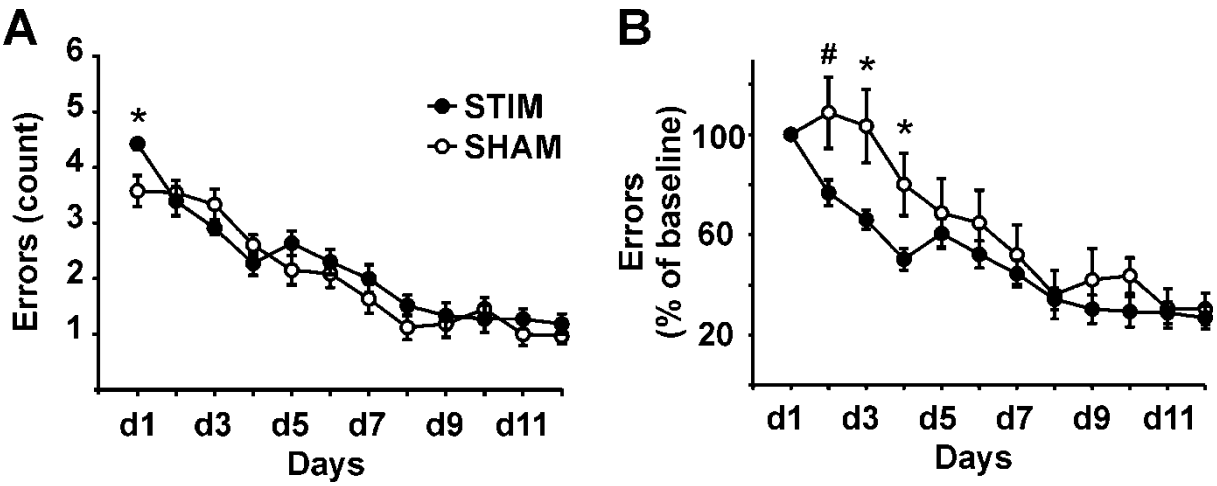

Figure 4.2. Reference memory errors, **A**, absolute error count (Mean +/- SEM per day), **B**, baseline corrected errors (Mean +/- SEM per day), conducted due to a significant baseline difference between conditions. ANOVAs for repeated measures followed by post-hoc t-tests. * $p < .05$, # $p < .1$.

The number of working memory errors also declined with training (time: $F(11, 220) = 10.35$, $p < .001$), without an overall group difference (condition: $F(1, 20) = 0.82$, $p = .778$). Again, a significant interaction across time effect could be detected (condition x time: $F(11, 220) = 2.45$, $p = .034$). Post -hoc tests revealed this effect to be due to a tendency of the STIM group towards poorer performance on day 1, but significantly better performance on subsequent days 3 and 4, (see Figure 4.3A). Since significant differences in working memory errors were found only up to day 4, the differential analysis of re-entries into baited and never baited arms was restricted to the first 4 days. STIM animals made less errors on baited arms than the animals in the SHAM group (condition: $F(1, 20) = 7.74$, $p = .011$), see figure 4.3B. There was no interaction effect (condition x time: $F(3, 60) = 2.08$, $p = .127$), and the decline of errors across these four days showed only a marginal trend (time: $F(3, 60) = 7.74$, $p = .098$). For re-entries into never baited arms a differential effect occurred between conditions (condition x time: $F(3, 60) = 3.52$, $p = .033$), although both groups revealing an overall decline in errors (condition: $F(1, 20) = 0.79$, $p = .782$; time: $F(3, 60) = 15.24$, $p < .001$). Post-hoc tests revealed STIM animals to perform more poorly on day 1, see Figure 4.3C.

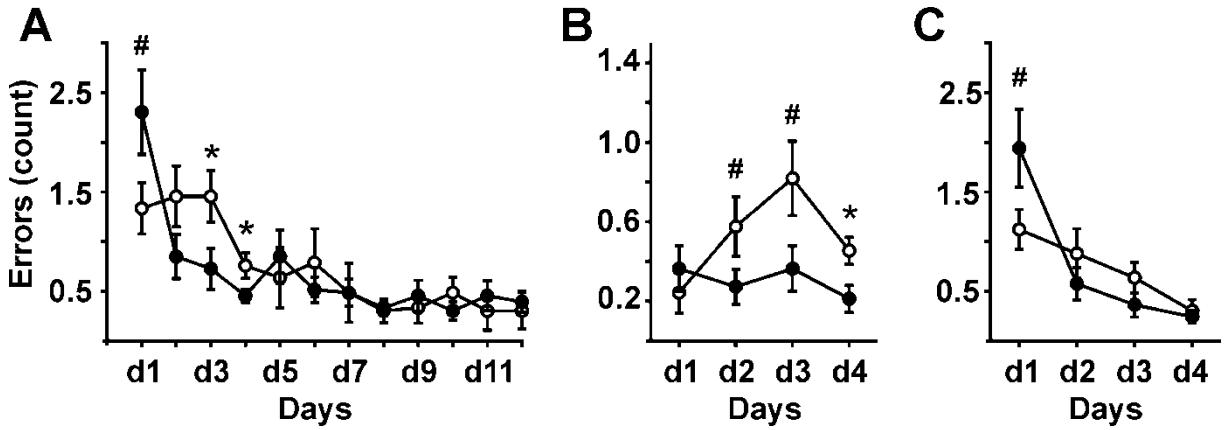

Figure 4.3. Working memory errors. **A**, absolute error count (Mean +/- SEM per day). **B**, absolute count for working memory errors made on baited arms (=re-entries into baited arms where the bait has been consumed already). **C**, absolute count for working memory errors made on never baited arms. ANOVAs for repeated measures, followed by post-hoc t-tests. * $p < .05$, $^{\#}$ $p < .1$.

Speed, expressed as ratio of total time for trial completion to number of arm entries, was higher in the STIM animals (condition: $F(1, 20) = 4.93$, $p = .038$), decreasing in both groups over the course of training (time: $F(11, 220) = 27.49$, $p < .001$; condition x time: $F(11, 220) = .892$, $p = .463$). The results are depicted in Figure 4.4A. Habituation trials did not differ between the groups, neither in regard to the number of consumed baits (STIM: 13.8 +/- 1.0, SHAM: 12.2 +/- 1.4; $T(20) = .94$, $p = .358$) nor duration of the trial (STIM: 19.1 +/- 1.6 min, SHAM: 18.5 +/- .6 min; $T(20) = .31$, $p = 760$). As shown in Figure 4.4.B, body weight did not differ between the groups (condition: $F(1, 20) = .59$, $p = .456$; condition x time: $F(3.6, 200) = 1.81$, $p = .143$).

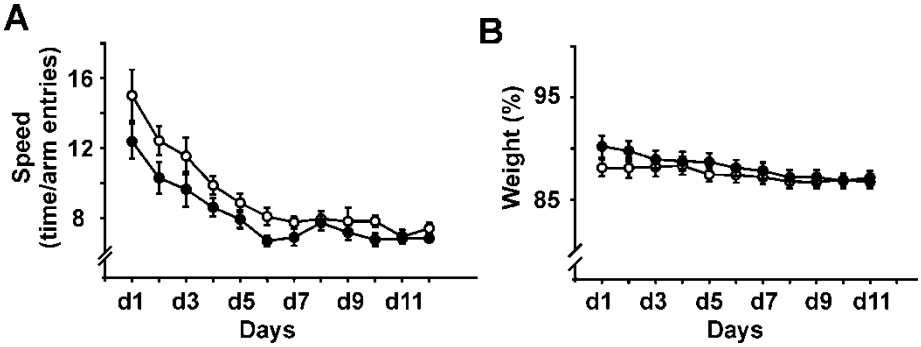

Figure 4.4. Unspecific measures. **A,** Speed of trial completion given as the division of time for trial completion in s by the number of arm entries. STIM animals complete trials significantly faster throughout the experiment ($p < .05$). **B,** Body weight given as percentage of presurgical weight. No difference between the conditions was found. ANOVAs for repeated measures.

4.2.2 Sleep architecture and stimulation

Shortly, there were no significant differences between the groups regarding any of the measures of sleep architecture and no interactions with time (Time spent awake, in NREM, REM and PreREM sleep, duration of Stim/Sham epochs, all expressed both in minutes and as

percentage of TST). During the course of the experiment, the amount of time animals spent awake increased at the expense of all sleep stages evenly (see Figure 4.5 and Table 4.1), but sleep latency and REM latency did not change over time (sleep latency: STIM: 19.8 +/-1.1 min, SHAM: 19.7 +/- 1.0 min; REM latency: STIM 54.9 +/- 1.2 min, SHAM: 53.9 +/- 1.5 min; means are given across the experimental days, $p < .05$).

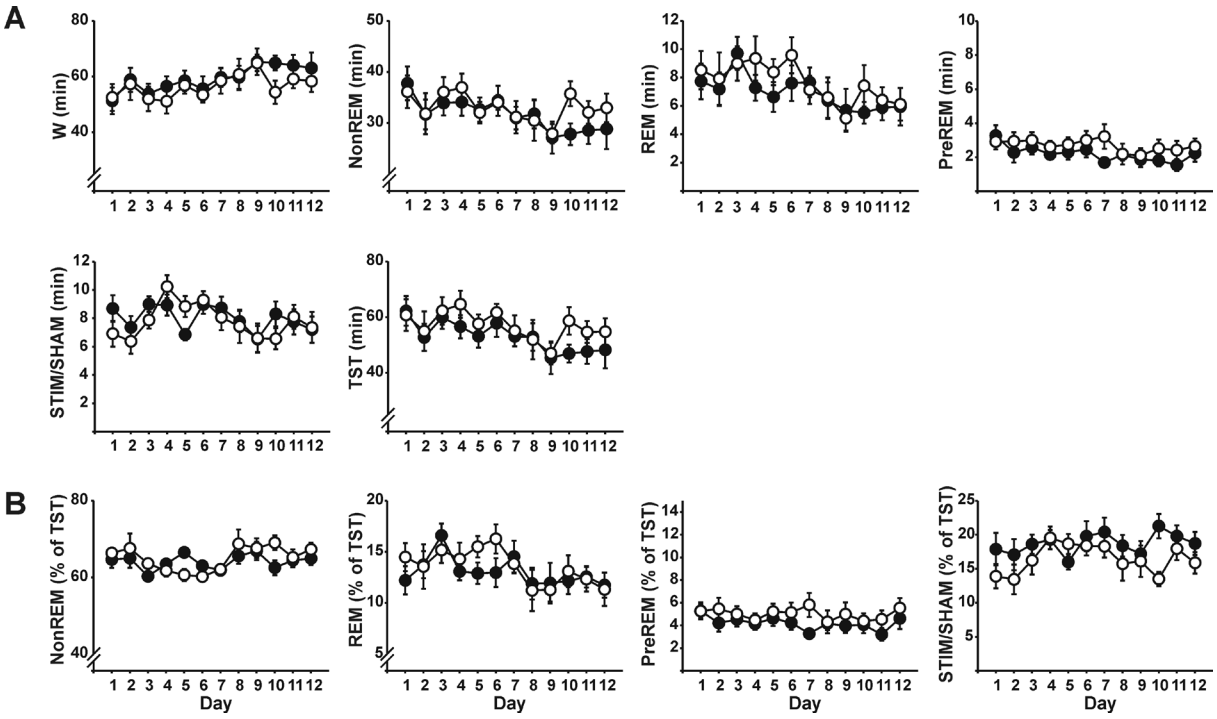

Figure 4.5. Sleep Architecture (mean +/- SEM) across the daily 2h-recording period. Black circles represent STIM condition, open circles SHAM condition. **A**, Different stages are given in minutes. There were no differences between the conditions. **B**, NREM, REM, PreREM sleep and STIM/SHAM epochs in percentage of TST. The amount of sleep stages did not differ between the conditions. ANOVAs for repeated measures.

Table 4.1 F-statistics on sleep architecture.

	Time (min)		% of TST	
	F	*p*	*F*	*p*
TST				
condition	.77	.392	-	-
time	2.98	.006**	-	-
condition x time	.48	.846	-	-
W				
condition	.56	.816	-	-
time	2.93	.007**	-	-
condition x time	.37	.919	-	-
NREM				
condition	.99	.331	.58	.545
time	2.29	.033*	2.56	.015*
condition x time	.88	.538	1.51	.167
REM				
condition	.58	.454	.17	.682
time	3.15	.001**	2.66	.007**
condition x time	.60	.830	.84	.580
PreREM				
condition	1.02	.325	.95	.342
time	2.61	.004**	1.30	.229
condition x time	1.20	.286	.98	.461
STIM/SHAM				
condition	.12	.736	1.83	.191
time	2.74	.003**	1.69	.080
condition x time	1.17	.313	1.45	.156
Sleep latency				
condition	< .01	.976	-	-
time	1.16	.330	-	-
condition x time	1.78	.104	-	-
REM latency				
condition	.02	.889	-	-
time	1.19	.314	-	-
condition x time	.51	.832	-	-

Note: Degrees of freedom: 'condition' $F(1,20)$, 'time' and 'condition x time' $F(11,220)$; Huynh-Feldt corrections were used if necessary. * $p < .05$, ** $p < .01$, ANOVAs for repeated measures.

SO-tDCS was only applied when animals were in NREM sleep. When signs of awakening as defined in section 4.1.7 were evident during the 30-sec stimulation period or sleep stage changed within 10s after stimulation ended, this stimulation period was regarded as 'not successful'. The number of (sham)stimulations did not differ between the conditions, neither if all applied (sham)stimulations were analyzed (STIM: 20.0 +/- 1.2, SHAM: 18.3 +/- 1.2; condition: $F(1,20) = 1.07$, $p = .313$; condition x time: $F(11,220) = 1.52$, $p = .126$) nor if only the percentage of 'successful' (sham)stimulations were considered (STIM: 48.0 +/- 3.7, SHAM: 50.2 +/- 3.7; condition: $F(1,20) = .18$, $p = .677$; condition x time: $F(11,220) = .77$, $p = .673$). An effect of time in both measures did not seem to be due to any systematic changes (total count: $F(11,220) = 2.22$, $p = .016$; percentage 'successful': $F(11,220) = 2.93$, $p = .001$).

See Figure 4.8 for total count of applied and amount of 'successful' (sham)stimulation epochs, and Appendix A for a comparison of stimulation epochs between study 2 and 3.

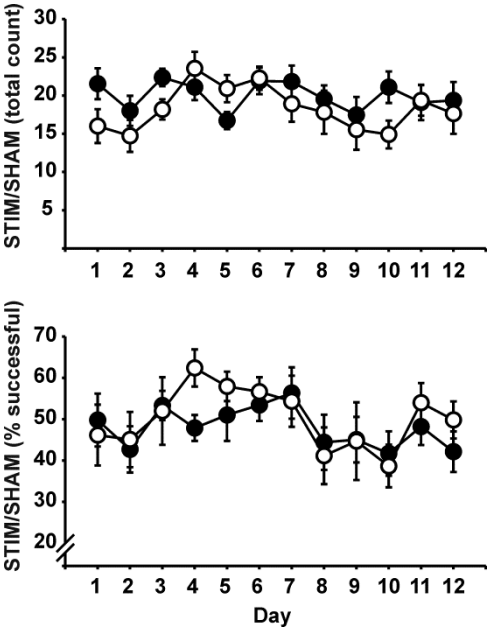

Figure 4.8. Stimulation epochs. The upper figure shows the total number of (sham)stimulations, the lower figure shows the amount of 'succesful' (sham)stimulations (= no change of sleep stage during (sham)stimulation and/or during a 10s interval afterwards). Black circles = STIM group, white circles = SHAM group. There were no differences between the conditions. ANOVAs for repeated measures.

4.2.3 EEG power analysis

The composition of NREM sleep during the complete 2 hour recording period did not differ between the groups in any of the examined frequency bands, neither during baseline recording (see Figure 4.7A and Table 4.2) nor during the experimental recording sessions (see Figure 4.7A and Table 4.3). Within the 10s-intervals of post-stimulation NREM sleep a trend towards an increased upper delta power in the STIM group could be seen (p = .054) together with a trend towards an interaction with time (p = .074). Post-hoc t-tests indicated higher power in the STIM than SHAM group on days 1, 7, 8 and 12. No difference was evident in any other frequency band examined. Detailed F-statistics are given in Table 4.3, and EEG power for both groups across all 12 experimental days is shown in Figure 4.7B.

Table 4.2. T-statistics for EEG power during NREM sleep within baseline recording

	Mean +/-SEM		T	p
	STIM	SHAM		
SO (0.85-2 Hz)	27.75+/- 1.51	25.78 +/- 1.63	.91	.375
Upper Delta (2-4 Hz)	26.31+/- .77	24.25 +/- 2.03	1.07	.308
Theta (5-9Hz)	18.95+/- .82	19.29 +/- 1.06	-.20	.846
Spindle (10.5-13.6 Hz)	7.19 +/- .55	6.84 +/- .50	.41	.686

Note: Means are given in percentage of total power between 0.85-35 Hz for descriptive purposes. T-Tests are conducted on logarithmized data. Degress of freedom: $T(20)$. In case of inhomogeneous variances, values were corrected.

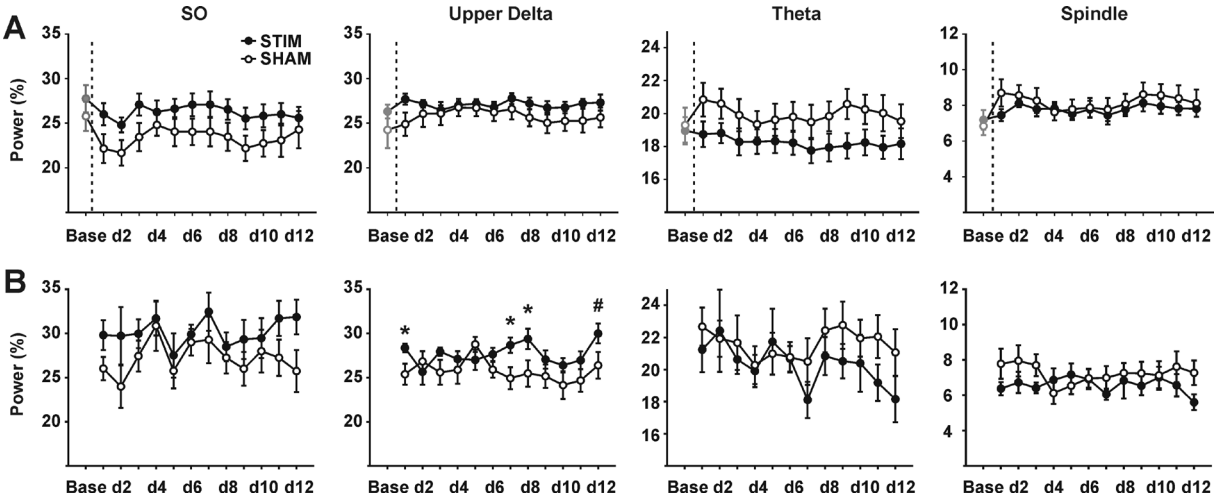

Fig 4.7. EEG power during NREM sleep. **A**, EEG power within the complete 2h-recording for all 12 experimental days and baseline recording (Base, depicted in grey). There were no significant differences between the conditions. **B**, EEG power within the first 10-s of the stimulation-free intervals for all 12 experimental days. A trend towards increased upper delta for the STIM group and a trend towards an interaction was seen but no group differences for the other investigated bands. SO: 0.85-2.0 Hz, Upper Delta: 2.0-4.0, Theta 5.0-9.0 Hz, Spindle: 10.5-13.6 Hz. ANOVAs for repeated measures followed by post-hoc t-tests. * $p < .05$, # $p < .01$.

Table 4.3. F-statistics for EEG power during NREM sleep

	complete 2h-recording		10s post stimulation	
	F	*p*	*F*	*p*
SO (0.85-2 Hz)				
condition	2.82	.109	1.72	.204
time	2.73	.014*	1.93	.071$^{\#}$
condition x time	.71	.642	.70	.672
Upper delta (2-4 Hz)				
condition	1.34	.261	4.18	.054$^{\#}$
time	1.64	.089$^{\#}$	1.34	.202
condition x time	1.56	.121	1.73	.074$^{\#}$
Theta (5-9 Hz)				
condition	2.15	.158	.82	.375
time	2.53	.005*	2.04	.039*
condition x time	.75	.686	.85	.595
Spindle (10.5-13.6 Hz)				
condition	.154	.699	.89	.356
time	2.14	.033*	.68	.741
condition x time	.01	.697	1.21	.283

Note: Degrees of freedom: 'condition' $F(1,20)$, 'time' and 'condition x time' $F(11,220)$; Huynh-Feldt corrections were used if necessary. * $p < .05$, $^{\#}$ $p < .1$, ANOVAs for repeated measures.

Since homeostatic effects (or temporal dynamics) have been reported in other studies with so-tDCS (Reato et al., 2013) and in the present study the total time period of stimulation

spanned across 1 hour, specifically effects of so-tDCS within the first and last post-stimulation intervals were compared. Upper delta power showed a significant interaction of position (first vs. last interval) x group (p = .015). Upper delta power was higher in the STIM group for the first but not the last post-stimulation interval, as revealed by two separate ANOVAS conducted for these two post-stimulation intervals on all 12 experimental days ($F(1,20) = 5.38$, p = .031). No other interactions with position of stimulation across the 2 h recording session were found. Significant effects of position indicated lower SO together with higher theta and spindle power within the first interval compared to the last, most probably reflecting deepening of sleep over time. Results are depicted in Figure 4.8 and F-statistics are given in table 4.4.

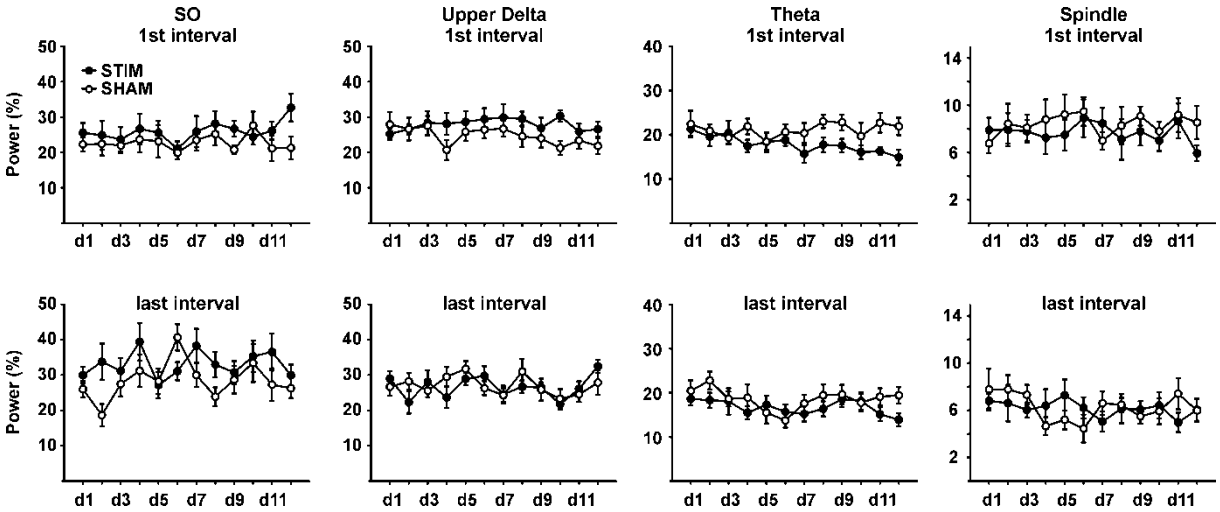

Figure 4.8. EEG power during NREM sleep for the first and the last 10s-interval after stimulation for all 12 experimental days. Upper delta power is significantly higher in the STIM group during the 1st interval. ANOVAs for repeated measures.

Table 4.4. F-statistics for EEG power during NREM sleep for the first and the last 10s interval after (sham)stimulation

	condition x time x position		First interval		Last interval	
	F	*p*	*F*	*p*	*F*	*p*
SO (0.85-2 Hz)						
condition	2.98	.100#	-	-	-	-
time	.84	.546	-	-	-	-
position	38.94	<.001*	-	-	-	-
condition x time	1.05	.407	-	-	-	-
condition x position	.29	.597	-	-	-	-
time x position	1.53	.121	-	-	-	-
condition x time x position	1.15	.321	-	-	-	-
Upper delta (2-4 Hz)						
condition	1.75	.201	5.38	.031*	.06	.805
time	1.23	.272	.68	.742	1.65	.086#
position	.58	.454	-	-	-	-
condition x time	.76	.676	.76	.669	1.25	.253
condition x position	7.15	.015*	-	-	-	-
time x position	1.10	.364	-	-	-	-
condition x time x position	1.25	.257	-	-	-	-
Theta (5-9 Hz)						
condition	2.89	.105	-	-	-	-
time	2.27	.012*	-	-	-	-
position	9.83	.005*	-	-	-	-
condition x time	1.98	.031*	-	-	-	-
condition x position	1.56	.227	-	-	-	-
time x position	.13	.708	-	-	-	-
condition x time x position	.35	.974	-	-	-	-
Spindle (10.5-13.6 Hz)						
condition	.21	.652	-	-	-	-
time	.64	.794	-	-	-	-
position	27.66	<.001*	-	-	-	-
condition x time	.79	.651	-	-	-	-
condition x position	.90	.353	-	-	-	-
time x position	1.13	.340	-	-	-	-
condition x time x position	.35	.316	-	-	-	-

Note: Degrees of freedom: 'condition', 'position' and 'condition x position' $F(1,20)$, 'time', 'condition x time','time x position' and 'condition x time x position' $F(11,220)$; Huynh-Feldt corrections were used if necessary. Separate analyses on first and last interval were only conducted in case of significant 'condition x position' interaction.* $p < .05$,# $p \leq .1$, ANOVAs for repeated measures.

For both conditions a significant difference between the training days could be seen for upper delta, theta and spindle band during the acute stimulation period (see Figure 4.9 and Table 4.5). FFT analysis revealed significantly lower theta power in the STIM group during the stimulation period starting from day 8 on (effect of condition and interaction $p < .05$). To investigate whether this difference was due to a decrease in theta power or a prevention of theta power rise, power within days 8 to 12 was compared with values obtained within comparable sham-stimulation intervals of baseline recording. Results indicate a significant decrease of theta power during day 9 and 10 ($p < .05$) and trends during days 11 and 12 ($p < .1$) for the STIM group, but no significant deviations from baseline for the SHAM group ($p > .1$).

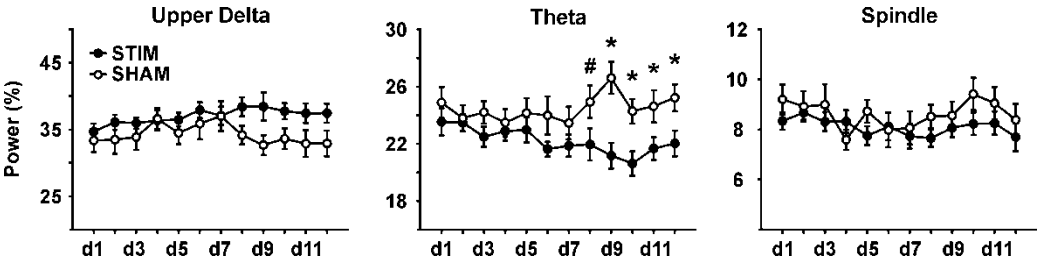

Figure 4.9. EEG power during the acute (sham)stimulation. A significant group difference and an interaction could only be seen for the theta band. Note SO power could not be analyzed due to frequency overlap with so-tDCS. ANOVAs for repeated measures followed by post-hoc t-tests. * $p < .05$, # $p < .01$.

Table 4.5. F-statistics for EEG power during acute (sham)stimulation.

	F	*p*
Upper delta (2-4 Hz)		
condition	2.72	.114
time	1.27	.242
condition x time	1.40	.172
Theta (5-9 Hz)		
condition	4.61	.044*
time	1.48	.143
condition x time	2.92	.002*
Spindle (10.5-13.6 Hz)		
condition	.60	.449
time	1.7	.089#
condition x time	.92	.511

Note: Degrees of freedom: 'condition' $F(1,20)$, 'time' and 'condition x time' $F(11,220)$; Huynh-Feldt corrections were used if necessary. * $p < .05$, # $p < .1$, ANOVAs for repeated measures.

Sleep spindle density during NREM sleep did not differ between the groups, neither for the whole recording period, nor if only the 10s post-stimulation intervals or acute stimulation periods of 'successful' stimulations were considered (condition: whole recording: $F(1,20) = 1.41$, $p = .248$; intervals: $F(1,20) = 1.91$, $p = .182$; acute stimulation: $F(1,20) = 1.13$, $p = .3$). There were no significant interactions, and spindle density did not differ between experimental days (all $p > .05$).

As depicted in Figure 4.10, for REM sleep during the total 2h-recording period no differences were found for theta power neither in baseline recording (STIM: 38.4 +/- 2.6%, SHAM: 39.7 +/- 2.0%; $T(20) = .38$, $p = .707$) nor between the conditions or between experimental days (condition: $F(1,20) = .01$, $p = .928$; time: $F(9.8, 196.5) = .86$, $p = .568$; condition x time: $F(9.8, 196.5) = .67$, $p = .747$).

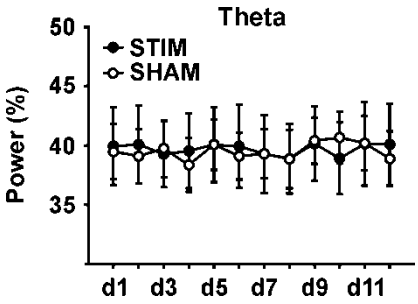

Figure 4.10. EEG power during REM sleep within the complete 2h-recording for all 12 experimental days. Conditions did not differ significantly. ANOVA for repeated measures.

5 DISCUSSION

5.1 Intact memory performance in the OPR task depends on sleep but not on circadian time of day

Experiment 1 of **study 1** investigated if memory performance in the object-place recognition task differs depending on whether the task is conducted in the early morning, a time of day when rats naturally show a high amount of sleep, or in the early evening, a time of day rats usually spent mostly awake. It was hypothesized that intact task performance depends critically on sleep during the 2-h retention interval, and therefore animals were expected to solve the task (i.e. perform above chance level within the Test trial) only if the task was conducted in the morning. Indeed, results indicate that rats in the morning after a retention interval presumably spent mostly asleep accomplished the object-place recognition task whereas they failed to do so when tested after an Evening retention interval which was presumably predominated by active wakefulness. Thus the results are in accordance with human data showing improved retention for hippocampus-dependent declarative memory after an interval of sleep in comparison to a period of wakefulness (e.g., Plihal & Born, 1997). However, Experiment 1, taking advantage of the regular 24-hour rest-activity cycle, did not dissociate effects of sleep from those of the circadian rhythm. It is known that activity and expression of some enzymes and genes involved in memory consolidation underlie circadian oscillations (Dolci et al., 2003; Eckel-Mahan et al., 2008; Wang et al., 2009). However, studies of circadian effects on learning and memory yielded mixed results, with some revealing better recall when subjects were trained and tested during the active phase whereas others found no effect of time-of-day of training on recall (Chaudhury & Colwell, 2002; Valentinuzzi et al., 2001). Indeed, many studies on circadian influences on memory functions did not aim at disentangling effects of sleep from the observed circadian changes (e.g., Devan et al., 2001; Van der Zee et al., 2008; Wisor et al., 2002).

To better distinguish between circadian effects and those of sleep, in Experiment 2 of **study 1** animals were sleep deprived and the effects on memory retention were compared with those following sleep during the same times of the day. It was hypothesized that test performance does not critically depend on time of day, but on sleep within the retention interval. The main finding is that a significant memory for the object place in the sleep as compared to the SD condition was only found for animals with undisturbed sleep in the Morning retention interval. Even if a contribution of circadian processes on memory retention cannot be ultimately excluded - e.g. by differential hormonal levels between Morning and

Evening sessions (Atkinson & Waddell, 1997; Bertani et al., 2010; Born & Wagner, 2009; Kalus, Kneib, Steiger, Holsboer, & Yassouridis, 2009) - these appear not to be essential for successful task completion: If circadian factors alone sufficed to enhance memory consolidation, intact performance after Morning SD should likewise have been observed.

The finding that sleep critically supports consolidation of a hippocampus-dependent task is consistent with previous studies investigating the influence of sleep on memory consolidation in rats (Graves et al., 2003; Hagewoud et al., 2010; Smith & Rose, 1996; Smith, 1996). Interestingly, some of these studies have in addition shown that other versions of the same task not relying on hippocampal processing did not benefit from sleep (Graves et al., 2003; Smith & Rose, 1996; Smith et al., 1998). A few studies in mice (Palchykova, Winsky-Sommerer, Meerlo, Durr, & Tobler, 2006; Palchykova, Winsky-Sommerer, & Tobler, 2009; Rolls et al., 2011) have revealed an effect of sleep deprivation on novel-object recognition performance, a task considered not to essentially involve hippocampal function (Bussey et al., 2000; Mumby et al., 2002). Whether differences in the species or in the methods (e.g., duration of the retention interval) are responsible for these divergent findings cannot be answered here. To my knowledge, of the many studies on object recognition by rats (reviewed in Dere et al., 2007), up until now only one study, which was also conducted in our lab, compared the effects of sleep and sleep loss in both tasks (OPR and NOR; Inostroza, Binder, & Born, 2013). Indeed it could be shown that while performance in the OPR task relies critically on sleep within the 80 min retention interval used there, animals were able to perform well in the non-hippocampus dependent NOR task, irrespective if the retention interval was dominated by sleep or wakefulness.

Taken together, the present results support the hypotheses on the sleep-dependency of intact memory consolidation in the OPR task: Only after a high amount of sleep within the retention interval animals were able to solve the task, and test performance depends critically on sleep within this retention interval, but not on time of day.

5.2 Slow oscillatory and spindle activity are enhanced after learning

In Experiment 2 of **study 1** EEG was recorded for proper sleep monitoring and to investigate putative differences in sleep-associated brain oscillations related to successful memory consolidation. It was hypothesized that SO and spindle activity during the retention interval spent asleep after learning are enhanced in comparison to a baseline sleep episode as well as to the Evening condition. Morning sleep as the essential factor mediating consolidation of object-place memory is indicated by the finding that the Morning retention interval with undisturbed sleep generated significant object-place memory, whereas in the undisturbed

Evening condition sleep did not prove sufficient to consolidate memory on object positions. In addition to the almost three fold longer time spent in sleep during the undisturbed Morning as compared to the Evening retention interval, the EEG in particular during NREM sleep differed markedly between the Morning and Evening retention intervals: Strikingly more slow wave activity (0.85-4 Hz) occurred during Morning intervals. Post-learning slow wave activity including the slow oscillations and delta activity have been revealed in prior studies to be critical for the consolidation of hippocampus-dependent memories as well as for providing the temporal framework for neuronal replay (Crunelli & Hughes, 2010; Marshall & Born, 2007; Marshall et al., 2006).

Interestingly, spindle power (10.5-13.5 Hz) was higher in the undisturbed Evening than Morning sleep interval. Considering evidence that increased spindle activity has shown to be a reliable indicator of "good learning" (Fogel et al., 2009; Schabus et al., 2004), at first glance, the higher spindle activity in the Evening retention interval appears to be contradictory to the chance level object recognition performance after this interval. However, the morning-to-evening increase in spindles may primarily reflect a circadian process which is similarly observed in humans showing increased stage 2 spindle activity towards the end of the nocturnal sleep period (Aeschbach, Dijk, & Borbely, 1997). Most importantly, session spindle activity after the Sample trial in the Morning was significantly higher than during a respective baseline morning interval. This pattern is in line with prior findings that learning can stimulate spindle activity during subsequent sleep, as shown in humans and rodents (Eschenko et al., 2006; Fogel et al., 2009; Gais, Mölle, Helms, & Born, 2002). The present results furthermore show that this increase in spindle activity after learning on the hippocampus-dependent object recognition task is confined to the slow wave sleep-rich period in the beginning of the rest as compared to the active period. Together these findings suggest that spindles contribute to hippocampus-dependent memory consolidation, but only when occurring conjointly with intense slow wave activity.

In sum, the present results support the hypothesis that SO and spindle power are enhanced within Morning sleep after learning in comparison to a morning baseline sleep episode.

5.3 No essential role of REM sleep for the OPR task

Study 1 additionally investigated if REM sleep is affected by learning in the OPR task. It was hypothesized that REM sleep is not changed by this type of hippocampus-dependent task low in emotional content, since a change in REM sleep (i.e., increased amount) after learning could be regarded as an indication for an important role of this sleep stage for memory

consolidation. Compared with baseline morning sleep, sleep in the morning retention interval after the Sample trial was characterized by a significantly lower amount of REM sleep, which does not support an essential role of REM sleep for consolidation of the object place memories. However, this comparison reveals contrary to the hypothesis a change in REM sleep due to prior learning in the OPR task. Interestingly, in **study 2** REM sleep duration was slightly reduced in the STIM condition ~ 8h after learning, together with an enhancement in memory consolidation. However, this effect was not detectable anymore if REM sleep was expressed as percentage of TST. Additionally, the present findings revealed rats spent more time in REM sleep in the morning sleep session than in the Evening session in **study 1**.

Many studies have shown the importance of REM sleep for successful memory consolidation in rats (Hennevin, Huetz, & Edeline, 2007; Smith, 1996). Furthermore, there is evidence that the sequential occurrence of both NREM and REM sleep are necessary for plasticity processes during sleep to take place (Ambrosini & Giuditta, 2001; Giuditta, 1985; Ribeiro & Nicolelis, 2004; Ribeiro et al., 2007). It could thus be argued that the increased REM sleep during the Morning sleep interval in comparison to the Evening session further added to the improved memory performance in this condition. However, an immediate contribution of REM sleep to the enhanced retention of object place memories appears to be unlikely: Firstly, in previous studies (Smith & Rose, 1996; Smith, 1996) the effects of REM sleep on memory processing appeared to be often restricted to “REM windows” which usually occurred with longer delays after learning, compared to the 2–hour post-learning retention interval of the present study. Secondly, most previous studies demonstrating an immediate contribution of REM sleep to memory consolidation used tasks with a strong emotional, i.e., aversive, component like fear conditioning (Datta, Mavanji, Ulloor, & Patterson, 2004) and it might be this emotional component that makes these tasks particularly sensitive to the effects of REM sleep (Born & Wagner, 2009; Nishida et al., 2009). Thirdly, and most importantly, compared with baseline morning sleep, sleep in the morning retention interval after the Sample trial was characterized by a significantly lower amount of REM sleep, which does not support an essential role of REM sleep for consolidation of the object place memories. Additional support for this assumption stems from the results of the post-hoc experiment of **study 2**, where animals received theta-tDCS during post-learning REM sleep. Here, no effect of theta-tDCS on memory performance was apparent.

The hypothesis that REM sleep is not changed by the OPR task has to be refuted, since REM sleep was decreased in the Morning sleep retention interval in comparison to the

baseline condition. However, this decrease suggests no essential role of REM sleep for memory consolidation in this task.

5.4 So-tDCS during SWS enhances hippocampus-dependent memory consolidation

In this section, mainly findings on the effect of so-tDCS on hippocampus-dependent memory consolidation (i.e., behavioral data) are discussed, while in-depth discussion of electrophysiological data will follow in section 5.6. However, as it is assumed that behavioral effects of so-tDCS are related to changes in electrophysiological parameters during prior sleep induced by so-tDCS, essential results of EEG data necessary to understand the line of discussion are mentioned and discussed in this section already.

Based upon the findings of **study 1**, showing sleep-dependency of memory consolidation in the hippocampus-dependent OPR task, **study 2** investigated the effects of so-tDCS applied during post-learning NREM sleep on memory performance after an increased retention interval in the same task. **Study 3** aimed at extending the findings of **study 2** to another hippocampus-dependent spatial task (radial arm maze) and investigating the effects of repeated application of so-tDCS over the course of multiple learning trials conducted on consecutive days. It was hypothesized that so-tDCS during SWS leads to enhanced hippocampus-dependent memory consolidation in both tasks.

Results of **study 2** indeed show an effect of so-tDCS during early SWS on memory performance in the OPR task after a 24h retention interval: Animals accomplished the task after receiving so-tDCS, while failing to do so without stimulation. Furthermore, analysis of EEG data indicates an enhancement of slow-oscillatory activity within a 10-s interval after cessation of so-tDCS, possibly pointing towards a facilitatory effect of so-tDCS on endogenous cortical activity. It does not appear that a general difference in sleep architecture contributed to effects on memory consolidation, since these parameters hardly differed between the conditions. A slight reduction initially measured in REM sleep duration in the STIM condition was no longer significant when expressed as amount of TST. Behavioral control measures (e.g. Sample trial duration, time spent in the 4 quadrants of the open field within Sample and Test trial) did not differ significantly between conditions nor did these behavioral measures within Sample trials systematically affected the Test trials, underlining that stimulation specifically influenced memory consolidation. Theta-tDCS during REM sleep, conducted within a post-hoc experiment, failed to improve memory performance after a

24h retention interval, thus supporting the specificity of so-tDCS during SWS for an improvement of memory consolidation in the OPR task. These findings are in line with results from human subjects where after so-tDCS at the transition into SWS an enhancement in hippocampus-dependent memory consolidation could be shown (Marshall et al., 2006).

However, a putative beneficial effect of so-tDCS on the hippocampus-dependent reference memory could not be stated so clearly for **study 3**, since a baseline difference between the groups made a comparison difficult (see below in section 5.5 for a discussion of possible reasons). Although a direct comparison of reference memory errors did not show the expected accelerated learning curve for the STIM animals, baseline corrected values (performance on day 1 set to 100%) point towards faster acquisition of the memory for baited locations after so-tDCS up to day 4. In contrast to **study 2**, no effect on SO power immediately after stimulation was seen, but post-stimulation enhancement in upper delta power and reduction in theta during acute stimulation within the last experimental days indicate an influence of so-tDCS on cortical activity, but see section 5.6 for detailed discussion on electrophysiological outcome.

The results of **study 2** extend the findings of **study 1** on the essential role of a high amount of slow-wave activity during early sleep for consolidation in the OPR task. Furthermore, while in that study a 2h-interval was used, a time range considered to be in the range of so-called "intermediate memory", by applying so-tDCS the retention interval could be extended to 24h, an interval which is commonly used to investigate long-term memory (Kesner & Hunsaker, 2010). It is to note that this was achieved without any major changes in the design of the task. The majority of other studies on the OPR task using 24-h or longer retention intervals usually increased the number of objects or the number of sample trials, and in this way change core-features of the task (Commins, Cunningham, Harvey, & Walsh, 2003; Paban, Jaffard, Chambon, Malafosse, & Alescio-Lautier, 2005; Gaskin, Tardif, & Mumby, 2009). To my knowledge, only one study by Ozawa, Yamada, & Ichitani (2011) could show intact performance after a 24h delay using the original one-trial task design by Ennaceur et al., 1997[3].

Several differences between **study 2 and 3** should be mentioned, concerning task-related and stimulation-protocol related issues, which may be relevant to explain the less pronounced effect of so-tDCS in **study 3** on the consolidation of spatial memory (i.e., effects

[3] It is to note that in object recognition tasks relying on spontaneous exploration patterns there are many contributing factors (e.g. animal strain, housing conditions, stress level, object types etc.) which can influence the exact duration of the retention interval for which intact memory performance can be seen, thus making comparisons on this aspect between laboratories difficult (for review, see Dere et al., 2007).

found only after baseline adjustment of reference memory errors and only up to day 4). First of all, the radial maze procedure is a task learned over several days and requires several trials per day, while the object-place recognition task being a one-trial task. This aspect alone may be not a critical point, since at least on the second day of the radial maze task (i.e. after the first stimulation session) an enhancement in memory consolidation through so-tDCS should be clearly detectable, even if one could speculate that repeated so-tDCS may later on lead to some kind of habituation effect, expressed behaviorally in reduced effectiveness on memory performance. Secondly, the motivation for the animals to solve the task is fundamentally different. While the radial maze task relies on food as a motivator and was conducted in food deprived animals, the OPR task uses the inborn tendency of rodents to prefer novelty over familiarity and is conducted under ad libitum access to food. This aspect could indeed make a critical difference, since it is known that food deprivation influences sleep architecture (Jacobs & McGinty, 1971; Dewasmes, Duchamp, & Minaire, 1989). In fact, total sleep time in **study 3** decreases over the course of the experiment (see Figure 4.5A), along with a decrease in weight on a descriptive level. Furthermore, although animals in **study 3** spent a slightly higher amount of TST in NREM sleep, sleep in the radial maze experiment was much more unstable than in the OPR study, which is reflected by the number of stimulations not usable for analysis due to short awakenings or sleep stage changes (**study 3**: between 40-50% successful stimulations, **study 2**: 75 % successful stimulations; see also Appendix: Table A2 and A3). Since this holds true for both conditions SHAM and STIM equally, this effect cannot be explained by an acute influence of so-tDCS in **study 3** on probability of sleep stage change. Furthermore, sleep latency was strikingly shorter in **study 3**, without a difference in total sleep time to **study 2**, which also speaks for decreased sleep stability (by means of undisturbed sleep cycles not interrupted by unusual numerous arousals). This is in line with Dewasmes et al. (1989), who could show a progressive reduction of TST together with increased number of awakenings and reduced length of SWS episodes in fasting rats, and interestingly also with a study in anorectic patients by Nobili et al. (2004), who found increased number of arousals and decreased slow wave activity in comparison to age matched healthy controls, with SWA correlating with body mass index. Besides affecting sleep quality, food deprivation is known to induce - together with its influence on other hormonal and immunological factors - elevations in stress-hormones (Nakamura et al., 1990; Armario, Montero, & Jolin, 1987; Guarnieri et al., 2012), and stress again is known to be able to impinge upon memory performance (Kim, Song, & Kosten, 2006; Beck & Luine, 1999). So one could speculate that the impact so-tDCS has on a stressed organism may be not as

effective as on an individual in a non-stressed state. However, further studies are necessary to elucidate this putative relationship and its possible mediators like sleep stability, hormonal and neuronal factors.

Findings of **study 2** suggest that the enhancement in memory consolidation resulted from boosting SO activity. Enhanced synchronization of cortical networks in the SO frequency band presumably facilitated the hippocampal-neocortical dialogue presumed to play an essential role for sleep-dependent memory consolidation (Sirota, Csicsvari, Buhl, & Buzsaki, 2003; Mölle et al., 2006; Takashima et al., 2006; Gais et al., 2007; Lesburgueres et al., 2011). Although a proper analysis of cortical activity in the SO range during stimulation was precluded in both studies applying so-tDCS due to a frequency overlap with endogenous SO, a trend towards an increment in broad band SO activity (0.85-2 Hz) within the 10 s following stimulation could be shown in **study 2**. This is consistent with the acute entrainment observed in animals in-vitro (Fröhlich & McCormick, 2010) and in-vivo (Ozen et al., 2010) and is consistent with the post-stimulation entrainment/increase in SO power previously observed in human data (Marshall et al., 2006; Antonenko, Diekelmann, Olsen, Born, & Molle, 2013). A recent modelling study suggests that weak-field stimulation may increase firing rates during the UP-state (high-gamma activity) regardless of stimulation polarity – but only if oscillatory stimulation entrains the endogenous slow-waves (Reato et al, 2013).

The putative enhancement of the hippocampal-neocortical dialogue by so-tDCS could in essence result from a positive impact on plasticity in PFC, as shown for endogenous as well as for exogenous triggered slow oscillatory activity (Chauvette, Seigneur, & Timofeev, 2012). Studies on spatial memory involving training over several days - as the radial maze task used here in **study 3** - conducted within the framework of the standard consolidation theory (Marr, 1971; Frankland & Bontempi, 2005) typically showed an involvement of PFC for the recall of remote memory (i.e., memories which already became independent of the hippocampus, operationally defined usually as delays > 10 days), but not for recent memory (still hippocampus dependent memories, usually operationally defined as delays < 3 days; Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999; Maviel, Durkin, Menzaghi, & Bontempi, 2004; Teixeira, Pomedli, Maei, Kee, & Frankland, 2006). These operational definitions stem mainly from experiments using alpha-CamKII-mutant mice showing deficient plasticity in neocortex, but not in hippocampus, concomitant with intact memory retention on day 1-3 following learning, but impaired memory if tested at longer delays (10-30 days; Frankland, O'Brien, Ohno, Kirkwood, & Silva, 2001) But, on the contrary, there is

also evidence for the recruitment of PFC for recent memory, as shown for trace fear conditioning in mice (delay of 2 days; Blum, Hebert, & Dash, 2006), but also for consolidation and retrieval of spatial memory in a massed training water maze protocol (delay of 1 day; Leon, Bruno, Allard, Nader, & Cuello, 2010). If these divergent findings regarding the role of the PFC for recent memory are due to inherent task differences, i.e. regarding complexity or richness of contextual cues (Lopez et al., 2012) or due to other differences in methodological approaches remains to be determined. To my knowledge there are however no systematic investigations on the relative contribution of hippocampus and neocortex over time to the one-trial OPR task, as applied in **study 2**. It has only been shown for another version of the OPR task using multiple Sample trials over several days, that performance can well be maintained for retention intervals greater than 24 h (Gaskin, Gamliel, Tardif, Cole, & Mumby, 2009) and become independent on intact hippocampus over time (Gaskin, Tardif, & Mumby, 2011; Gaskin et al., 2009).

Taken together, the present results essentially support the hypothesis that so-tDCS during SWS leads to an enhancement in memory consolidation, as measured by above chance performance in the Test trial in **study 2** and by accelerated decline in reference memory errors in **study 3** up to day 4. However, in **study 3**, the expected accelerated decline in errors was only detectable after baseline correction and only within the first experimental days, which may be related to task-specific differences and/or differing stimulation protocols (the latter will be discussed in greater detail in section 5.6).

5.5 Differential effects of so-tDCS on unspecific behavior and working memory performance

To underscore the specific effect of so-tDCS on hippocampus-dependent memory consolidation, possible effects of so-tDCS on other behavioral processes beside hippocampus-dependent memory consolidation were analyzed. In **study 2**, a pilot study was conducted prior to the main study consisting of 3 trials in novel OFs. This pilot study took place after the first application of so-tDCS following the 3 habituation sessions to the OF used for the main experiment, assessing long-term effects on locomotion, exploration and emotionality. More precisely, in a within subjects design, ~ 30h following so-tDCS or sham-stimulation and again 7-9 days afterwards, animals were placed in a novel OF and the above mentioned behaviors were assessed (see also section 3.1.8 for detailed description of procedure, Figure 3.2 for a timeline and section 3.2.2 for results). This pilot study on OF behavior was intertwined with the pilot study on retention intervals, where 3 different intervals (2h, 5h, 7-8h) were applied.

The OF trials followed the first 5h session, i.e., the session where so-tDCS or sham-stimulation was applied for the first time. In **study 3**, working memory and speed of trial completion were evaluated (see also Figures 4.3 and 4.4 for results). It was hypothesized that so-tDCS does not affect these unspecific behaviors or working memory processes.

Animals in **study 2** showed reduced grooming activity in the OF trials ~ 30h after the application of so-tDCS compared to sham-stimulation, but grooming did not differ between conditions during the Test trial of the main experiment. In this pilot study, no further parameters of exploration, locomotion and emotionality were changed. Grooming activity, viewed as a displacement response in reaction to stressful events, has been shown to decrease under the influence of anxiolytics (as benzodiazepines and other $GABA_A$ agonists) and habituates over several trials within the same apparatus (Espejo, 1997; Prut & Belzung, 2003; van Gaalen & Steckler, 2000). Altogether, this behavior can be regarded as an emotional response in terms of anxiety or reaction to stress, although there is also evidence that under specific circumstances grooming activity can be a sign of the animal shifting into a more restful state after a stressor was removed (van Erp, Kruk, Meelis, & Willekens-Bramer, 1994). However, most studies so far found changes in grooming in conjunction with changes in other measures of emotionality, like rearing (Prut & Belzung, 2003). Therefore, an interpretation of the effects so-tDCS may have on grooming alone cannot be given unequivocal, especially in the light of a missing replication of an influence on this parameter following so-tDCS in the main experiment. In this context it is to note that rearing activity and wall-near time decreased while center time increased between the first OF habituation trial (in the OF used for the OPR sessions) and the following 3 trials in the novel OFs. This indicates that habituation took place, either to the general procedure of placing the animals into a novel enclosure, or that the different OFs and extra-maze cues used in the pilot study did not sufficiently differ from each other, allowing for between trial habituation. However, as grooming was not affected by this habituation over time, and most importantly the order of condition SHAM and STIM was balanced, between trial habituation could not have accounted for the reduced grooming activity seen after first exposure to so-tDCS.

In **study 3**, so-tDCS enhanced in the beginning of the experiment the working memory component, more precisely reduced the number of re-entries into baited arms. Furthermore, the speed to obtain all baits was enhanced in the STIM group over the whole experiment (see Figure 4.4).

The enhanced working memory for baited arms on day 2 to 4 indicates that mPFC activity was affected: Besides being a memory storage, the PFC also plays a prominent role as

mediator of executive functions by supporting processes associated with working memory, temporal processing of information, rule learning and decision making (Kesner & Churchwell, 2011; Laroche, Davis, & Jay, 2000; Velazquez-Zamora, Gonzalez-Ramirez, Beas-Zarate, & Gonzalez-Burgos, 2011; Hayton, Lovett-Barron, Dumont, & Olmstead, 2010). An interesting fact is that working memory performance was not overall changed, but that the improvement here was limited to the baited arms, i.e. it seems animals receiving so-tDCS were better in acquiring the rule "if a bait is eaten already it won't come back within the same trial" and behave according to this rule. Therefore, the enhancement of working memory for baited arms in the STIM group could possibly be explained by an effect of so-tDCS on executive functions. Pairing of constant tDCS of the PFC in the rat with training on working memory and skill learning was reported to have a long term benefit on skill retention and spatial working memory (Dockery, Liebetanz, Birbaumer, Malinowska, & Wesierska, 2011; de Souza Custodio, Martins, Lugon, Fregni, & Nakamura-Palacios, 2012) though in these studies tDCS was applied irrespective of the behavioral state of the animal. Common mechanisms could be responsible for the effects seen by these two studies and in study 3 presented here, as rule-learning and acquisition of working memory tasks depend on plasticity processes in prefrontal regions (Velazquez-Zamora et al., 2011; Laroche et al., 2000), and tDCS was shown before to trigger plasticity processes, as described in detail below in section 5.6. Taken together, in can be argued that the enhanced performance of the STIM animals in the working memory component for baited arms could be associated with so-tDCS induced plastic changes in the PFC. This association furthermore implies that improved retention of spatial memory in the OPR task (**study 2**) and the accelerated reference memory acquisition in the radial maze (**study 3**) may also be related to plasticity processes in PFC initiated by so-tDCS.

The difference between the two groups in **study 3** in reference memory already on baseline day 1 needs to be commented. If this difference was anything but chance, the only systematic variation between the groups at day 1 lies in the baseline so-tDCS session, 48 h before training day 1. Here STIM animals, but not the SHAM group, received so-tDCS. Although an effect of so-tDCS on memory tested 48 h later cannot be ultimately excluded, the following argue against it: Firstly, exploratory activity - measured as distance travelled and rearings - in a novel open field was identical in SHAM and STIM animals ~ 30 hours after a single so-tDCS session in **study 2**. And secondly, during the habituation trial to the radial maze one day prior to the first memory task, no systematic differences between the groups were detected regarding bait consumption or trial duration.

A similar behavioral pattern in the radial maze measured by means of increased speed to trial completion as presented by the STIM group here was described before for high-rearing activity (HRA) rats (Görisch & Schwarting, 2006). These rats are distinguished from otherwise identical animals - in regard of strain, age, sex, and housing conditions - by increased rearing response to novel environments. Görisch & Schwarting (2006) hypothesize that hunger and the incentive value of food had a stronger impact on HRA rats expressed in higher speed of trial completion in the radial maze, and relate this to differences found between HRA and low RA animals in specific neurotransmitter concentrations in brain regions associated with emotional and motivational aspects of learning (Thiel, Müller, Huston, & Schwarting, 1999). As no difference in body weight between the SHAM and STIM animals were found in **study 3**, a difference in incentive value of food due to differential levels in hunger between both groups appears unlikely. However, as discussed above, tDCS can exert its effects not only through direct action on neuronal excitability, but also through changes in neurotransmitter concentrations at the stimulation site (Nitsche et al., 2004; Stagg et al., 2009), which could explain the similar behavioral pattern of STIM and HRA rats. Interestingly, HRA rats also show increased reference and working memories on day 1 of learning. It is however questionable how the worse performance on day one could be related to neurotransmitter changes due to so-tDCS here, since the last sot-DCS session was 72 h before day 1 of radial maze training.

In sum, the hypothesis that so-tDCS has no influence on unspecific behavior in the open field in **study 2** and locomotion and working memory in **study 3** has to be refused. While the reduction of grooming activity following so-tDCS is difficult to interpret and needs further investigation to examine the replicability of this effect, so-tDCS enhanced speed of trial completion and decreased working memory errors for baited arms in **study 3**.

5.6 so-tDCS effects on brain oscillations may depend on stimulation parameters and on species studied

Based upon findings in human subjects, it was hypothesized that so-tDCS would enhance SO- as well as spindle activity in comparison to sham-stimulation. As already described above in section 5.4, in line with this hypothesis, SO EEG activity in the frequency bin 1.4-1.5 Hz was transiently enhanced immediately after so-tDCS in **study 2** within a 10s interval of NREM sleep following so-tDCS, but not in **study 3**. For the broad SO band (0.8-2.0 Hz) in study 2 only a trend was revealed. In **study 3**, within the same time interval, upper delta was enhanced. Comparisons of the first vs. last stimulation of the day indicated this effect contained a temporal component, as this effect was only apparent after the first stimulation of

the day. An effect across experimental days also appeared to be involved, since increases in upper delta were found to be significant only on day 1, 7, 8 and by trend on day 12. Thus, effects of so-tDCS on upper delta activity are most pronounced at the beginning of the 2 h recording session as well as during later experimental days. Within the last days of the experiment during the period of acute stimulation theta power was decreased, indicating a long-term effect of so-tDCS. In contrast to the hypothesis, neither in **study 2** nor in **study 3** an effect of stimulation on spindle activity was found.

In support of the hypothesis, the findings of **study 2** suggests a synchronizing effect of so-tDCS on cortical SO activity, as shown before in studies on human subjects (Marshall et al., 2006; Antonenko et al., 2013), rats (Ozen et al., 2010) and ferret brain slices (Fröhlich & McCormick, 2010). As discussed above in section 5.4, it can be suggested that this boosting effect on SO promoted the enhanced consolidation of place memory. The restriction of enhancement in post-stimulation SO power to a small bin (1.4-1.5 Hz) resembles the effects found by Marshall et al. (2006), where similarly only a narrow band (0.5-1 Hz) was enhanced by so-tDCS. However, there a strong increase in slow spindle activity was found concomitantly, which was not detected here, neither in **study 2** or **study 3**.

Although the working hypothesis is that so-tDCS promotes systems consolidation by the same route as endogenous slow oscillation activity, which processes exactly are involved in the effects induced by so-tDCS still remains to be determined. So-tDCS may have exerted its effects on the hipppocampo-neocortical system at least on two levels: On the one hand, SOs could have influenced hippocampal activity such as sharp-wave ripples (Isomura et al., 2006), which play an essential role in memory consolidation (Girardeau & Zugaro, 2011). On the other hand, hippocampal inputs may have arrived at a neocortex which was more susceptible to these inputs due to enhanced synchronized SO activity and/or modified thalamo-cortical state (Wierzynski, Lubenov, Gu, & Siapas, 2009; Molle, Bergmann, Marshall, & Born, 2011). In fact relevance of the neocortical network in the hippocampal-neocortical dialogue has already been indicated. It was found that neuronal ensembles in PFC that were active during an actual learning experience were targeted by hippoccampal replay during SWS (Peyrache et al., 2009). Furthermore, non-oscillatory cortical polarisation can lead to plastic changes in cortical network: tDCS was shown to enhance presynaptic and BDNF-dependent plasticity as well as calcium accumulation (Moriwaki, 1991; Islam et al., 1995; Islam et al., 1995; Fritsch et al., 2010; Marquez-Ruiz et al., 2012), and pharmacological experiments in human subjects suggested an implication of NMDA receptors on enhanced excitability by tDCS (Nitsche et al., 2003). The after effects of anodal tDCS were furthermore

shown to depend on modulation of GABAergic and glutamatergic synapses (Stagg & Nitsche, 2011). For oscillatory tDCS, data on effects on cortical plasticity is still sparse. However, a recent study by Chauvette et al. (2012) suggests endogenous or induced slow oscillatory activity promotes postsynaptic calcium-dependent plasticity. Thus, the effect of so-tDCS may indeed have primarily resulted from a modulation of cortical plasticity. For long-term memory to be established, plasticity processes have to take place (Bailey et al., 1996). In fact the efficiency of so-tDCS at such a long (~24 h) retention interval as used here could be viewed as reflecting a strong contribution of cortical network plasticity. In addition, tDCS can cause local changes in neurotransmission (Stagg & Nitsche, 2011). Changes in neurotransmission, are known to be critically involved in synaptic transmission and plasticity (Stagg & Nitsche, 2011). A third possibility could be that the efficiency of so-tDCS on memory was primarily due to a direct effect on the hippocampus. Transcranial stimulation of rats at field strengths of > 1mV/mm was shown to induce volume conduction and thereby entrain hippocampal units to the applied oscillation and in this way changing their natural phase in relation to cortical SO activity (Ozen et al., 2010). According to electric field calculations for **study 2,** maximum subcortical field strength at the height of the anodes induced by so-tDCS was at most 0.2 mV/mm. Although along these lines entrainment of hippocampal-neuronal firing seems unlikely, a direct effect on hippocampal activity cannot completely be excluded (Francis et al., 2003; Reato, Rahman, Bikson, & Parra, 2010).

On the contrary, from the findings of **study 3** a boosting effect of so-tDCS on post-stimulation endogenous SO activity cannot be concluded, since within 10s post-stimulation no difference in this frequency band between SHAM and STIM animals was detected. However, accelerated decline of reference memory errors (after correcting for the baseline difference on day 1) and the improved working memory performance on baited arms up to day 4 indicate a behavioral effect of so-tDCS. One could speculate these effects being the consequence of enhanced SO activity induced during *acute* so-tDCS, as acute entrainment of cortical activity to slowly oscillating weak electric fields have been shown before in vivo and in vitro (Ozen et al., 2010; Fröhlich & McCormick, 2010).

Factors related to the so-tDCS protocol may have influenced the failure to enhance post-stimulation SO activity resulting in a less pronounced effect on hippocampus-dependent memory consolidation in **study 3**. Firstly, in **study 2**, a trapezoidal pattern of so-tDCS was used, analogue to the human study conducted by (Marshall et al., 2006), while in **study 3** a sinusoidal pattern was applied. How the form of the applied current influences cortical activity and memory consolidation has not been systematically investigated yet in vivo,

however slice data indicates steeper slopes of applied weak electrical fields are more successful in entraining network activity (Fröhlich & McCormick, 2010). Secondly, the stimulation protocol differed slightly: While a time window for application of so-tDCS was used in **study 3** (stimulation was applied within 1 hour after having reached stable NREM sleep for the first time), in the OPR experiment (**study 2**) a constant number of 20 successful stimulations (i.e. no awakenings/movements during so-tDCS, no sleep stage change immediately afterwards) were applied. This difference in protocols yielded a variable number of stimulations in the experiment on radial maze performance (**study 3**), which could have possibly reduced their overall effectiveness. It is to note, however, that the time window of stimulation was in the end similar in both studies, so that the variable number of stimulations in **study 3** could most probably be related to the reduced sleep stability of the animals (see section 5.4 above, Table A2 and A3 in the Appendix, section 8.3).

The decrease in **theta** power during acute stimulation starting from day 8 on in **study 3** may at first seem at odds to the hypothesized facilitatory effect of so-tDCS on SO and its associated facilitation of memory consolidation during NREM sleep. Particularly in rodents, theta rhythm is characteristic of exploratory behavior and REM sleep, but not of NREM sleep during which slower frequencies prevail. However, since deep NREM sleep is associated with increased power in slow frequencies (as SO) and reduced faster frequencies (Bjorvatn, Fagerland, & Ursin, 1998; Grasing & Szeto, 1992), one could hypothesize the observed reduced theta in the STIM group may have been related to an increase in SO. In fact, facilitation or entrainment of SO had been expected here, and was shown before to be induced by so-tDCS (Ozen et al., 2010; Marshall et al., 2006; Fröhlich & McCormick, 2010). Similarly, even if only on a descriptive level, during acute stimulation upper delta seems to be slightly enhanced concomitant with decreased theta power, and a similar pattern is observed within post-stimulation intervals on days when upper-delta power is transiently increased following so-tDCS. Furthermore, relations between presumed frontal cortical theta in humans and so-tDCS were previously observed as well (Kirov et al., 2009; Marshall et al., 2011), indicating a possible relationship between both oscillatory bands. A direct measurement of SO activity during acute so-tDCS with cortical EEG only was however precluded due to frequency overlap of endogenous SO and so-tDCS. The delayed occurrence of theta reduction in response to so-tDCS starting from day 8, in comparison to controls as well as to own baseline values, could be interpreted as a kind of plasticity or learning within the cortical network, even if it was devoid of any measured behavioral correlate (Shahaf & Marom, 2001; le Feber, Stegenga, & Rutten, 2010). Cortical theta networks during wakefulness were shown

to play an important role in attention, exploration, working memory as well as for encoding and retrieval processes (Kawamata, Kirino, Inoue, & Arai, 2007; Young & McNaughton, 2009; Klimesch, 1999; Nyhus & Curran, 2010; Colgin, 2013). Thus, a functional relevance of the observed theta reduction during acute so-tDCS for memory processes cannot ultimately be excluded.

Interestingly the only modification in EEG activity occurring in temporal proximity to changes in memory in **study 3** was enhanced upper delta activity at the beginning of the post-training stimulation intervals, but no effect on a narrow (1.4-1.5 Hz as in **study 2,**) or broad (0.8-2 Hz) SO band was found. This seems at first to contrast the hypothesis, that so-tDCS would enhance post-stimulation endogenous slow oscillatory activity as reported in humans (Marshall et al., 2006), although a failure of a mode of slow oscillation stimulation to increase post stimulation EEG in elderly has also been reported (Eggers et al., 2013). On the other hand, Antonenko et al. (2013) showed enhancing effects of so-tDCS on a broad delta band (0.5-4 Hz) during an afternoon nap in human subjects, but no details on spectral composition are given. Furthermore, the relation between slower and faster components of the delta band (0.5-4 Hz) are still not fully understood, and there have been suggestions that delta waves at EEG level, especially their hyperpolarizing phase, may represent a faster equivalent of SO (Buzsaki, 2006). Additionally, it is conceivable and in need for further investigations, that the increase in upper delta activity seen in **study 3** can to some extend be described by mechanisms responsible for a frequency shift in resonant activity, not uncommon in biological systems (Lau & Zochowski, 2011).

The failure to enhance spindle activity by so-tDCS, as hypothesized based on findings in human subjects (Marshall et al., 2006), may be related to species specific differences. In comparison to humans, rats do not show a pronounced spindle peak in the power spectrum during NREM sleep (see Figure 3.7A). Multiple studies including **study 1** presented here showed enhanced spindle activity after successful learning in rats (Fogel et al., 2009; Eschenko et al., 2006; Binder et al., 2012), and spindles in humans and rats were shown to couple under baseline conditions to the up-states of the SO (Mölle et al., 2009; Mölle et al., 2006). On the other hand, learning-induced increased spindle activity concentrated to up-states only in humans, but not in rats (Mölle et al., 2009). Mölle et al. (2009) speculate that this difference may be task-related, as the odor discrimination task applied there is probably less dependent on thalamo-neocortical circuitry as the declarative tasks used in human subjects. However, if tasks relying stronger on this circuit would elicit a stronger effect on coupling of spindles with up-stated needs further investigations. Beside this, other studies

usually find learning-induced spindle increase in comparison to baseline sleep or in comparison to exposition to training context without any learning requirements. In **study 2 and 3,** the learning experience was identical in both STIM and SHAM conditions, so further spindle enhancement may not to be expected given that compared to humans rats' spindle activity is in general less pronounced. Furthermore, due to the trapezoidal pattern of so-tDCS spindle activity could not be examined during acute stimulation in **study 2**, where effects on spatial memory were more pronounced than in **study 3**.

Taken together, the hypothesis that so-tDCS leads to a post-stimulation increase in SO activity is supported by findings of **study 2**, where SO power was enhanced within a narrow bin of 1.4-1.5 Hz within a 10s post-stimulation interval. This hypothesis has however to be refuted in its strict sense for **study 3**, as the prior defined SO band of 0.85-2 Hz was not changed in a post-stimulation interval by so-tDCS. Likewise, the hypothesis that spindle activity is enhanced post-stimulatory in both studies and during acute so-tDCS in **study 3** has to be refuted, too.

6 CONCLUSIONS

In conclusion, this work could show that memory consolidation of hippocampus dependent memory in a rodent model using a task closely comparable to tasks applied in humans (OPR task) relies critically on sleep within the retention interval rich in SWS and sleep spindles. Additionally, a contribution of confounding purely circadian factors as well as a strong contribution of REM sleep could be excluded. Based on these results, the effectiveness of so-tDCS in rats for improving sleep-associated hippocampus-dependent memory consolidation could be shown in this task, together with an accelerating effect on reference memory acquisition and a positive impact on rule-learning over several days in the radial maze task. However, results on EEG level were not unequivocal, as the expected enhancement of SO activity was only found in the one-trial learning OPR task, but not in the radial maze study requiring learning over several days. But in the latter, upper delta was transiently enhanced in a post-stimulation interval, and theta power was decreased during acute stimulation within the last days of the experiment. If sleep stability, differences in stimulation pattern (trapezoidal vs. sinusoidal) or other factors modulated this differential outcome needs further investigation. Similar, the underlying mechanisms of the unexpected long term effects on theta power encourage follow-up studies on the impact of multiple so-tDCS sessions on cortical activity.

As within this work only epidural EEG activity was measured, future studies are necessary to investigate more closely the contribution of PFC and hippocampus on the effects

of endogenous brain oscillations and applied weak electric fields. Possible methods to gain a more detailed insight into the underlying mechanisms could involve the use of using intracranial recording techniques, molecular analysis of brain tissue as well as selective activation and inactivation of brain circuits and neuronal subtypes, e.g., by using optogenetic techniques or genetically modified animals models.

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8 Appendix

8.1 List of Abbreviations

ANOVA	Analysis of Variance
CSF	Cerebrospinal fluid
DC	Direct current
EEG	Electroencephalography
EMG	Electromyogram
EPSP	Excitatory postsynaptic potential
FEM	Finite element method
FFT	Fast Fourier transformation
FIR	Finite impulse response
GABA	Gamma-Aminobutyric acid
HRA	High rearing activity
Hz	Hertz
IEG	Immediate early gene
IIR	Infinite impulse response
IPSP	Inhibitory postsynaptic potential
LFP	Local field potential
LTD	Long-term depression
LTM	Long-term memory
LTP	Long-term potentiation
LTP	Long-term potentiation
M	Arithmetic mean

mPFC	Medial prefrontal cortex
MRI	Magnetic resonance imaging
NaCl	Sodium chloride
NMDA	N-methyl-D-aspartate
NOR	Novel object recognition
NREM	Non rapid eye movement
O2	Oxygen
OF	Open field
OPR	Object-place recognition
PFC	Prefrontal cortex
P-Index	Preference-Index
preREM	Pre-rapid eye movement sleep
RE	Reticular nucleus thalami
REM	Rapid eye movement
rms	Root mean square
s.c.	Subcutaneous
SD	Sleep deprivation
SDev	Standard deviation
SEM	Standard error of mean
SEM	Standard error of mean
SO	Slow oscillations
so-tDCS	Slow oscillatory transcranial current stimulation
SPW	Sharp-wave ripple
SWA	Slow wave activity
SWA	Slow-wave activity

SWS	Slow wave sleep
tACS	Transcranial alternating current stimulation
TC	Corticothalamic neuron
tDCS	Transcranial direct current stimulation
TES	Transcranial electric stimulation
TMS	Transcranial magnetic stimulation
tRNS	Transcranial random noise stimulation
TST	Total sleep time
WM	Working memory

8.2 Additional data: Spindle frequencies

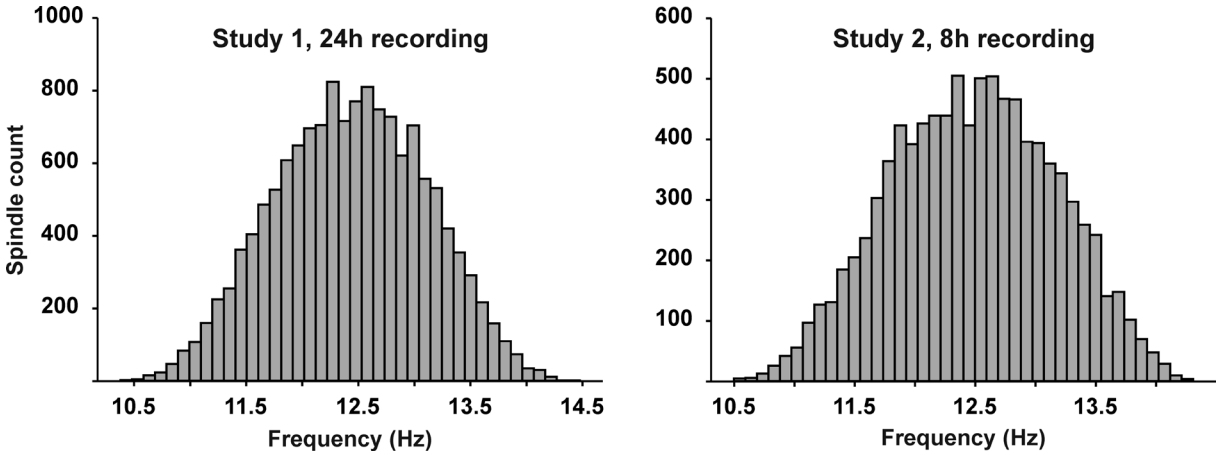

Figure A1. Histograms of spindle peak-to-peak frequency. **Left**: Distribution of spindles in NREM sleep for animals of study 1 (N=14) within the ~ 24h Baseline recording. **Right**: Distribution of spindles in NREM sleep for animals of study 2 (N=12) within the ~ 8h SHAM recording of the main experiment. See Table A1 for further information.

Table A1. Spindles in NREM sleep for study 1 (24 h Baseline recording) and 2 (~8h recording, SHAM, main experiment)

	Study 1	Study 2
Total number	14,080	9626
Mean frequency (Hz)	12..43 +/- .01	12.48 +/- .01
Spindles < 10.5 Hz (%)	0.04	0.01
Spindles > 14.0 Hz (%)	0.69	0.53
Minimum frequency (Hz)	10.38	10.50
Maximum frequency (Hz)	14.47	14.31

Note: Spindles were automatically detected within a 10-15 Hz range.
See method section of study 2 (3.1.11.3) for further information

8.3 Additional data: Comparison of stimulation epochs and sleep architecture between study 2 and study 3

Table A2. Number of stimulations

	Study 2 (OPR task)			Study 3 (RAM task)			*p*
	STIM	SHAM	All N=12	STIM	SHAM	All N=22	
Total number	23.75 +/- 1.21	25.25 +/- .88	24.50 +/- .88	20.00 +/- .57	18.32 +/- .86	19.16 +/- .56	<.001*
'successful' (total number)	17.75 +/- .55	18.83 +/- .21	18.29 +/- .26	10.21 +/- .47	10.08 +/- .76	10.14 +/- .49	<.001*
'successful' (%)	75.94 +/- 2.65	75.78 +/- 3.27	75.86 +/- 2.47	48.00 +/- 1.48	50.24 +/- 2.18	49.12 +/- 1.66	.013*

Note: 'successful' refers to stimulations characterized by no EMG activity/awakenings during acute stimulation and by a post-stimulation interval of at least 10s filled with NREM sleep only. For study 3, means over all 12 experimental days are given. No significant differences were detected within the studies between STIM and SHAM condition. *p*-values refer to a comparison between both studies, T-tests for independent measures. * $p < .05$.

Table A3. Sleep architecture

	Study 2 (OPR task) N = 12	Study 3 (RAM task) N = 22	*p*
TST (min)	57.7 +/-2.6	55.0 +/-2.2	.459
W (min)	62.3 +/- 2.6	63.2 +/- 2.2	.793
NREM (min)	35.6 +/-1.9	35.0 +/- 1.3	.816
REM (min)	8.1 +/- .7	7.9 +/- .6	.791
PreREM (min)	2.2 +/- .3	2.7 +/- .3	.292
Stim (min)	11.9 +/- .4	9.5 +/- .4	<.001*
NREM (% TST)	61.4 +/- .8	63.9 +/- .5	.008*
REM (% TST)	13.9 +/- .8	13.9 +/- .6	.969
PreREM (% TST)	3.7 +/- .4	4.7 +/- .4	.125
Stim (% TST)	21.0 +/-1.1	17.6 +/- .8	.017*
Sleep Latency (min)	51.9 +/-2.5	34.4 +/-3.1	<.001*
REM Latency (min)	68.0 +/-3.5	54.4 +/-3.6	.019*

Note: For study 3, means over all 12 experimental days are given. No significant differences were detected within the studies between STIM and SHAM condition. *p*-values refer to a comparison between both studies, T-tests for independent measures. * $p < .05$.

8.4 Publications

Original Articles

Zlomuzica A, Viggiano D, Degen J, **Binder S**, Ruocco LA, Sadile AG, Willecke K, Huston JP, Dere E (2012). Behavioral alterations and changes in Ca/calmodulin kinase II levels in the striatum of connexin36 deficient mice. *Behavioural Brain Research*, 226(1):293-300.

Tress O, Maglione M, May D, Pivneva T, Seyfarth J, **Binder S**, Zlomuzica A, Theis M, Dere E, Kettenmann H, Willecke K (2012) Panglial gap junctional communication is essential for maintenance of myelin in the central nervous system. *Journal of Neuroscience*, 32(22):7499 – 7518

Sartorius, T., Ketterer, C., Kullmann, S., Rotermund, C., **Binder, S**., Hallschmid, M., Machann, J., Schick, F., Preissl, H., Fritsche, A., Häring, H.-U., and Hennige, A.M. (2012) Mono-unsaturated fatty acids prevent the deleterious effects of obesity on locomotion, brain activity and sleep behavior, *Diabetes*, 61(7): 1669-1679.

Binder, S., Baier, P.C., Mölle, M., Inostroza, M., Born, J., Marshall, L. (2012). Sleep enhances memory consolidation in the hippocampus-dependent object-place recognition task in rats. *Neurobiology of Learning and Memory*, 97(2):213-219.

Zlomuzica A, Tress O, **Binder S**, Rovira C, Willecke K, Dere E (2012). Changes in object recognition and anxiety-like behaviour in mice expressing a Cx47 mutation that causes Pelizaeus-Merzbacher-like Disease. *Developmental Neuroscience*, 34(2-3): 277-287.

Inostroza, M., **Binder, S.**, Jan Born.(2013). Sleep-dependency of episodic-like memory consolidation in rats, *Behavioural Brain Research*, 237*,* 15-22

Book Chapters

Zlomuzica, A., **Binder, S.** & Dere, E. (2012). Ch 1: Gap Junctions in the Brain. In Dere E. (Ed.) Gap Junctions in The Brain: Physiological and Pathological Roles, Academic Press Inc

Conference Contributions

Binder, S., Baier, P.C., Mölle, M., Born, J. & Marshall, L. (2011). Effects of anodal slow oscillation transcranial direct current stimulation (tDCS) in the rat. 9th Göttingen Meeting of the German Neuroscience Society, March 23-27 2011, Göttingen, Germany (Poster)

Gasca, F., Marshall, L., **Binder, S**., Schlaefer, A., Hofmann, U., and Schweikard, A. (2011). Finite element simulation of transcranial current stimulation in realistic rat head model. 5th International IEEE EMBS Conference on Neural Engineering, April 27 - May 1 2011, Cancun, Mexico (Proceeding article)

Binder, S., Baier, P.C., Mölle, M., Born, J., Marshall, L.; Sleep enhances memory consolidation in the hippocampus-dependent object-place recognition task in rats (2011), European Brain and Behaviour Society Meeting, September 9-12 2011 Sevilla, Spain (Poster)

Binder, S., Rawohl, J., Born, J. & Marshall, L. (2012). Anodal slow oscillatory transcranial direct current stimulation (sotDCS) during sleep in rats: Effects on radial maze performance and cortical activity. 8th FENS Forum of Neuroscience, July 14-18 2012, Barcelona, Spain (Poster)

Binder, S., Berg, K., Gasca, F., Born, J. & Marshall, L. (2013). Boosting sleep slow oscillations by oscillatory transcranial direct current stimulation enhances memory consolidation in rats. 5th International Conference on Non-invasive Brain Stimulation, 19-21 March 2013, Leipzig, Germany (Selected oral presentation)

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