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When It's Clicking Overnight

Auditory Stimulation of Sleep Slow Oscillations to Enhance Memory

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It is a common experience that a problem difficult at night is resolved in the morning after the committee of sleep has worked on it.

John Steinbeck

Abstract

Brain rhythms reflect synchronized activity that regulates information processing and communication in neuronal networks during different states and thereby enables learning and memory formation. The < 1 Hz sleep slow oscillation is the largest of these oscillations. It hallmarks the electroencephalogram during slow-wave sleep and has been suggested to causally contribute to the consolidation of memories during sleep. Importantly, the slow oscillation groups other brain rhythms of higher frequency within its cycle, e.g. thalamo-cortical sleep spindles and hippocampal sharp-wave ripples, and thus orchestrating a dialogue between cortical and subcortical structures assumed to be essential for the strengthening of memories. This function in particular has raised the question whether slow oscillations can be induced by external stimulation in order to promote memory consolidation.

While previous attempts have successfully implemented transcranial electrical, transcranial magnetic and intracranial electrical stimulation to induce slow oscillations, the aim of the present work was to explore the potential of sensory stimulation, namely auditory stimulation, as a less invasive and more natural interventional means to trigger slow oscillations.

For this purpose, a series of experiments was performed in healthy humans. First, it was examined in a pilot study whether slow oscillations can be induced in general by rhythmic auditory stimulation mimicking the slow oscillatory rhythm in comparison to random and no-stimulation. Results indicate that rhythmic auditory stimulation effectively enhances slow oscillation activity, but that this effect depends on the current brain state, i.e. is restricted to stable NonREM sleep.

In the second and main experiment of this project, a novel closed-loop stimulation approach was developed and tested for its ability to improve the sleep-dependent consolidation of declarative memory. This study revealed that the efficacy of auditory stimulation critically depends on its timing: stimulation applied in phase but not out of phase with the ongoing rhythmic occurrence of slow oscillatory positive half-waves profoundly increases the functional efficacy of slow oscillations. It generated a strong resonance of both slow oscillatory and fast spindle activity and, consequently, improved the consolidation of declarative memory.

The third experiment aimed at testing the limits of auditory closed-loop stimulation against the background of findings that slow oscillations, in particular their positive

half-waves, represent phases of strong synchrony and reduced inhibitory signalling which favour epileptic activity. Here, the implementation of a new pressing stimulation algorithm in which auditory stimuli are presented as long as ongoing slow oscillatory activity is present was intended to overdrive the closed-loop stimulation with the aim of unravelling possible mechanisms that counteract induced hyper-synchrony and thereby limit the enhancing effect of stimulation. This approach yielded a rapidly fading response in fast spindle activity during persisting closed-loop stimulation which presumably prevents the brain from potential over-excitation, i.e. seizure activity, thus limiting the enhancing effect of auditory stimulation on sleep rhythms and memory formation.

In sum, the present findings demonstrate that auditory stimulation reliably induces slow oscillations. Closed-loop auditory stimulation in particular enhances endogenous brain rhythms in humans, specifically sleep slow oscillations and their functional contribution to memory consolidation. The closed-loop approach may not only be a tool to ameliorate sleep slow oscillations, but could also be transferred to other rhythmic domains such as theta oscillations during wakefulness. Eventually, it might permit the identification of mechanisms underlying brain rhythms and the development of new therapeutic interventions for disorders such as epilepsy or insomnia.

Zusammenfassung

Gehirnrhythmen reflektieren synchronisierte Aktivität, welche die Verarbeitung von Informationen und die Kommunikation zwischen neuronalen Netzwerken in unterschiedlichen Zuständen reguliert und auf diese Weise Lernen und Gedächtnisbildung ermöglicht. Unter den rhythmischen Oszillationen ist die langsame Oszillation < 1 Hz ("slow oscillation") die stärkste. Sie ist das Hauptmerkmal des Tiefschlafs im Elektroenzephalogramm und spielt nach heutigem Kenntnisstand eine tragende Rolle in der schlafabhängigen Gedächtnisbildung. Insbesondere gruppieren sich innerhalb des Zyklus einer langsamen Oszillation höherfrequente Hirnrhythmen wie thalamo-kortikale Schlafspindeln und hippokampale "sharp-wave ripples", so dass die langsame Oszillation den Dialog zwischen kortikalen und subkortikalen Strukturen dirigiert, welcher grundlegend für die Festigung von Gedächtnisspuren ist. Diese basale Funktion wirft die Frage auf, inwiefern es möglich ist, langsame Oszillationen mittels externer Stimulationstechniken auszulösen und mithin die Gedächtnisbildung zu unterstützen.

Während frühere Arbeiten bereits zeigen konnten, dass trans- und intrakranielle elektrische sowie transkranielle magnetische Stimulation langsame Oszillationen herbeiführen können, erkundet die vorliegende Arbeit die Möglichkeit, mit sensorischer Stimulation, im speziellen auditorischer Stimulation, als einer weniger invasiven und natürlicheren Methode langsame Oszillationen auszulösen.

Zu diesem Zweck wurde eine Reihe von Experimenten an gesunden Probanden durchgeführt. Zunächst wurde in einer Pilotstudie die Tauglichkeit einer der langsamen Oszillation nachempfundenen periodischen auditorischen Stimulation im Vergleich zu zufälliger oder Nicht-Stimulation untersucht. Die Ergebnisse zeigten, dass die rhythmische Stimulation zu einer signifikanten Verstärkung langsamer oszillierender Aktivität führt, dieser Effekt jedoch vom gegebenen Gehirnzustand abhängt, d.h. stabilen Non-REM-Schlaf voraussetzt.

Im zweiten Experiment, dem Kernstück der vorliegenden Arbeit, wurde ein neuartiges Verfahren der Stimulation im geschlossenen Regelkreis ("closed-loop") entwickelt und daraufhin überprüft, ob es die schlafabhängige Konsolidierung deklarativer Gedächtnisinhalte verbessern kann. Diese Studie offenbarte, dass die Wirksamkeit der auditorischen Stimulation entscheidend vom genauen Timing abhängt: die Stimulation in Phase, aber nicht außer Phase zu den rhythmisch auftretenden positiven Wellenbergen langsamer Oszillationen führte zu einer erheblichen Verstärkung ihrer Funktionalität,

d.h. einer resonanten Verstärkung der langsamen Oszillationen und der schnellen Spindelaktivität und damit zu einer verbesserten Konsolidierung von deklarativen Gedächtnisinhalten.

Langsame Oszillationen, insbesondere positive Halbwellen stellen Phasen verstärkter Synchronizität und verringerter Hemmung neuronaler Signalübertragung dar, die das Auftreten epileptischer Aktivität begünstigen. Deshalb lotete das dritte und abschließende Experiment die Grenzen der auditorischen closed-loop-Stimulation aus. Zu diesem Zweck wurde eine neue, sogenannte treibende Stimulation entwickelt, die auditorische Stimuli präsentiert, solange langsame Oszillationen auftreten. Dadurch sollte die rhythmische Aktivität des schlafenden Gehirns übersteuert werden, um mögliche Mechanismen aufzudecken, welche einer derart induzierten Hypersynchronizität entgegenwirken und positive Stimulationseffekte begrenzen. Diese Überstimulation führte zu einer raschen Abnahme der induzierten schnellen Spindelaktivität, welche das Gehirn vermutlich vor Übererregung, d.h. epileptischer Krampfaktivität, schützt und somit die Wirkung auditorischer Stimulation auf Schlafrhythmen und Gedächtnisbildung einschränkt.

Die erbrachten Ergebnisse zeigen, dass auditorische Stimulation zuverlässig langsame Oszillationen hervorruft. Insbesondere die Anwendung eines closed-loop-Systems zur auditorischen Stimulation verstärkt endogene Gehirnrhythmen beim Menschen, speziell langsame Oszillationen und deren Beitrag zur Gedächtnisbildung. Der vorgestellte closed-loop-Ansatz ist nicht nur eine Methode zur Verbesserung langsamer Schlafoszillationen, sondern könnte auch auf andere Hirnrhythmen wie beispielsweise Theta-Oszillationen im Wachzustand Anwendung finden. Darüber hinaus könnte er die Aufklärung von Mechanismen, die spezifischen Hirnrhythmen zugrunde liegen, und die Entwicklung neuer therapeutischer Maßnahmen bei Störungen wie Epilepsie oder Schlaflosigkeit ermöglichen.

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Abbreviations

AEP	**A**uditory **e**voked **p**otential
EEG	**E**lectro**e**ncephalo**g**ram
EMG	**E**lectro**m**yo**g**ram
HEOG & VEOG	**H**orizontal and **v**ertical **e**lectro**o**culo**g**ram
ISI	**I**nter-**s**timulus **i**nterval
REM	**R**apid-**e**ye **m**ovement
RMS	**R**oot **m**ean **s**quare
SO	**S**low **o**scillation
SWA	**S**low **w**ave **a**ctivity
SW-R	**S**harp-**w**ave **r**ipple
SWS	**S**low **w**ave **s**leep
tDCS	**T**ranscranial **D**irect **C**urrent **s**timulation
tMS	**T**ranscranial **m**agnetic **s**timulation
TST	**T**otal **s**leep **t**ime

Chapter 1

Introduction

As long as one can remember the mystery of sleep has fascinated mankind. It has continuously been embraced in art, literature and science and is an inevitable element of our daily lives. Sleep is a recurrent and very specific behaviour characterised by a certain body posture with physical inactivity, a lowered reactivity to stimuli from the environment and a temporary loss of consciousness (Aserinksy & Kleitman, 1953). Several assumptions as to why we need to sleep have been developed. These hypotheses have focused on the energy-saving properties of sleep (Berger & Phillips, 1995), the regeneration of energy resources (Oswald, 1980), body tissue and bodily functions during sleep and sleep's role in the evolutionary adaptation to the environment (Siegel, 2009). Among all of these functions, the role of sleep for memory consolidation, which was first described in the seminal work of Jenkins & Dallenbach (1924), is of particular relevance. In these early studies it was demonstrated that subjects were able to remember more non-sense syllables if allowed to sleep after learning in comparison to recall performance after a wake period of equal length.

With the development of the electroencephalogram (EEG) in 1933 by Hans Berger, which enabled the recording of brain activity as electrophysiological signals, the foundation for experimental brain research was laid and a new era of modern sleep science began. Over time it was revealed that the brain exhibits distinct rhythms (Buzsáki & Draguhn, 2004) and that the rhythms hallmarking sleep contribute to the formation of memory (Diekelmann & Born, 2010, Rasch & Born, 2013). The so-called slow oscillation takes a leading role in mediating processes that enable the strengthening of memory traces. Due to its functional importance, the sleep slow oscillation has been extensively investigated in a variety of studies, and methods to manipulate it have attracted growing

interest (Marshall *et al.*, 2006, Landsness *et al.*, 2009, van der Werf *et al.*, 2009)

Along this line, the present work focused on the sleep slow oscillation and explored whether auditory stimulation is a suitable means to manipulate slow oscillations (Chapter 3), how its efficacy can be improved with regard to an enhancement of memory consolidation (Chapter 4) and the limitations entailed by this approach (Chapter 5).

This chapter will first outline the theoretical background of this work. I will start with a general introduction on memory and its different types and states. This is followed by an overview over sleep and in particular its electrophysiology. Subsequently I will give a detailed introduction on the sleep slow oscillation and current knowledge of its relationship to memory consolidation, also touching on other sleep rhythms involved in this process. After a brief overview of the auditory system this chapter concludes with an elaboration of the main questions and the fundamental hypotheses of this work.

1.1 Memory

Simply speaking, memory comprises the storage of information for later access. It is a distinguishing feature of consciousness in both humans and animals and essential for the survival of every organism in our ever-changing world. However, there is much more to memory than it seems at first. Depending on how long information is stored or, rather, held accessible, memories are categorized into three different types: (i) sensory or ultra-short-term memory, (ii) short-term or working memory and (iii) long-term memory (Baddeley, 1997). Among these forms, sensory memory operates on the shortest time-scale. By storing information obtained from a sensory source, e.g. auditory or visual, for a certain time beyond the actual stimulus exposure, it allows for the integration across several stimuli and thus provides the basis for any following processing of sensory information (Hennevin *et al.*, 2007).

Short-term memory, as a memory with a conscious component, retains information for a few seconds up to one minute and exhibits a limited capacity (Miller, 1956). Unlike sensory memory, information transfer into short-term memory depends on attention during acquisition (Cowan, 1993, Deco & Rolls, 2005). Long-term memory enables the permanent storage of information with an unlimited capacity. While short-term memory has been associated with pre-frontal brain structures (Wiegersma *et al.*, 1990, Pribram & Tubbs, 1967), long-term memory cannot be localized to a single brain region but involves plastic changes across the whole brain. The present work focused primarily on

long-term memory because it displays the strongest dependence on sleep and has been studied most intensively.

1.1.1 Declarative vs. Non-Declarative Memory

Long-term memory can be subdivided into declarative and non-declarative memory based on the actual nature of the contents and the corresponding brain structures in which they are preserved or processed (Squire & Zola, 1996). As shown in Figure 1.1, non-declarative memory comprises a diverse range of different subtypes, e.g. procedural memory of skills and habits or classical conditioning. These forms are all implicit, i.e. they operate in an unconscious manner and depend on structures like the basal ganglia system and cortical motor areas.

Explicit memory for facts and events on the other hand belongs to the declarative type of memory. Encoding of declarative information relies on medial temporal structures, like the hippocampus, where this information is stored only temporarily prior to being transferred into the neocortex for permanent storage (Squire & Zola, 1991). To assess the impact of auditory stimulation on memory consolidation, I focused on hippocampus-dependent declarative memories which have been shown to be sensitive to sleep-related mechanisms (Marshall *et al.*, 2004, Plihal & Born, 1997).

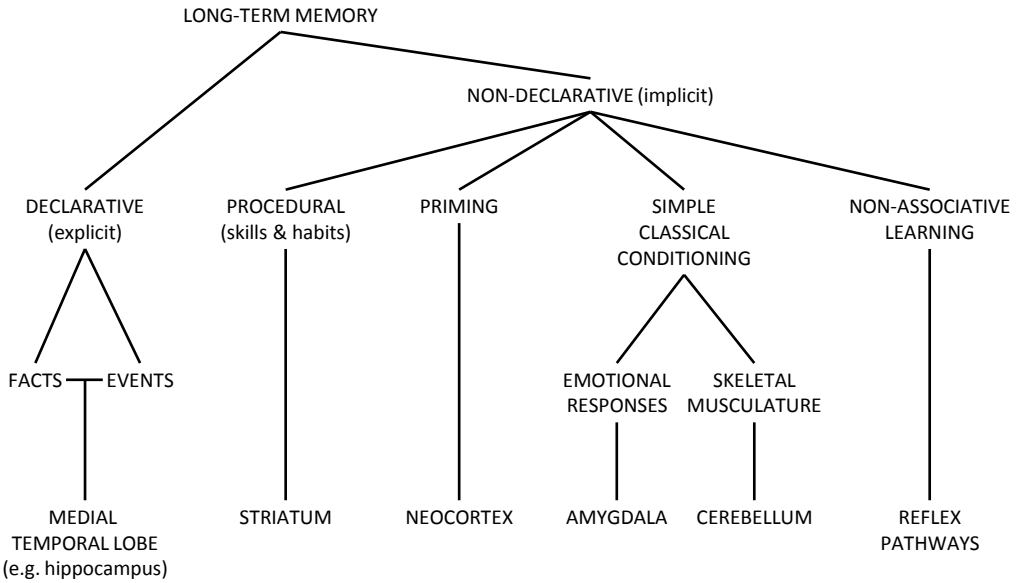

Figure 1.1 – The Long-Term-Memory System.
Overview of the different subsystems of long-term memory. Each system is classified according to the nature of its content and the brain structures involved. The figure was adapted from Squire & Zola (1996).

1.1.2 The Stages of Memory Formation

The processes which take place until a stable memory trace is established in long-term stores comprise three different stages (Figure 1.2): In the initial phase a new memory trace is acquired via exposure to a stimulus and is *encoded* as a temporary trace in the hippocampus (in the case of declarative memories). At this point the information is still labile and susceptible to interference. It is only after the second stage, i.e. *consolidation*, that corresponding memory traces in the neocortex become stable and resistant to interference. Eventually, the consolidated memory traces can be *retrieved*. As already mentioned, how long a certain memory trace is accessible determines whether it is of sensory, short-term or long-term nature. In general and in the following, the interval between initial encoding and a retrieval phase is referred to as the *retention interval*.

A large body of evidence indicates that the consolidation phase takes place primarily during sleep (Diekelmann & Born, 2010): Comparing the performance on declarative memory tasks after periods of wakefulness and sleep revealed a significant advantage when subjects were allowed to sleep (Plihal & Born, 1997, Tucker *et al.*, 2006, Lahl *et al.*, 2008). Interestingly, procedural or emotional memory tasks have also revealed a beneficial effect of sleep (Walker *et al.*, 2003, Korman *et al.*, 2007, Payne *et al.*, 2008), although in contrast to declarative memory contents, these forms of memory appear to benefit from REM sleep rather than slow wave sleep (please see Section 1.2), while performance on a visual discrimination task is supported by both REM sleep and slow wave sleep (Stickgold *et al.*, 2000). Ground-breaking animal studies demonstrated that

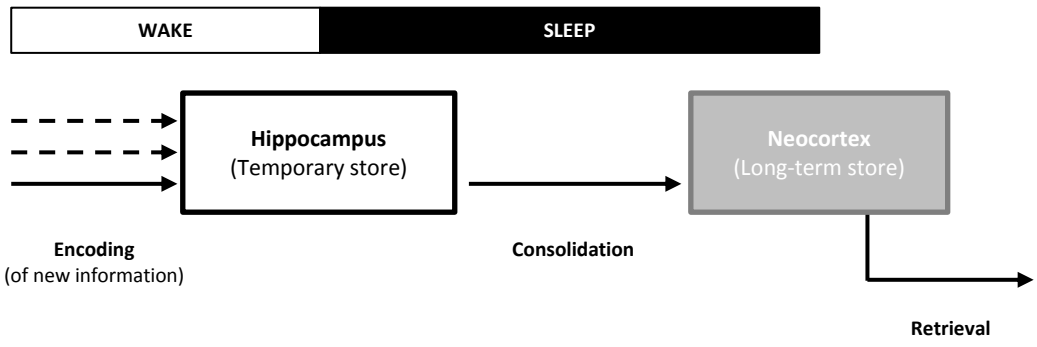

Figure 1.2 – Stages of Memory Formation & Two-Stage Model.
New information acquired during wakefulness is initially encoded in the hippocampus. This information is labile and only temporarily accessible. For long-term storage, these memory traces have to be consolidated, i.e., gradually transferred to the neocortex, where they are stabilized and can then be retrieved at any time. Whereas encoding and retrieval are performed during wakefulness, consolidation presumably takes place during sleep.

encoding of information is associated with distinct neural firing patterns and the subsequent consolidation in fact comprises a repeated reactivation of these patterns which appears to mediate the gradual transfer from hippocampal to neocortical sites (Wilson & McNaughton, 1994, Nadasdy *et al.*, 1999, Ji & Wilson, 2007).

That encoding and consolidation of memory take place in two different structures was first proposed by Marr (1971) in the so-called two-stage model, which provided an elegant theoretical framework for the assumption that consolidation necessarily coincides with sleep: the hippocampus is a fast-learning structure which, however, has a limited capacity to buffer information. Since encoding takes place primarily during wakefulness and as a result we continuously receive input, 'off-periods', i.e. sleep, are required for the accumulated information to be processed and consolidated.

1.1.3 Synaptic vs. Systems Consolidation

The process of consolidation is assumed to comprise synaptic and systems consolidation which differ mainly with regard to the respective time-scale. Synaptic consolidation refers to all underlying processes on a neuronal level with a time-scale ranging from seconds to several hours, relying on actual plastic changes of synaptic connections between neurons within neural networks encoding a certain memory trace. These changes operate on a basis of long-term potentiation or long-term depression and depend on the causal firing pattern between pairs of neurons to determine whether their connection is strengthened or attenuated (Collingridge *et al.*, 2010, Hebb, 1949, Tsanov & Manahan-Vaughan, 2008, Bi & Poo, 1998).

'Memory consolidation' as outlined above refers to the so-called systems consolidation. Building upon the synaptic changes, systems consolidation comprises the recurrent reactivation of network representations of memories during retention intervals, thus driving the reorganization of these traces into neocortical networks for long-term storage (Frankland & Bontempi, 2005). In contrast to synaptic consolidation, systems consolidation occurs over a much larger time-scale but in turn leads to longer-lasting memory retention.

1.2 The Electrophysiology of Sleep

Unlike what a lot of people would assume, the sleeping brain is very active. With regard to electrophysiology, the brain in fact exhibits the largest neural activity during sleep.

In their manual published in 1961, Rechtschaffen & Kale introduced a segmentation of sleep into several stages based on the EEG, eye movements (Electrooculogram, EOG) and the muscle tone (Electromyogramm, EMG). The waking brain is dominated by a high-frequent EEG with low amplitudes and a high level of muscle activity. A prevailing rhythm during resting with eyes closed is the alpha rhythm of 8 - 12 Hz. When the brain falls asleep, it first passes through the transitional sleep stage S1, characterized by rolling eye movements and a diminished amount of alpha. With gradually increasing sleep depth, the EEG rhythms become larger and slower during sleep stages S2 to S4. In humans, about $50 - 60\%$ of total sleep time is spent in S2 which is hallmarked by sleep spindles - brief oscillations of 9 - 15 Hz with a unique pattern of activity fading in and out - and K-complexes (solitary large negative deflections followed by positive half-waves of 0.5 - 2 Hz). Sleep stages S3 and S4 represent the periods of deepest sleep. They are typically subsumed as slow wave sleep (SWS) and are dominated by slow delta waves with frequencies between 0.5 and 4 Hz. Together, S2, S3 and S4 are also referred to as Non-REM sleep, while rapid-eye movement sleep (REM) with its wake-like brain activity, lowest levels of EMG and characteristic sharp eye movements represents a very unique sleep pattern. REM sleep is therefore also labelled paradoxical sleep. Stage S1 is not included in Non-REM sleep because it usually marks brief intervals between wakefulness and light sleep and exhibits a spectral composition distinct from S2 and SWS. An overview of all sleep stages and their characteristic electrophysiological signals is given in Figure 1.3*A*. It should be noted that in 2007 a new sleep scoring manual was published by Iber *et al.*. The main difference is that S4 has been dropped as an independent sleep stage but is now combined with S3 to a stage N3 of deep sleep instead and Non-REM is therefore comprised of the sleep stages N1 to N3. Nevertheless, since classification of stage N3 relies on identical criteria as for stages S3 and S4 (or SWS), performing the EEG-analyses according to the old guidelines did not confound the results presented in the following chapters.

The architecture of sleep in humans exhibits a distinct pattern that changes with ongoing sleep (Figure 1.3*B*). In principle, a single sleep cycle comprising all sleep stages occurs with an approximate length of 60-90 minutes. A cycle begins with S1 or S2, progresses into SWS and returns to S2 or is immediately followed by REM sleep. While early sleep, which is usually defined as the first half of sleep, is dominated by SWS, the amount of SWS decreases or is fully absent in the second half that is characterized by a strong increase in REM sleep.

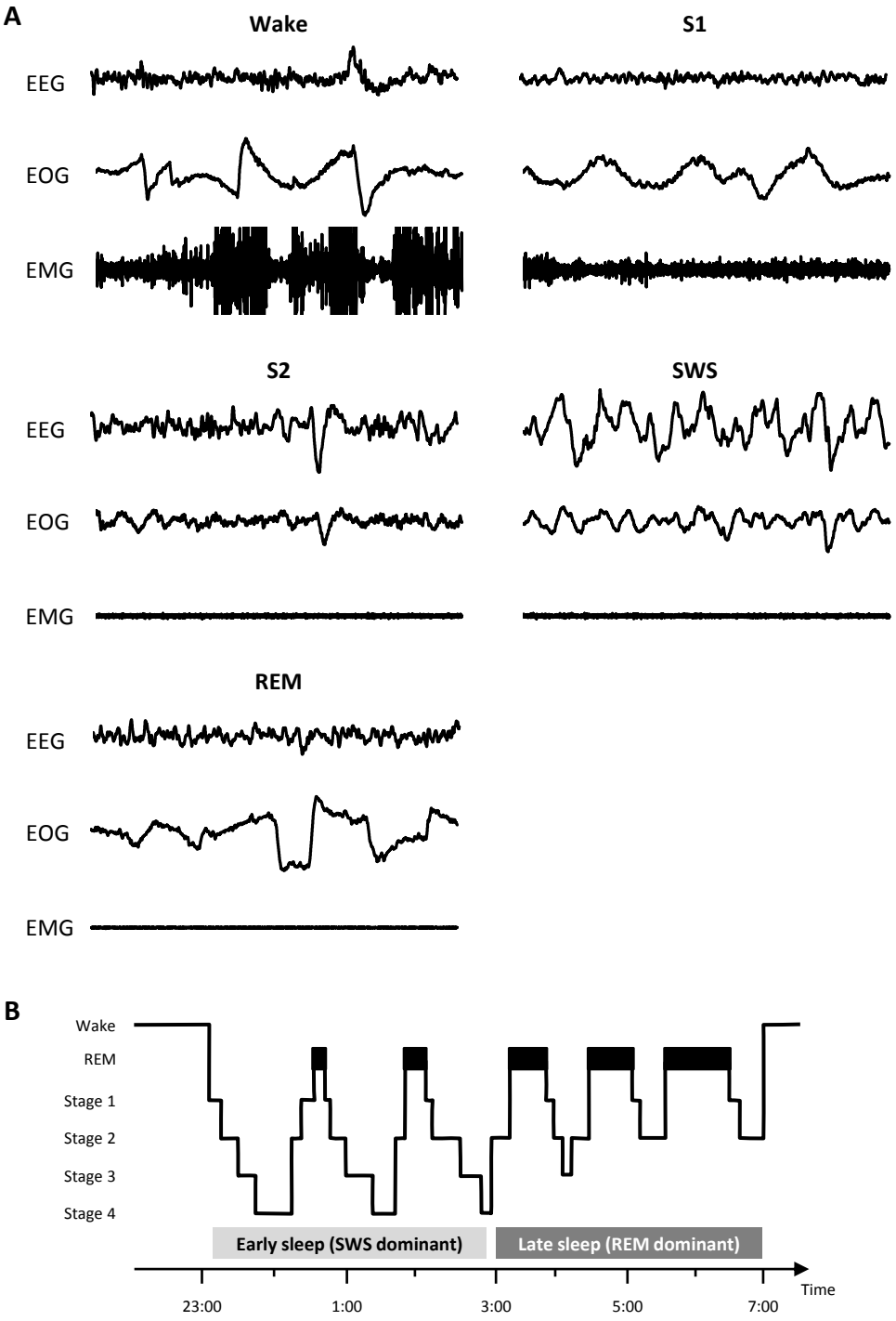

Figure 1.3 – The Different Stages of Sleep and Sleep Profiles

(*A*) Excerpts (10 s) of representative EEG, EOG and EMG traces for wake, S1, S2, SWS and REM sleep. (*B*) A hypnogram, i.e. the pattern of transitions between sleep stages during the course of a night which take place in repeated cycles. Early sleep which is generally defined as the first half of sleep is dominated by SWS, whereas late sleep exhibits mainly REM sleep.

1.3 Sleep Slow Oscillations and Active Systems Consolidation

Sleep slow oscillations (SOs) in humans, i.e. distinct delta activity with a spectral peak frequency of ∼0.8 Hz (Steriade *et al.*, 1993, Achermann & Borbély, 1997), represent the largest oscillatory events (amplitude $> 75\ \mu$V) recorded in the EEG and are the quintessential hallmark of SWS. They emerge from highly synchronized cortical neuronal networks undergoing alternations between phases of membrane depolarization with increased firing activity (up-states) and phases of hyperpolarized membrane potentials and neural quiescence (down-states), spreading across the neocortex, but also involving subcortical structures like thalamus and hippocampus (Steriade *et al.*, 1993, Sanchez-Vives & McCormick, 2000, Massimini *et al.*, 2004, Isomura *et al.*, 2006). SOs have likewise been observed in animals such as mice, rats and cats (Rattenborg *et al.*, 2011, Steriade, 2006). Some studies argue that thalamic oscillations play a major role in SO generation (Crunelli & Hughes, 2010, Nir *et al.*, 2011), but a large body of evidence suggests a cortical origin, possibly as a consequence of ongoing miniature excitatory post-synaptic potential activity in these networks (Huber *et al.*, 2004, Bazhenov *et al.*, 2002).

SOs critically contribute to the major cognitive functions of sleep: On the one hand, they mediate a global down-scaling of synapses potentiated by the encoding of information during wakefulness, and thereby renew the network's capacity to encode new information during succeeding wake phases (van der Werf *et al.*, 2009, Antonenko *et al.*, 2013, Tononi & Cirelli, 2014). On the other hand, SOs group ensembles of subcortical rhythms composed primarily of sleep spindles and hippocampal sharp-wave ripples. Sleep spindles are further separated into cortical slow spindles ($\sim 9-12$ Hz) located in frontal regions and thalamo-cortical fast spindles ($\sim 12-15$ Hz) with a centro-parietal dominance. While slow spindles are grouped within the up-to-down transition of SOs, fast spindles usually coincide with SO up-states (Mölle *et al.*, 2011). Sharp-wave ripples (SW-Rs) are brief depolarizations superimposed by fast oscillations of $100-300$ Hz that are not only temporally aligned to SO up-states but are also phase-locked to the troughs of fast spindles (Siapas & Wilson, 1998, Clemens *et al.*, 2007).

This pattern of top-down coherence among SOs, spindles and SW-Rs forms the foundation for memory consolidation during sleep and is a widely accepted feature of the theoretical model of active systems consolidation (Figure 1.4). In this framework,

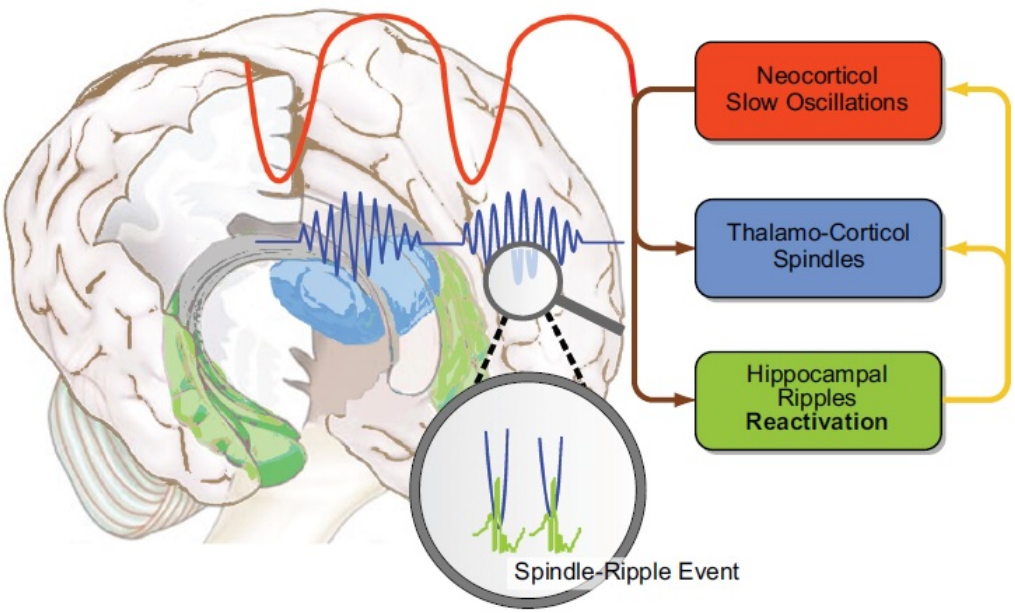

Figure 1.4 – Active Systems Consolidation.
Illustration of the interaction between the main structures and sleep rhythms contributing to memory consolidation. During slow wave sleep, slow oscillation up-phases mediate the transfer of information from the hippocampus to the neocortical long-term store. Within these short time windows, memory representations within the hippocampus are reactivated by sharp-wave ripples nested in the troughs of thalamo-cortical spindles. The figure was adapted from (Rasch & Born, 2013).

consolidation is presumably conveyed via the SO up-states driving the repeated reactivation of memory traces in hippocampal networks within SW-R events, which temporarily strengthens the hippocampal representation itself and simultaneously supports the redistribution towards neocortical long-term storage sites (Ji & Wilson, 2007, Diekelmann & Born, 2010, Wilhelm *et al.*, 2012). This process is orchestrated by the SO and transmitted via SW-Rs nested in troughs of spindle events. In this view, the SO up-states represent a short time window for neural processes and occurrence of synaptic plasticity (Steriade, 2006, Destexhe *et al.*, 2007, Compte *et al.*, 2008, Mölle & Born, 2011). In fact, studies employing electrical and transcranial magnetic stimulation have confirmed the notion of depolarizing up-phases as periods of enhanced excitability and emphasized the role of SOs as central elements in memory consolidation (Timofeev & Steriade, 1996, Massimini *et al.*, 2003, Bergmann *et al.*, 2009, 2012). First evidence that reactivation takes place in humans and is causally linked to SWS was gathered in seminal studies of Rasch *et al.* (2007). By exposing participants to an odour during encoding and once again during subsequent SWS, they demonstrated a boost in retrieval performance on a

declarative memory task in comparison to both an odourless placebo stimulus and odour presentation during REM or wakefulness, indicating that the conditioned enhancement of SWS-associated memory reactivation benefits consolidation processes.

1.4 Auditory System

The main function of the auditory system is capturing auditory stimuli and transforming them into sensory perception. Even though acoustic stimuli basically represent pressure waves, frequency and amplitude are only two examples of a variety of sound parameters permitting the transmission of information as an elementary component in the communication between life forms. Impairments of the auditory system are severe burdens for daily living that can lead to social isolation or even disturb the development of children (Stevenson *et al.*, 2010, Polley *et al.*, 2013, Mick *et al.*, 2014).

The auditory system consists of three main structures, i.e. outer, middle and inner ear (Figure 1.5*A*). Composed of the visible pinna and the ear canal, the outer ear captures the sounds waves and focuses them on the middle ear. The outer ear thus also acts as a spatial buffer between the highly sensitive interior auditory structures and potential sources of harm. In addition, frequency components between 3 and 12 kHz (i.e., the range of human speech) of sound waves travelling along the ear canal are further amplified (Purves *et al.*, 2004). In the middle ear, incoming sound waves first hit the eardrum which directly forwards changes in air pressure to a chain of three so-called ossicles (hammer, anvil and stirrup), resulting in an additional mechanical amplification of the sound waves before they arrive at the inner ear.

The inner ear transforms the mechanical waves into electrochemical signals which are then transported to the brain via the auditory nerve (Schnupp *et al.*, 2012, Figure 1.5*B*). This transformation takes place in the cochlea, the main structure of the inner ear, which receives the amplified waves from the middle ear. Travelling along the basilar membrane within the cochlea, the waves induce vibrations of the membrane and its sensory cells which eventually generate electro-chemical signals. Since the location where the acoustic stimulus is processed by the sensory cells depends on its frequency, the cochlea moreover performs a pre-selection of frequencies before they are transmitted to higher brain centres. Once the transformation into a neuronal signal has been accomplished, the information passes through several nuclei in the brainstem and thalamus,

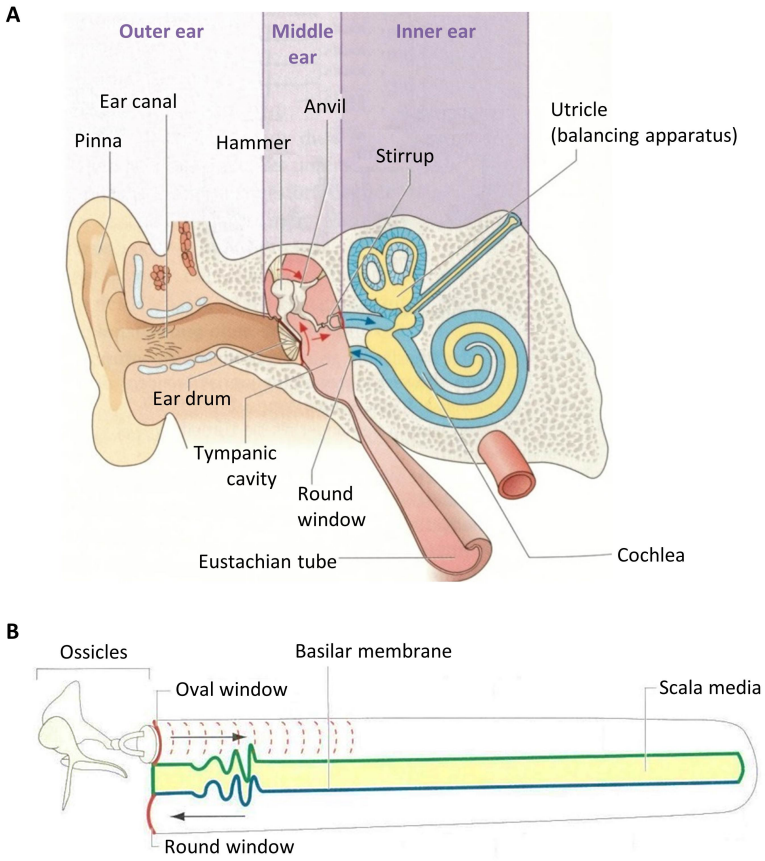

Figure 1.5 – The Auditory System.
(*A*) Anatomy of the human ear. The auditory system comprises three main structures: outer ear, middle ear and inner ear. Incoming sound waves are captured by the pinna and passed through the ear canal to the middle ear. In the middle ear the sound waves are mechanically amplified. In the cochlea of the inner ear, they are transformed into an electrochemical signal which is forwarded to the brain for further processing. (*B*) Schematic illustration of an unrolled cochlea. Sound waves that have been amplified by the ossicles in the middle ear reach the cochlea through the oval window and elicit a travelling wave. Depending on the frequency composition, this wave leads to deflections at specific locations along the basilar membrane. Sensory cells attached to the basilar membrane finally translate the (mechanical) deflections into a electrochemical signal. The figure was modified from Behrends *et al.* (2010).

each of them serving a different function, until it reaches the primary and secondary auditory cortices where it is integrated into a complex auditory perception.

The effect of auditory stimulation on the sleeping brain has been thoroughly investigated. The majority of these studies, however, focused on the amplitudes and latencies of specific components of the auditory evoked potential (AEP) response during sleep in comparison to wakefulness in order to examine the extent to which information is processed during Non-REM and REM sleep (Bastuji *et al.*, 1995, 2002, Campbell & Colrain, 2002, Dang-Vu *et al.*, 2011), in particular in clinical settings for the diagnosis of auditory disorders (Colrain & Campbell, 2007, Miller *et al.*, 2008). Intriguingly,

these studies showed that in contrast to responses with fast components during wakefulness, AEP waveforms during SWS are composed of a large depolarization followed by hyperpolarization, thus resembling a slow oscillation (Amzica & Steriade, 1998, Nielsen-Bohlman *et al.*, 1991). Moreover, brief auditory stimulation has been associated with the induction of K-complexes, which are considered as forerunners of the SO (De Gennaro *et al.*, 2000, Cash *et al.*, 2009). These observations suggest that acoustic stimulation could serve as an effective and elegant means to modulate endogenous sleep SO activity.

Stimulation of the sleeping brain by transcranial direct current or transcranial magnetic approaches generates artefacts in the EEG which constrain subsequent analyses or necessitate complex methods to correct the EEG signal for these disturbances without distorting the phenomena of interest. Electrical stimulation also raises ethical concerns either due to potential risks often greeted with considerable scepticism or the opposite case, in which it is thought as a simple way to enhance cognitive functions applied with improvised devices and not under controlled conditions in a scientific or clinical environment (Bikson *et al.*, 2013). The precise application of tMS, on the other hand, demands the fixation of the participant's head, which can have a huge impact on sleep comfort and quality. In contrast, auditory stimulation (or stimulation via other sensory modalities) is non-invasive, completely artefact-free and does not induce any side effects. Finally, it does not impose any practical constraints on participants because it only requires them to wear headphones, which subjects can get accustomed to very quickly. Therefore, the approach to manipulate or, more specifically, to evoke SOs via auditory stimulation seems very feasible and promising.

1.5 Overarching Goal and Hypotheses

Sleep consolidates memory and the SO plays a major role in the underlying processes, which has raised interest in the induction of SOs by means of external stimulation. Against this background, the leading question of this thesis was to explore the feasibility and efficiency of auditory stimulation to enhance SO activity in humans. For this purpose a set of experiments was conducted to test the following hypotheses:

Hypothesis 1 - Facilitation of Sleep Onset:
Auditory stimulation mimicking the slow oscillation rhythm presented during wakefulness accelerates the transition to sleep.

Hypothesis 2 - Feasibility of Auditory Stimulation:
Auditory evoked potentials during Non-REM sleep resemble SOs and exhibit the same characteristics, e.g. grouping of spindle activity.

Hypothesis 3 - Entrainment of SOs:
Rhythmic auditory stimulation entrains SOs to the external pace.

Hypothesis 4 - Phase-dependency of Auditory SO Stimulation:
A stimulation in phase with SO up-phases prolongs the on-going SO and enhances memory consolidation, whereas a disruptive out-of-phase stimulation results in an impairment of memory performance.

Hypothesis 5 - Limits of SO stimulation:
The capacity to prolong on-going SOs via repetitive in-phase stimulation is limited.

The first three hypotheses were addressed in Experiment I and will be presented in Chapter 3. Building upon these findings, the study design and stimulation protocol in Experiment II (Chapter 4) were adapted to examine the efficacy of auditory stimulation and its relevance for the consolidation of declarative memory as proposed in Hypothesis 4. A final experiment was conducted to test Hypothesis 5 and the corresponding results will be presented in Chapter 5. In the final Chapter 6 I will discuss the findings of these experiments and conclude with an outlook of some ideas for future studies.

Chapter 2

Material and Methods

The present work is based on different methods used for the acquisition and analysis of both electrophysiological and behavioural data. This current chapter is therefore designed to be an overview of the general study design and procedures of the proposed experiments examining the above mentioned hypotheses and common methods of data analysis employed in the following chapters.

2.1 Participants

Overall 63 volunteers participated in the experiments, of which 24 were male. Their age ranged from 18 to 30 years and was on average ($\pm$ SEM) 23.6 ± 0.4 years. All participants were non-smokers, free of medication and native German speakers. Prior screening ensured no history of neurological or psychiatric disease. Subjects had followed a normal sleep-wake rhythm, i.e. no shift work, for at least 4 weeks leading up to the experiments. Before an experimental night, participants were not allowed to ingest alcohol on the day before and were asked to refrain from caffeine 8 hours before the scheduled sleeping time. Moreover, they were instructed to get up at 07:00 a.m. and not to take a nap during these days. Prior to the experiments, subjects were accustomed to sleeping under laboratory conditions during an adaptation night, including EEG and polysomnographic recordings as well as wearing headphones (but without stimulation). The experiments were approved by the ethics committee of the University of Lübeck and Tübingen, and all subjects gave written informed consent prior to their participation.

Data from 5 participants were discarded because of missing SWS (2 subjects) during an adaptation night or prolonged awakenings during the stimulation nights (3 subjects).

Additionally in Experiment III, EEG data from two subjects had to be excluded from the EEG analysis due to technical problems with the overnight recording. Nevertheless, they were included in the analysis of behavioural aspects, since technical problems did not affect the auditory manipulation and therefore any performance on the memory task.

2.2 Study Designs and General Procedure

Each participant was examined according to a within-subject design on either three (Exp. I) or two conditions (Exp. II & III), with the respective experimental nights separated by at least 7 days. The order of conditions was balanced across participants.

On experimental days, participants arrived at the laboratory between 8:00-9:00 p.m. and were prepared for EEG and polysomnographic recordings. In the first experiment, subjects went to bed directly afterwards and auditory stimulation commenced already during wakefulness 2 minutes before lights were turned off (at 11:00 p.m.). During this 2-min interval subjects lied in bed with eyes open fixating a point at the ceiling. After lights off, subjects were allowed to sleep until $\sim$ 7:00 a.m. with the auditory stimulation continuing for further a 90 min. Contrary, participants in Experiments II and III additionally performed a declarative memory task (word-pair associates) approximately between 9:15 and 10:45 p.m. (encoding phase) before they went to bed. Furthermore auditory stimulation started 5 minutes after the subjects displayed stable sleep stage 2 (or deeper) for the first time after sleep onset and discontinued 210 min later. If SWS was ongoing at this time, stimulation was continued until the end of this SWS period. Subjects were awakened after 7 hours of sleep ($\sim$ 6:00 a.m.) the next morning, whenever they had entered light Non-REM sleep, i.e. stages 1 or 2. About 30 min after awakening, recall of memories was examined (retrieval).

Before and after sleep, the participant's mood and tiredness were assessed using the Positive and Negative Affect Schedule (PANAS) and the Stanford Sleepiness Scale (SSS). To control for general alertness and vigilance, all subjects performed on Psychomotor Vigilance Task (PVT) before encoding and retrieval testing based on Dinges & Powell (1985). In this task, a counter appeared at the center of a computer screen randomly every 2-10 seconds for about 5 minutes and participants had to respond as quickly as possible by pressing a button. After the retention interval, participants filled out a questionnaire to measure sleep quality and feeling of being well-rested. Additionally, in Experiment III after retrieval testing, a digit span test as well as a word fluency test were

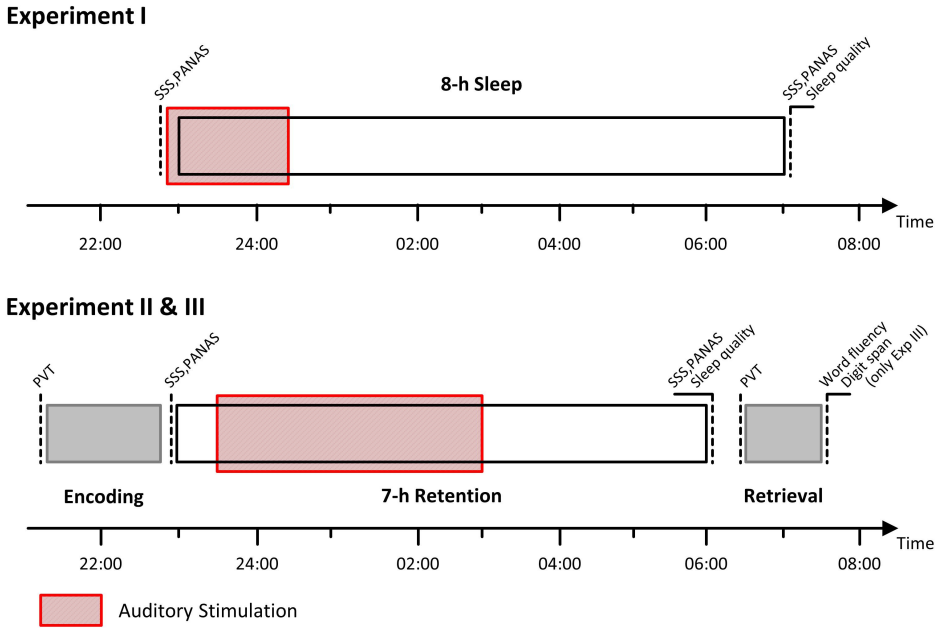

Figure 2.1 – Study Design of Experiments I-III.
Overview of the procedure for all experiments. Unlike in Experiment I, in which auditory stimulation (indicated in red) commenced in wakefulness two minutes before the lights were turned off and continued for another 90 min, during Experiment II and III auditory stimulation was applied while subjects displayed stable Non-REM sleep and lasted for about 210 minutes. Furthermore, these participants were additionally tested on a declarative learning task before retention (Encoding) and again afterwards in the morning (Retrieval). Time points of control tests and questionnaires are indicated by vertical dashed lines. SSS: Stanford Sleepiness Scale, PANAS: Positive and Negative Affect Scale, PVT: Psychomotor Vigilance Test.

performed to exclude confounds by general changes in working memory and executive (retrieval) functions, respectively. For the digit span test, subjects were asked to repeat lists of orally presented digits forward and backward. The word fluency task required the subject to write down as many kinds of either jobs or hobbies as possible denoted by words starting with either the letter 'M' or 'P' within 2-min periods.

Every experiment included one night serving as a control condition, in the following referred to as 'Sham', in which participants slept with headphones, but no auditory stimuli were presented. The exact protocols concerning how the stimulations were performed in the remaining experimental nights will be introduced in the following within each corresponding chapter. The overview in Figure 2.1 illustrates the study design for all three experiments.

2.3 Auditory Stimulation

Auditory stimuli were clicks (bursts of pink $1/f$ noise) of 50 ms duration, with a 5-ms rising and falling time, respectively, to elicit a broadband auditory stimulation over the entire frequency band following (Gao *et al.*, 2009). Pink instead of white noise was used because it sounds softer and is therefore more comfortable to hear. The sound volume was measured directly at the in-ear headphones and calibrated prior to each experimental night using a Voltcraft sound level meter SL-400 (Conrad Electronic SE, Hirschau, Germany) to 60 dB SPL in Experiment I and 55 dB for Experiment II and III, respectively. Stimuli were presented binaurally via commercially available in-ear headphones (Exp I: Philips RP-HJE170 (The Netherlands), Exp II and III: Sony MDR-EX35 (Germany)). The equipment to deliver the auditory stimuli and to record the electrophysiological data was located in a surveillance room, which was located adjacent to the sleeping room and allowed to control the experiment without disturbing the subject during the retention period (Figure 2.2).

Since Experiment II and III focused on auditory stimulation during SWS, subjects

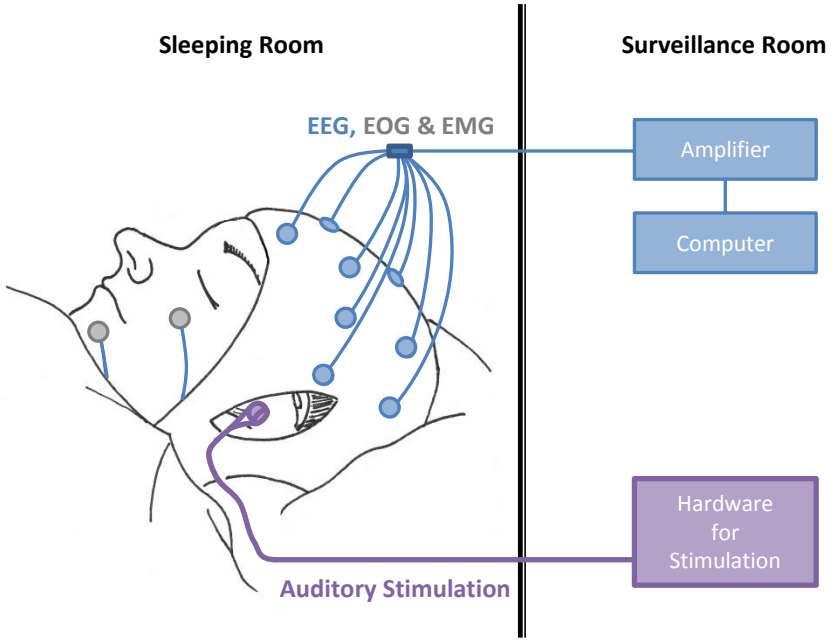

Figure 2.2 – Schematic Set-up for Auditory Stimulation and EEG recordings
Adjacent to the room where subjects slept during the retention period was a surveillance room with all the equipment to record and monitor both EEG and polysomnography (blue and grey circles) as well as to control the auditory stimulation. Subjects' in-ear headphones were connected via an audio extension cable to the corresponding hardware through a caulked hole in the separating wall.

who participated in those experiments were asked in the mornings after retention whether they had noticed the stimulation. For Experiment II, 11 out of 19 subjects and 7 of the 30 subjects of Experiment III reported perception of auditory stimuli during the night. This might reflect that the presence of sleep stage 2 (and SWS) does not completely preclude any awareness of stimulation or that during stimulation sudden transient arousals occurred before stimulation was halted, which happened in rare cases.

2.4 Declarative Memory Task: Paired Associate Learning

To assess declarative memory in Exp. II and III, a paired-associates learning task was used which had previously proven sensitive to the effect of sleep (Marshall *et al.*, 2004, Plihal & Born, 1997) (Figure 2.3). The task consisted of the sequential presentations of 120 pairs of nouns on a monitor, each for 4 s and with an interstimulus interval (ISI) of 1 s. Each word pair was composed of moderately semantically related words (e.g. 'brain - consciousness' or 'solution - problem'). Different word lists were used in different experimental conditions of one subject, with the order of word lists balanced across all subjects and conditions. At learning before sleep, presentation of the list was followed by an immediate cued recall test, in which the subject had to respond by naming the second word on presentation of the first word of each pair, with word pairs presented in random order. The time to respond was unlimited. Immediately after a response, the correct answer was revealed on the screen for 2 s. At retrieval testing in the morning after sleep, cued recall was repeated in the same manner as immediately after learning, except that no feedback was provided after the participant's response. Overnight memory retention was determined by the difference in the number of correctly recalled words between retrieval testing in the morning after sleep and immediate recall performance after learning but before sleep.

2.5 EEG Recordings and Polysomnography

In all experiments, EEG was recorded continuously from 19 channels according to the extended 10-20 system (Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2) referenced to linked mastoids (M1, M2). A ground electrode was placed on the forehead. Ag-AgCl ring electrodes were used and impedances were kept < 5 kΩ. Vertical and horizontal eye movements (VEOG, HEOG) as well as electromyogram from

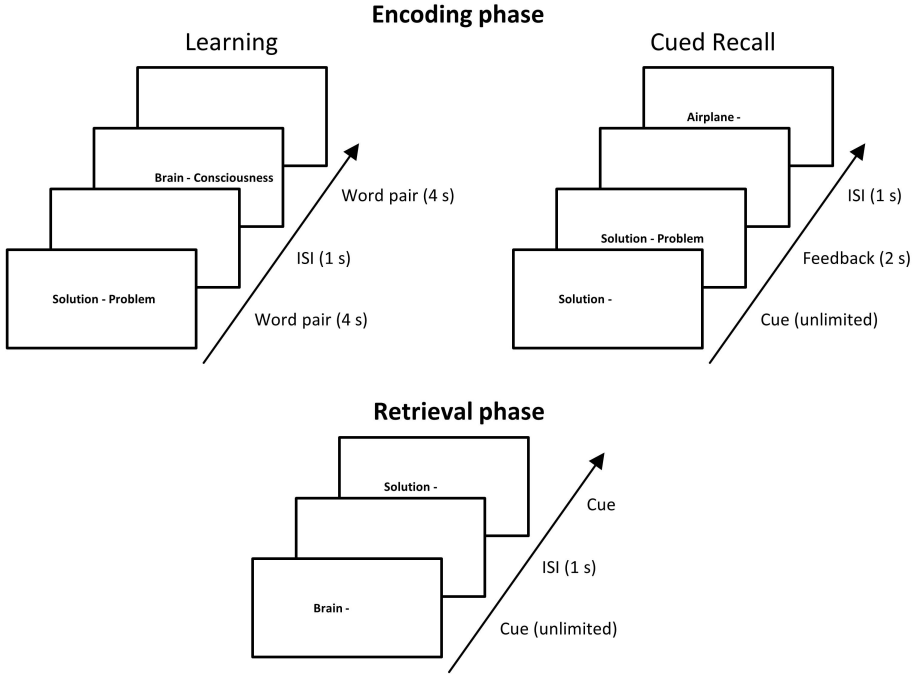

Figure 2.3 – Declarative Memory Task.
Illustration of the procedure for the declarative memory task. The task was comprised of an encoding phase before sleep and retrieval following sleep. During encoding subject first learned 120 word pairs, shown in random order for 4 s with an interstimulus interval of 1 s. Immediately afterwards a cued recall followed in which subjects were presented with the first word of a randomly drawn pair and had to name (with no time limit) the corresponding second word. After a response was given, the correct answer was shown for 2 s. Cued recall at the retrieval phase in the morning took place in the same manner except that no feedback was given. Retention was measured as the difference of correct responses in the morning from the number obtained in the evening before sleep.

the chin (EMG) were obtained for standard polysomnography and for artifact detection. In Experiment I, EEG was recorded with a Neurofax EEG-9200 (Nihon Kohden, Japan) and the recording filters were set to a bandpass between 0.08 and 120 Hz for all channels. A BrainAmp DC amplifier (Brain Products, Germany) was used in Experiment II and III. Signals were recorded in DC-mode (i.e. without high pass filtering) and low pass filtered at 150 Hz. All recordings were acquired during the whole retention interval from lights off (∼11:00 p.m.) until subjects were awakened in the morning with a sampling rate of 500 Hz and stored on a PC for later off-line analyses. In addition, time points of auditory stimulation or corresponding time points during Sham conditions were marked in the EEG to allow an event-related analysis.

2.6 Sleep Scoring and Analyses of Sleep Measures

EEG (at C3 and C4), EOG and EMG recordings were used for off-line scoring of sleep stages by two experienced raters who were blinded with regard to the experimental condition. Scoring was done for subsequent 30-s recording epochs according to standard criteria (Rechtschaffen & Kale, 1968). Prior to scoring, the EEG and both EOG channels had been band-pass filtered between 0.3 to 30 Hz, and EMG channels had been high-pass filtered at 5 Hz. Total sleep time (TST), time spent in the different sleep stages (wake, S1, S2, SWS and REM) and movement arousals were determined for the total nights as well as for the periods of auditory stimulation (and for corresponding periods during Sham condition) and remaining sleep during the late night. Also, sleep onset latency was defined as the first occurrence of stage 1 sleep followed by stage 2 sleep with reference to lights off.

2.7 EEG Analyses

Pre-Processing

Analyses were performed with Spike2 (Cambridge Electronic Design, United Kingdom) and Brain Vision Analyzer 2 (Brain Products, Germany). EEG data had been pre-processed with a band pass filter of 0.3 - 30 Hz and EMG data with a high pass filter of 5 Hz. Stimuli evoking or occurring during arousals and awakenings were marked by visual inspection in order to discard them from any following analyses.

Because SOs are most pronounced during SWS, analyses of SOs as well as accompanying EEG analyses reported in the next chapters concentrate on SWS epochs, although results essentially did not change with additional inclusion of stage 2 sleep epochs. All EEG analyses were based on generic scripts which treated every data set equally and thereby prevented a biased interpretation.

Spectral Power

For the analysis of spectral power, Fast Fourier Transformation (FFT) was applied in the EEG data using a Hanning window with 4,096 data points ($\sim$ 8.2 s) and 50% overlap, resulting in a frequency resolution of 0.122 Hz. The power spectra were averaged across all 8.2 s windows and subsequently smoothed with a three-point moving average. To account for individual variability, we normalized the power spectra for each EEG channel by its cumulative power up to 30 Hz. Power of different frequency bands was determined

by summation of the values obtained from the corresponding frequency bins: SO ($0.5-1$ Hz), slow-wave activity (SWA, $0.5-4$ Hz), theta ($4-8$ Hz), slow ($9-12$ Hz) and fast spindle ($12-15$ Hz) bands.

Auditory Evoked Potentials

To assess immediate effects of auditory stimulation, EEG signals were averaged with reference to stimulus onset. In Sham conditions, averaging was performed across corresponding periods, time-locked to markers representing the time points when a stimulus would have been presented in a stimulation condition. The number of averaged stimuli was statistically tested between conditions. If a significant difference was present, the condition with the lowest absolute number of stimuli was taken as a reference to randomly draw subsets with the same amount of stimuli from the remaining conditions to allow a comparison. Identical averaging analyses were performed for slow and fast spindle activity. Prior to this analysis, the pre-processed EEG signal had been down-sampled to 100 Hz, filtered in the respective frequency bands ($9-12$ Hz and $12-15$ Hz), and the root mean square signal (RMS signal) had been determined, which extracts a measure similar to the envelope of the spindle activity and therefore represents spindle power.

Off-line Detection of Discrete SO Events

For a fine-course analysis of SOs and how they are affected by the auditory stimulation, an off-line detection of discrete SO events was performed according to a custom-made algorithm described in (Mölle *et al.*, 2002). At first, the EEG was band-pass filtered between 0.25 and 30 Hz, and afterwards down-sampled to 100 Hz. To extract the SO component a low-pass filter of 3.5 Hz was applied. Then, negative and positive peak potentials were derived from all intervals between consecutive positive-to-negative zero crossings (i.e. one negative and one positive peak between two succeeding positive-to-negative zero crossings). However, only intervals with a length ranging between 0.8 and 2 s, which corresponds to a frequency of $0.5-1.25$ Hz, were included in the next steps. The mean values of the negative peak and negative-to-positive peak across all pre-selected intervals were then calculated and an interval was identified as an actual SO if both its absolute negative and negative-to-positive peak potential was larger than 1.25 times the respective average value. This last step introduces an adaptation of both detection thresholds to each individual participant. This is necessary since threshold

parameters are relatively stable within one participant but usually vary between different participants. In the following these identified SO events will be characterised by the position of the negative peak as the negative half-waves typically show a more distinct peak, whereas positive half-waves have a greater variability and broader shape (Mölle *et al.*, 2002). Figure A.1 in the Appendix summarizes the SO detection algorithm with step-by-step illustrations. Importantly, the detection in Experiment I was performed on each of the 19 EEG channels whereas for Exp. II and III the algorithm was mainly based on a virtual channel representing the mean EEG signal recorded from F3, Fz, F4, C3, Cz, C4, P3, Pz and P4, which resulted in a detection of SO events of a more global nature. Only for the analysis of the spreading behaviour of SOs in Experiment II a detection was additionally performed for each individual channel.

Following the identification procedure, an evaluation first of all involved general properties covering absolute number of detected SOs, negative-to-positive peak amplitude, slope from the negative peak to the following zero crossing and the duration (i.e. time between the two succeeding positive-to-negative zero crossings) (Figure 2.4). Analogue to the previously mentioned AEP analysis, averaging was performed with respect to the negative peak for the conventional EEG band as well as on the slow and fast spindle RMS-signal. To examine whether auditory stimulation affected the temporal occurrence of SOs, event correlation histograms were calculated on the detected SO negative peaks time-locked either to the stimulation markers or to the SO events themselves then representing an auto-event correlation. These histograms were afterwards normalized by the number of SO events to yield the event density.

2.8 Statistical Analyses

Unless stated otherwise, all data are presented as mean ± SEM. In the following statistical analyses were performed with SPSS Statistics software (SPSS Inc., U.S.) and are generally based on repeated-measures Analysis of Variance (ANOVA) as well as a Student's paired *t*-Test for *post-hoc* comparison if indicated. Consistent within-subject factors for repeated measures analyses in all experiments were the condition and topography, representing the 19 different recording channels. Additional factors necessary for an analysis specific to each experiment will be introduced in the corresponding chapters. In general, an exact testing of significant differences between conditions regarding a time series, e.g. SO waveform or event-correlation was performed on succeeding 300-ms

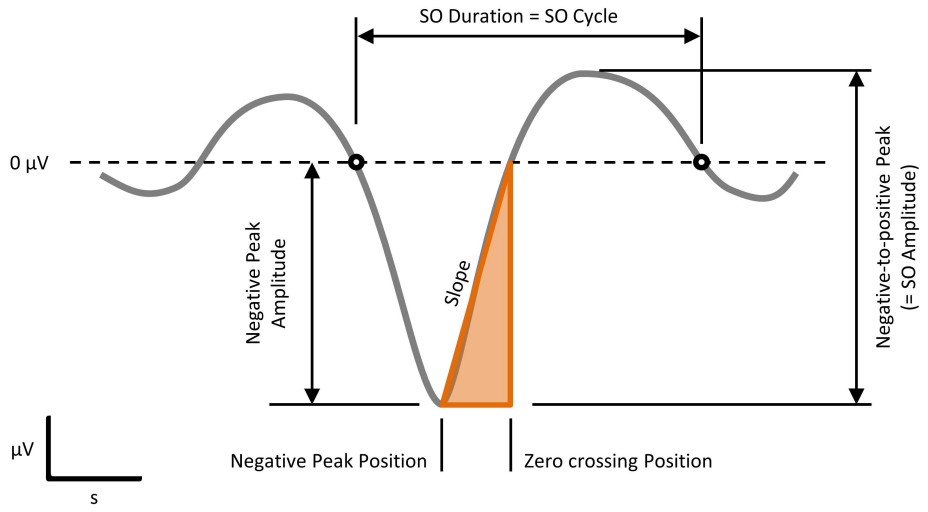

Figure 2.4 – Illustration of General Properties of a SO Event.
Overview of the SO properties used to examine the effects of auditory stimulation. Each SO (cycle), illustrated by the grey line, was characterized as an event by its negative peak position between two consecutive positive-to-negative zero crossing (black circles) marking also the onset and end of the SO. The SO amplitude was derived from the absolute value of the negative peak value plus the following positive peak amplitude. As a measure of synchrony the rising SO slope of the down-to-up transition was considered, i.e. the quotient of negative peak amplitude and the time between a negative peak and the succeeive (negative-to-positive) zero crossing.

intervals. To account for multiple testing, a Greenhouse-Geisser correction of degrees of freedom was applied where appropriate. Correlation analyses were performed with a two-sided Pearson-Correlation and always reported as the correlation coefficient and significance value. For all statistical tests a P-value < 0.05 was considered significant.

Chapter 3

Experiment I: Induction of Slow Oscillations by Rhythmic Auditory Stimulation - A Pilot Study

3.1 Introduction

The theoretical background reviewed in Chapter 1 revealed that slow oscillations are involved in processes of memory formation. This functional importance has attracted growing interest regarding their manipulation in terms of a general enhancement or suppression of SO power (Marshall *et al.*, 2006, Landsness *et al.*, 2009, van der Werf *et al.*, 2009, Tononi *et al.*, 2010). With regard to the triggering of SOs, this has been successfully attempted using transcranial direct current stimulation (tDCS, Marshall *et al.*, 2006), transcranial magnetic stimulation (tMS, Massimini *et al.*, 2007) or intracortical electrical stimulation (Vyazovskiy *et al.*, 2009). By the current state of knowledge, a rhythmic sensory stimulation has not been tested in detail as a tool to induce SOs in humans, although acoustic stimuli are well known to induce K-complexes, which are considered a forerunner of the SO (Cash *et al.*, 2009, De Gennaro *et al.*, 2000, Riedner *et al.*, 2011). In animals, on the contrary, Gao *et al.* (2009) demonstrated in anaesthetized guinea pigs that a regular sound stimulation produced an entrainment of SO activity in thalamic neurons. Based on this evidence and the overall simplicity of such an approach, a pilot

study was conducted to probe the capacity of rhythmic auditory stimulation in the 0.8-Hz SO frequency band to induce SOs in the human brain. Of particular interest was whether such regular stimulation had the capability to entrain endogenous SO rhythms to an external drive. Effects were tested while subjects were awake, transited into sleep and during stable Non-REM sleep. Of additional interest was the question whether rhythmic 0.8-Hz stimulation would accelerate onset of sleep and SWS. A similar effect was achieved in a previous work by instrumental conditioning of the sensorimotor rhythm (Hoedlmoser *et al.*, 2008).

3.2 Stimulation Protocol

In this first experiment 10 participants were examined under three experimental conditions (Figure 3.1): A 0.8-Hz stimulation condition in which sound bursts were presented with a constant interstimulus interval (ISI) of 1.25 s, corresponding to a frequency of 0.8 Hz as an approximation to the SO frequency. In a Random stimulation condition, sounds occurred randomly, with ISIs ranging from 0.125 to 5 s, excluding intervals between 0.5 and 2 s in order not to overlap with effects of the 0.8-Hz stimulation. Random ISIs were generated such that the average ISI was also 1.25 s in this condition, and that in both stimulation conditions the same total number of sounds ($n = 4416$) was presented. Of

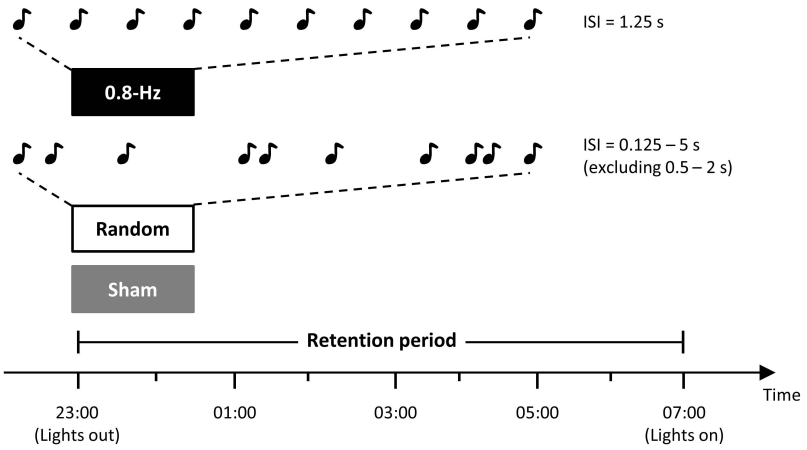

Figure 3.1 – Stimulation Protocol.
Subjects were tested under three conditions: A rhythmic 0.8-Hz stimulation (black), a Random stimulation (white) with stimuli presented between 0.125 to 5 s apart, however excluding intervals between 0.5 and 2 s, and a Sham condition (grey) without any click presentations during the first 90 min of the retention period (from lights out at 11:00 p.m.). In all conditions stimulation commenced during wakefulness 2 min before lights were turned off. Musical notes, each representing a click presentation, serve as a schematic illustration of the two different rhythms.

note, this procedure implies a higher amount of short ISIs during the Random stimulation than in the 0.8-Hz Stimulation. The auditory stimulation commenced 2 min before lights were turned off (at 11:00 p.m.) and the participants were allowed to sleep (Please refer back to Chapter 2.2 for an overview of the study designs and procedures). During this 2-min interval subjects lay in bed with eyes open fixating a point at the ceiling. After lights off, auditory stimulation continued for a further 90 min. The third condition served as a Sham control condition and comprised a periodic acoustic presentation only within the 2 min prior to lights off and none thereafter.

3.3 Results

3.3.1 Rhythmic 0.8-Hz Stimulation Delays Sleep Onset

Figure 3.2 shows the average time subjects needed to transition between stages, beginning from wakefulness to the first occurrence of stage 1 sleep, from stage 1 to stage 2 sleep, and from stage 2 sleep into SWS. Contrary to our expectations, in the 0.8-Hz stimulation condition subjects needed significantly more time to reach stage 1 compared with both Random stimulation ($P = 0.006$) and Sham ($P = 0.027$) condition, indicating a delayed sleep onset. The transition from stage 1 to stage 2 sleep was not affected by 0.8-Hz stimulation, although it revealed to by delayed with Random stimulation ($P = 0.023$, compared with Sham). Differences in the transition time from state 2 sleep into SWS were not significant ($P > 0.253$).

To assess the temporal dynamics of the fast spindle activity, beginning with the 2-min wake interval before lights off, a Fast Fourier Transformation (Hanning window, 16 384 data points) of the EEG signal was calculated on subsequent 33-s windows that was moved in 10-s steps in time for a total of 32 min and the mean spectral power between

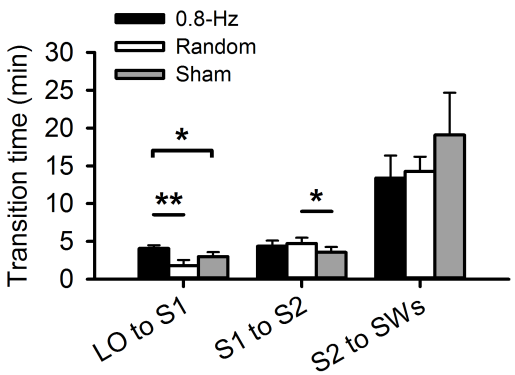

Figure 3.2 – 0.8-Hz stimulation delays sleep onset.
Transition times for the three stimulation conditions (0.8-Hz stimulation, Random stimulation, Sham) to reach S1 from wakefulness (*left*), S2 from S1 (*middle*), and SWS from S2 (*right*). Wakefulness refers to the time when lights were turned off, and only transitions to S1 which were followed by S2 were considered. $^*P < 0.05$, $^{**}P < 0.01$, for paired t-tests.

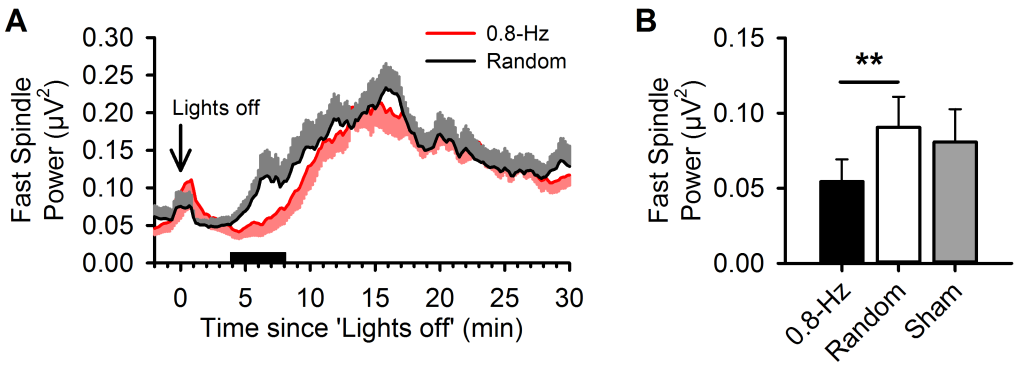

Figure 3.3 – Rhythmic Stimulation Suppresses Fast Spindle Power during Sleep Onset.

(*A*) Time course of fast spindle power ($12-15$ Hz, at Cz), with reference to lights off (time = 0 min) for the 0.8-Hz stimulation condition (red line) and for the Random stimulation condition (black line). The black horizontal bar indicates an interval from $4-8$ min after lights off where spindle power differed significantly ($P < 0.01$) between conditions. (*B*) Mean spindle power at Cz for the 0.8-Hz stimulation (black), Random stimulation (empty) and Sham stimulation (grey bar) conditions over the $4-8$ min interval marked in (*A*). $^{**}P < 0.01$ for paired *t*-test.

$12-15$ Hz was calculated. This analysis was limited to the 32-min time interval, as individual sleep courses became highly divergent with ongoing sleep resulting in large inter-individual variance. The resulting time course of fast spindle power confirmed that stimulation, and particularly 0.8-Hz stimulation, delayed the occurrence of stable Non-REM sleep (Figure 3.3*A*). A few minutes after subjects were allowed to sleep, fast spindle power started to increase. However, this increase was delayed during rhythmic 0.8-Hz stimulation. Thus, spindle power (at Cz, averaged time-locked to lights off) was reduced during 0.8-Hz stimulation, most consistently if compared with Random stimulation ($P = 0.007$; Figure 3.3*B*).

3.3.2 0.8-Hz Stimulation Enhances SO Activity Once Stage S2 Sleep Has Manifested

Contrary to the initial hypothesis, an analogue analysis of the temporal dynamics of power in the SO frequency band ($0.5-1$ Hz) revealed that 0.8-Hz stimulation, compared with Random stimulation and Sham, did not affect SO power during the waking period before lights off, nor thereafter in the beginning of the sleep period (Figure 3.4*A*). However, rhythmic 0.8-Hz stimulation had an impact once the subject advanced into Non-REM sleep stage 2. Averaging SO power time-locked to the onset of sleep stage 2 revealed that 0.8-Hz stimulation produced a distinct increase in SO power $10-25$ min later, with this effect coinciding with the occurrence of SWS (Figure 3.4*B*). This was

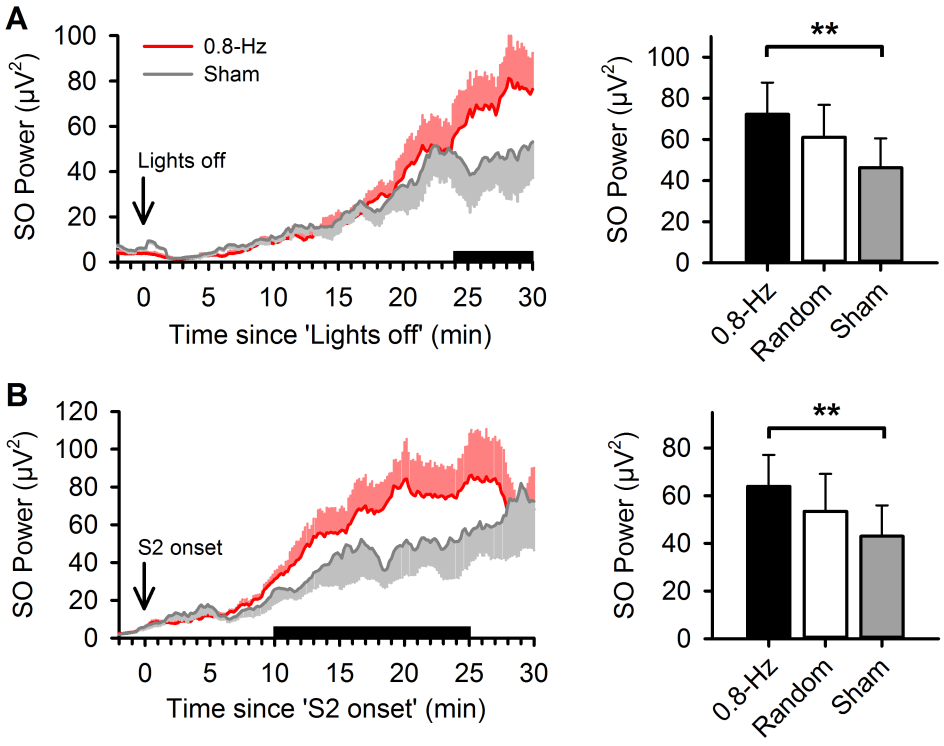

Figure 3.4 – Rhythmic Stimulation Enhances SO Activity only during Non-REM. Time course (*left panels*) of SO power (0.5 – 1 Hz, at Fz) averaged with reference to lights off (*A*) and with reference to the onset of sleep stage 2 (*B*), for the 0.8-Hz stimulation condition (red line) and for the Sham condition (grey line). Black horizontal bars indicate intervals where SO power differed significantly ($P < 0.01$) between conditions. Mean SO power for these intervals is indicated for all experimental conditions in the right panel. $^{**}P < 0.01$ for paired *t*-test.

most consistently observed at Fz when compared with the Sham condition ($P = 0.010$, for analyses with reference to lights off, Figure 3.4*A*; $P = 0.004$, with reference to S2 onset, Figure 3.4*B*). Changes in sleep onset latency during the 0.8-Hz stimulation condition (with reference to Sham condition) and SO power were not correlated ($r = 0.266$, $P = 0.458$), excluding that increases in SO power were an immediate consequence of the delaying effect of the stimulation on sleep onset. Effects of 0.8-Hz stimulation on SWA ($0.5 - 4$ Hz) were less consistent than those on SO activity and overall revealed only marginal significance. Effects on theta activity as well as on slow spindle activity remained non-significant.

3.3.3 Auditory Stimulation Modulates Slow and Fast Spindle Activity

Averaged AEPs to the stimulation were determined separately for the 0.8-Hz stimulation and the Random stimulation condition, with the responses for the Random stimulation condition additionally divided into stimuli which were solitary or not separated by more

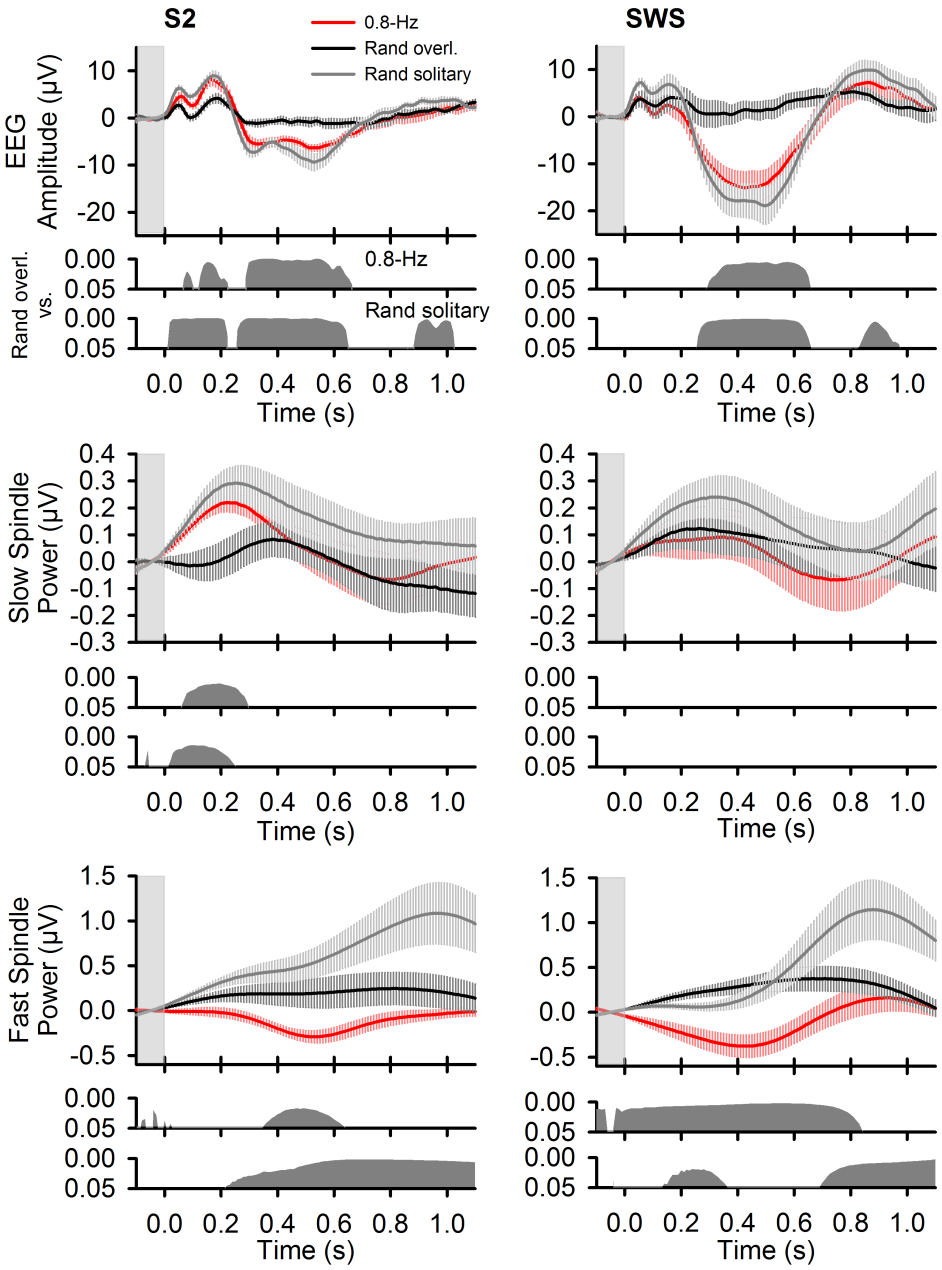

Figure 3.5 – Auditory Stimulation Modulates Slow and Fast Spindle Activity.
AEPs derived from 0.8-Hz stimulation (red line) and Random stimulation conditions (black line for overlapping and grey line for solitary stimuli) and categorized by sleep stage S2 (*left column*) and SWS (*right column*), as well as conventional EEG band (0.3-30 Hz, Fz, *top row*), slow spindle band (9-12 Hz, Fz, *middle row*) and fast spindle band (12-15 Hz, Cz, *bottom row*). Bottom panels indicate point-wise statistical comparison between the 0.8-Hz stimulation condition and the overlapping random stimulation (Rand overl.), and between the overlapping and solitary (Rand solitary) stimuli of the Random stimulation condition. Vertical grey bars indicate intervals used for baseline normalization. AEPs during S2: $n = 211.4 \pm 13.8$ and during SWS: $n = 194.5 \pm 18.6$ stimuli.

than 1.25 s from the previous and following click presentations ('non-overlapping' vs. 'overlapping' responses). Consistent with previous studies (Colrain & Campbell, 2007), AEPs during stage 2 sleep revealed a positive component about 200 ms post-stimulus onset followed by a double-peaked negative component 300-600 ms post-stimulus onset (Figure 3.5, *upper panel*). The two peaks of the latter component complex tended to merge into a single broad hyperpolarization during SWS, which was then followed by a depolarization at 900 ms post-stimulus. In fact, during the SWS this late negative-to-positive AEP complex (300-900 ms post-stimulus) showed some similarity with a SO. Generally, the AEP potential components were smallest for the 'overlapping' responses, as compared with the 'non-overlapping' responses and with the responses to the 0.8-Hz stimulation (see Figure 3.5 for statistical comparisons), reflecting the refractoriness of the AEP with shorter ISIs (Durrant & Boston, 2006). AEPs in the Random stimulation condition were also significantly smaller than those during the 0.8-Hz stimulation condition, when AEPs were averaged across all stimuli (overlapping and non-overlapping stimuli of the random stimulation condition, and all stimuli of the 0.8-Hz stimulation condition; Figure A.2 in Appendix A).

Root mean square slow spindle activity averaged with reference to stimulus onset during SWS showed a maximum shortly before the AEP negativity 300-600 ms post-stimulus, which only differed between the random overlapping and non-overlapping conditions, due to the aforementioned refractoriness of APEs (Figure 3.5, *middle row*). Fast spindle activity was suppressed during the AEP negativity (300-600 ms post-stimulus), particularly during the 0.8-Hz stimulation condition (see Figure 3.5 and A.2, *bottom row*, for statistical comparisons). This decrease in the 0.8-Hz stimulation condition was also significant when compared with responses averaged across all stimuli of the Random stimulation condition (Figure A.2). Fast spindle activity was enhanced during late AEP positivity (900 ms post-stimulus), in particular after non-overlapping stimuli of the Random stimulation condition. This late increase in fast spindle activity was also significant when all stimuli of the Random stimulation condition were compared with the 0.8-Hz stimulation condition (Figure A.2).

3.3.4 SOs are Modulated and Entrained by 0.8-Hz Stimulation

Figure 3.6 depicts averaged SOs identified during epochs of SWS occurring in the 90-min period of stimulation and during the corresponding periods of Random and Sham

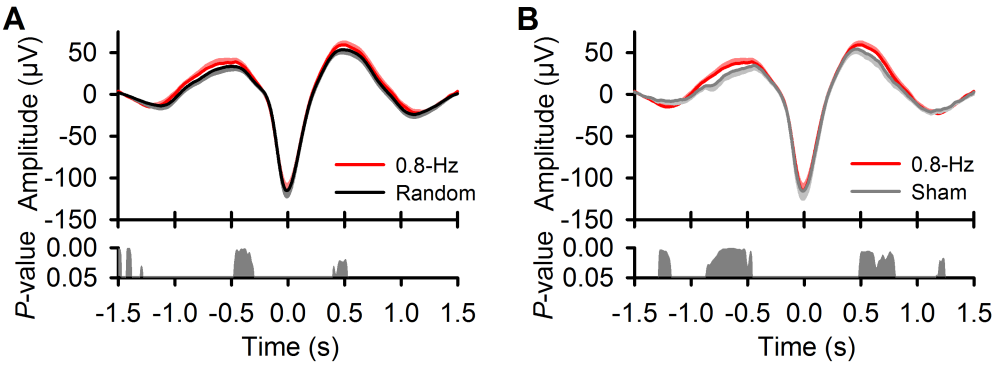

Figure 3.6 – Auditory Stimulation Modulates SOs.
Average SO waveform (at Fz) during SWS periods of the 90-min stimulation interval for (*A*) the 0.8-Hz stimulation condition (red line) and Random stimulation (black line), and (*B*) 0.8-Hz stimulation and Sham conditions (grey line). Differences in the potential level are indicated by point-wise statistical comparisons positioned below each average.

conditions. Notably, the 0.8-Hz stimulation did not significantly change the number of detected SO events during the 90-min stimulation period ($n = 779.2 \pm 124.6$ vs. Random stimulation $n = 730.9 \pm 54.2$ and vs. Sham $n = 732.0 \pm 123.5$, $P > 0.661$), underlining that the effect of the stimulation was primarily on the temporal entrainment of SOs. Averaging was performed with reference to the negative half-wave peak of the SOs. Comparison of the SOs in the three stimulation conditions shows a significantly stronger depolarization of the up-states before and after the negative half-wave of a SO event for the 0.8-Hz stimulation condition, in comparison with both Random and Sham conditions during intervals of greatest difference (see Figure 3.6 for statistical comparisons).

To analyse the occurrence of SO in trains (i.e., in close temporal succession) during different stimulation conditions, auto-event correlation histograms were calculated that visualize the timing between successive SOs. These auto-event correlation histograms indicated that rhythmic acoustic 0.8-Hz stimulation indeed induced more regular trains of SOs during SWS (Figure 3.7*A*). This was apparent by significant (all $P < 0.049$) increases in the frequency of SO peaks around time points being multiples of 1.25 s during the 0.8-Hz stimulation condition, i.e. the emerging SOs adapted to the external drive. Again this entraining effect of 0.8-Hz stimulation on the SOs was significant in comparison with both Random and Sham condition. Note the first peak in the auto-event correlation histogram (at 1.25 s) is identical for both the 0.8-Hz stimulation and Random stimulation conditions, reflecting that randomly presented stimuli also evoked two succeeding SOs. However, the succeeding peaks of the histogram at 2.5 and 3.75 s were significantly higher for the 0.8-Hz stimulation condition than the random

stimulation condition. This pattern is further illustrated in Figure 3.7*C* depicting the event correlation of detected SO negative peaks with respect to the click presentations for the 0.8-Hz stimulation condition and all random stimuli. While in both conditions a click is followed by a SO (with a negative peak at 500 ms post-stimulus) only the 0.8-Hz stimulation revealed an increase of SO events together with a decrease prior to a stimulus at approximately 800 ms and 500 ms, respectively, indicating that the 0.8-Hz stimulation induced regular trains of succeeding SOs. Notably, the present entrainment implies also a temporal alignment of the stimulus presentation to SO up-states that occur naturally about 500 ms before the (induced) negative half-wave.

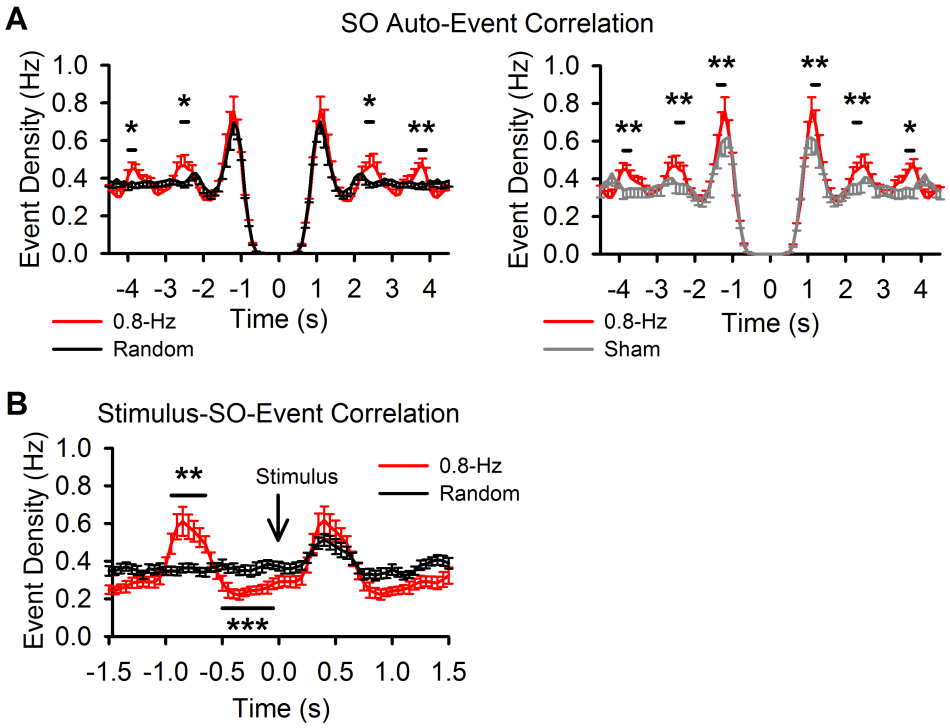

Figure 3.7 – Rhythmic Auditory Stimulation Entrains SOs.
(*A*) Auto-event correlation of detected SOs for recordings from Fz during SWS periods of the 90-min stimulation interval, for the 0.8-Hz stimulation condition (red lines), and the Random stimulation condition (black line, *left*) and the Sham condition (grey line, *right*). Analyses were performed for 9-s windows centred around the detected negative half-wave peaks with a 0.1-s bin-size. The x-axis indicates time intervals between successive SO events; the y-axis indicates the rates of SO events occurring at a given time interval. Thus, y-values represent the likelihood of a SO event occurring at a specific time before or after an identified SO event. Black bars denote 0.3-s intervals centrally positioned at multiples of 1.25 s, corresponding to the 0.8-Hz rhythm of acoustic stimulation. (*B*) Event correlation of detected SOs for Fz during the stimulation interval and SWS for the 0.8-Hz (red line) and Random stimulation (black line) conditions time-locked to the auditory stimuli (occurring at time $t = 0$). Black bars denote intervals for statistical comparison. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, for paired *t*-tests.

Table 3.1 – Sleep Stage Distribution for Different Time Intervals and Conditions

	Stimulation period			Remaining sleep		
Parameter	0.8-Hz	Random	Sham	0.8-Hz	Random	Sham
TST (min)	90.0 ± 0.0	89.7 ± 0.1	90.0 ± 0.0	387.9 ± 4.0	386.4 ± 4.9	392.0 ± 1.2
W (%)	4.6 ± 0.6	3.5 ± 1.4	3.6 ± 0.6	1.3 ± 0.5	0.9 ± 0.3	1.0 ± 0.3
S1 (%)	5.6 ± 0.7	5.6 ± 1.1	6.3 ± 0.8	2.9 ± 0.7	2.2 ± 0.4	2.0 ± 0.5
S2 (%)	46.6 ± 6.0	43.7 ± 3.0	50.2 ± 6.0	55.9 ± 1.8	52.6 ± 2.0	58.1 ± 2.0
SWS (%)	39.1 ± 6.1	38.0 ± 3.3	36.8 ± 5.9	10.0 ± 1.4	13.4 ± 1.3	10.6 ± 1.4
REM (%)	1.1 ± 1.1	4.5 ± 1.9	0.0 ± 0.0	23.3 ± 1.5	23.9 ± 1.3	23.0 ± 1.1
Arousal (%)	3.1 ± 0.5	4.6 ± 0.7	3.1 ± 0.6	5.9 ± 0.5	6.7 ± 0.5	5.7 ± 0.7

Percentage of time spent in different sleep stages for the three stimulation conditions (0.8-Hz stimulation, Random stimulation and Sham) and two time intervals: the 90-min stimulation period (measured from lights off) and the remaining stimulation-free sleep period (until awakening). There were no significant differences between conditions.
REM, rapid eye movement; S1, sleep stage 1; S2, sleep stage 2; SWS, slow-wave sleep; TST, total sleep time; W, wake.

3.3.5 Sleep Architecture Remained Unchanged during and After Stimulation

An analysis of the sleep stage distribution following the first 90 min was performed to examine whether continuing effects were present and affected remaining sleep. Table 3.1 lists sleep parameters for the 90-min period of stimulation as well as for the remaining sleep epoch, and did not indicate any difference between the stimulation conditions in time spent in the different sleep stages for both periods, i.e. during and after stimulation ($P > 0.096$, for all comparisons). Also, number of arousals did not significantly differ between conditions, excluding the possibility that auditory stimulation disturbed sleep.

3.4 Summary

The presented findings indicate that rhythmic auditory stimulation with a slow 0.8-Hz frequency mimicking the frequency of natural EEG SOs does not enhance SO activity in the waking brain, but leads to a distinct delay of sleep onset, which was paralleled by a suppression of spindle power. However, periodic 0.8-Hz stimulation increases spectral power in the SO band during Non-REM and SWS, although there was no earlier onset of SWS. Averaging of AEPs and, in parallel, evoked spindle activity revealed a stimulus-induced modulation of fast spindle activity reminiscent to that during spontaneous SOs,

specifically in the 0.8-Hz stimulation condition. Amplitude and auto-correlation analyses of SOs revealed that the 0.8-Hz stimulation not only increased the depolarizing up-phase of SOs, but effectively entrained these oscillations to the 0.8-Hz rhythm of stimulation.

Chapter 4

Experiment II: Closed-loop Nudging of Sleep Slow Oscillations to Enhance Memory

4.1 Introduction

The main outcome of Experiment I is that an auditory stimulation is indeed a very straight-forward tool to induce SOs but requires the presence of Non-REM or SWS. Not only does the general waveform of an auditory evoked response resemble a SO, it is also accompanied by an identical modulation of slow and fast spindle activity which is hypothesized to be critical for the functional role of the SO in memory consolidation.

As a logical consequence, the next step was to integrate a suitable learning task before sleep to the current experimental design and examine whether the previous findings can be used to improve memory overnight. More importantly, if one takes a closer look at the AEP during SWS shown in Figure 3.5, the average response of the negative component is about 20 μV and thus rather small compared to a SO with a negative peak of at least 75 μV per definition. This observation implies that even though the rhythmic stimulation leads to an entrainment of the endogenous SO rhythm, the individual responses are either too variable resulting in a temporal jitter of the negative peak position or are very low in general. In fact, the stimulation in Experiment I as well as in other studies imposed rhythms on the brain disregarding the phase of ongoing endogenous oscillatory activity, which might limit enhancement in cognitive brain functions accompanying SO induction. Hence the stimulation protocol itself should be optimized: According to the observations

from the Stimulus-SO-event correlation displayed in Figure 3.7*C*, the probability of a negative SO half-wave peak following a stimulus was highest about 500 ms after stimulus onset, which on the other hand means that a stimulus automatically coincided with the preceding SO positive half-wave. It was hypothesized that a targeting of this particular phase would lead to an enhanced resonating response.

The experiment presented in this chapter reflects the core of this thesis: A closed-loop feedback system was set up that utilizes the ongoing oscillatory EEG activity to detect spontaneous slow oscillations and time the auditory stimulation to a specific phase of a detected SO resulting in an adaptive stimulation in synchrony with the brain's endogenous rhythm.

4.2 Stimulation Protocol

In this experiment, a total of 18 participants were examined. In a main study, 11 (8 women; age: 24.2 ± 0.9 years) of those 18 participants were subjected to a stimulation in-phase with the rhythm of SO up-states to amplify the slow oscillatory activity and promote memory consolidation, whereas the remaining 7 subjects (5 women, age 21.1 ± 0.7 years) were tested in a control study with an Out-of-phase stimulation aiming at a completely opposite effect, i.e. a disruption of SOs and consequently an impairment of their functionality. In both studies, each subject took part in an additional Sham condition for a within-subject comparison to assess the effects of the two stimulation protocols.

4.2.1 On-line Detection of Slow Oscillations and In-Phase Auditory Stimulation

To accomplish the on-line detection of SOs and a precise timing of auditory stimulation, an additional EEG recording system was used (Figure 4.1), composed of a 'Digitimer D360' EEG amplifier (Digitimer, United Kingdom) and a 'Power 1401 mk 2' high-performance data acquisition interface (Cambridge Electronic Design, United Kingdom) connected to a separate PC. With this set-up, the pre-frontal EEG was recorded from an electrode placed at AFz and referenced to the average potential from linked electrodes attached to the earlobes. The EEG was acquired and hardware filtered between 0.25 and 4 Hz (2nd order Butterworth filter) by the D360 amplifier and afterwards digitized by the Power 1401 with 200 Hz. A custom-made script running under Spike2 (Version

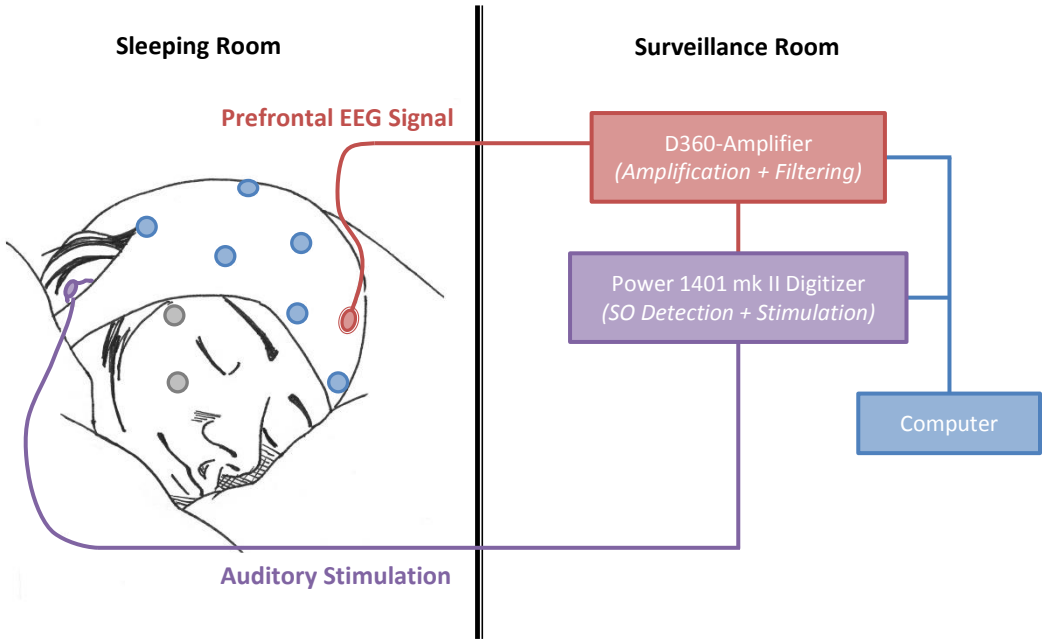

Figure 4.1 – Schematic of the Set-up for Closed-loop Auditory Stimulation.
In addition to the EEG (blue circles), EOG and EMG (grey circles), one electrode was placed at AFz to perform the on-line detection of SO events. This signal was fed into a D360-Amplifier for amplification and bandpass filtering in the SO frequency range ($0.25 - 4$ Hz). Afterwards the filtered signal was digitized by a Power 1401 digital acquisition interface for a real-time analysis. The Power 1401 executed both the detection of SOs and the stimulus presentation, which could still be controlled by the experimenter with a connected computer.

7, Cambridge Electronic Design, United Kingdom) together with a sequencer integrated within the Power 1401 then enabled responding to the incoming pre-frontal EEG data in real-time. The detection was based on a threshold criterion and responded to the negative half-wave of a SO: Each time the EEG signal crossed an adaptive threshold from above towards larger negative values, auditory stimulation was triggered. On default, the threshold was set to -80 μV. Every 0.5 s, it was updated to the minimal (i.e. largest negative) instantaneous EEG amplitude within the preceding 5 s interval, however, only if this value exceeded (in negativity) -80 μV. This adaptation ensured a reliable way to continuously detect SOs of increasing and decreasing amplitudes within several sleep cycles, which can show a large variability of their negative amplitudes over the course of a whole night.

For each subject, the on-line SO detection algorithm was also applied to the first SWS epoch of the adaptation night (without any auditory stimulation) in order to determine the subject's individual delay time between the detected negative half-wave peak and the succeeding depolarizing up-state, i.e. the mean time between the SO

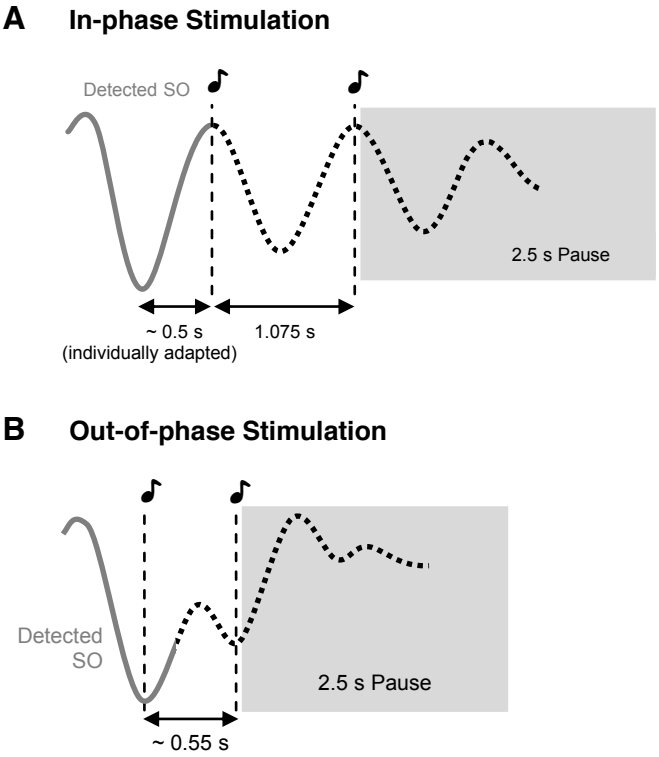

Figure 4.2 – In-phase and Out-of-phase Stimulation Protocols.
Overview of the stimulation protocols employed in Experiment II. (*A*) A first group of subjects, was stimulated with an In-phase protocol: following a detection of a spontaneous SO (by its negative half-wave, grey line), two stimuli separated by a fixed interval of 1,075 ms were delivered such that they coincided with the predicted up-states after a detection. (*B*) In a control study, the stimulation aimed at a disruption of the SO rhythm. In this case, the first stimulus was presented immediately upon SO detection, i.e. during the down-state, and the second click was individually adjusted to the resulting negative deflection from the first $\sim$ 550 ms afterwards. In both protocols, the second stimulus was followed by a 2.5 s pause. During Sham condition, corresponding stimulation time points were recorded but no auditory stimuli presented.

negative peak and the following positive peak. This time (on average 508.2 ± 18.3 ms across subjects) was used to individually adapt the stimulation such that the auditory stimuli were most likely to occur in phase with the SO up-states. Upon detection of an SO, the first stimulus was delivered after the subject's individual delay time. The second stimulus then followed after a fixed interval of 1,075 ms. Thereafter, stimulation was discontinued for 2.5 s (Figure 4.2*A*). Stimulation focused on inducing only two succeeding SO cycles based on previous observations (Mölle *et al.*, 2011), indicating that spontaneous SOs typically occur in trains of two to three SO cycles. The detection algorithm was applied throughout the stimulation period of 210-min but halted whenever a subject left Non-REM sleep stage 2 or SWS or arousals occurred. Triggers were sent to the EEG-System to mark the starting points of the first and second stimulus in the EEG

for later analysis. During Sham conditions, SO detection was performed in the same way, and the respective time points were marked in the EEG, but no auditory stimuli were delivered. SO detection, auditory stimulation, and presentation of triggers to the EEG recording system were all controlled by the Power 1401 system and required a constant time interval of 2.4 ms, i.e. the equipment enabled a precise timing of stimulus delivery.

4.2.2 Out-of-Phase Auditory Stimulation in Control Experiments

To evaluate to what extent changes in SO activity and memory performance depended on the phase presentation of auditory stimuli within a SO cycle, an initial control study was conducted (4 women, age: 22.0 ± 0.4 years) that aimed at a disruption of the SO rhythm by merely shifting the auditory stimuli within the SO cycle while the ISI (of 1,075 ms) was kept constant. However, changing only the phase of the stimulation from the up-state to the down-state, by presenting the first stimulus immediately upon a SO detection, did not effectively suppress SO activity. As shown in Figure A.3 for a representative subject, in this case the first stimulus delayed emergence of the succeeding depolarization such that the second stimulus coincided already with this emergent depolarization, thereby promoting, rather than disrupting, a further SO.

Against this backdrop, a second control study was performed, in which the interval between both auditory stimuli was additionally changed such that it did not correspond to the natural SO period length (Figure 4.2*B*). This Out-of-phase stimulation was generated by reducing the ISI to about half of the SO period (in addition to presenting the first stimulus immediately upon detection of the negative half-wave peak). The exact length of the ISI was individually fine-tuned to the timing of the hyperpolarization phase after the first click, based on an on-line assessment of the response to the first five stimulation trials. Across subjects, the interval averaged 550.0 ± 19.7 ms. With this Out-of-phase stimulation, the second stimulus indeed tended to coincide with the emerging hyperpolarization which effectively suppressed the development of SO trains. Identical to the In-phase condition, the second auditory stimulus was followed by a 2.5-s pause until the next detection was allowed.

4.3 Results

4.3.1 Auditory In-Phase Stimulation Induces SO Activity and Enhances Memory Consolidation

For the first group tested under In-phase stimulation, averaging the EEG time-locked to the first auditory stimulus revealed a clear increase in slow oscillatory activity, in comparison with the corresponding Sham condition (Figure 4.3*A*). Whereas in the Sham condition an individual SO cycle occurred, the two auditory stimuli in phase with the predicted SO up-states formed a sequence of three succeeding SO cycles. This suggests a resonating response of the network induced by the In-phase stimulation. The decrease in SO amplitude across these trains might reflect that the second auditory stimulus did not always hit the optimal SO up state phase (due to jitter in the SO rhythm) or some kind of network refractoriness.

Spectral analysis performed on SWS epochs during the stimulation period showed that In-phase stimulation increased power of SO band ($0.5 - 1$ Hz, $F_{1,10} = 20.4$, $P < 0.001$, Figure 4.4), i.e. in the frequency band matching the 1,075 ms interval between the two auditory stimuli. Of note, whereas examination of the entire slow-wave range ($0.5 - 4$ Hz) revealed no significant difference ($F_{1,10} = 0.04$, $P = 0.849$), power in the neighbouring delta band ($1 - 4Hz$) was concurrently decreased ($F_{1,10} = 8.8$, $P =$

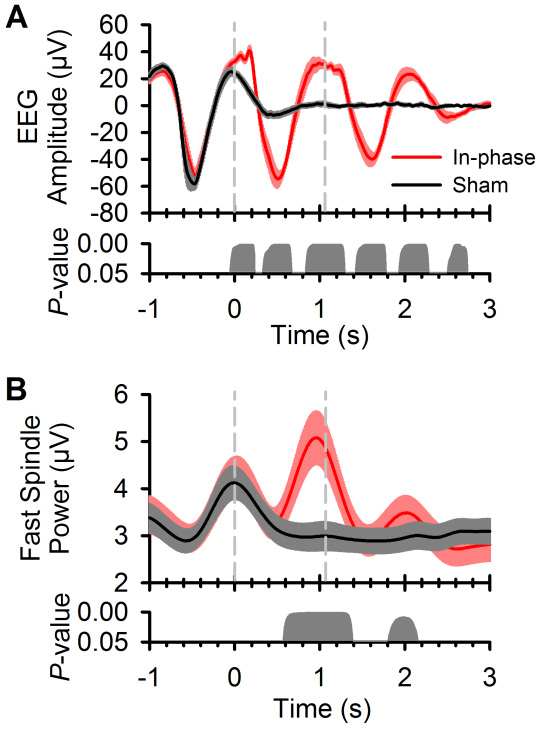

Figure 4.3 – Closed-Loop Auditory Stimulation In-Phase with SO Up States Induces Trains of SOs.

Averaged EEG signal of (*A*) the conventional EEG range ($0.3 - 30$ Hz) and (*B*) fast spindle power (at Cz) time-locked to the first auditory stimulus ($t = 0$ s) for the In-phase stimulation (red line) and Sham (black) conditions. Bottom panel indicates significant differences between conditions and vertical dashed lines mark first and second auditory stimuli.

Note that the criterion electrode site used for on-line detection of negative half-wave peaks was AFz, which explains that depicted negative half-wave amplitudes, which were recorded from more posterior sites, are smaller than the -80 μV threshold used for on-line detection.

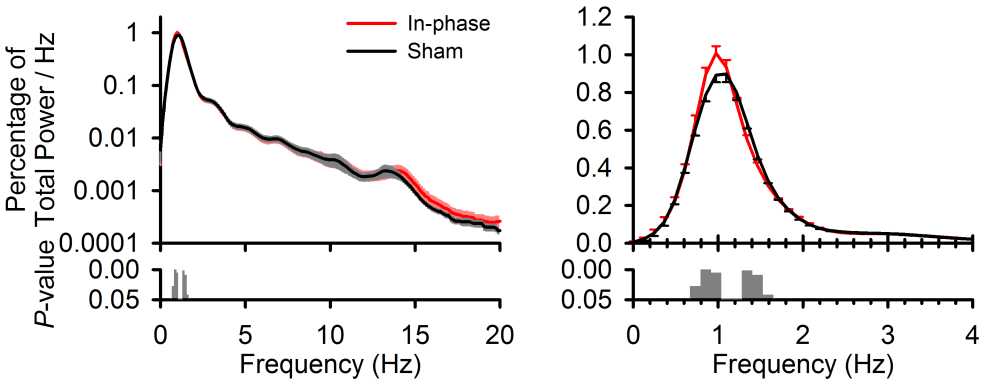

Figure 4.4 – Auditory In-phase Stimulation enhances Slow Oscillation Activity. Spectral power during In-phase stimulation (across all EEG channels) determined for the SWS epochs of the 210-min stimulation period for Stimulation (red line) and Sham conditions (black) up to 20 Hz (*left*) and separately for frequencies up to 4 Hz (*right*). Bottom panels indicate significance between the effects of Stimulation and Sham conditions. Note logarithmic scaling of y-axis in (*left*).

0.014). Together these findings suggest that In-phase stimulation entrained ongoing slow rhythms to the external frequency. Additional analyses on slow spindles ($9-12$ Hz), fast spindles ($12-15$ Hz) and beta band ($18-30$ Hz) did not reveal any significant difference between Stimulation and Sham conditions ($P > 0.331$, Figure). Furthermore, spectral analyses performed in the same way for the remaining sleep period following the 210-min period of stimulation showed no significant difference in power between Stimulation and Sham condition ($P > 0.364$).

To assess effects of stimulation on overnight memory consolidation, subjects performed the previously introduced paired-associates learning task before sleep. Generally, the subjects recalled more paired associates after sleep than at the immediate testing before sleep, which enabled re-encoding as feedback of the correct response word was provided. Strikingly, in the In-phase stimulation condition, the retention rate, defined by the difference in recall performance after sleep minus immediate recall performance before sleep, was distinctly higher than in the Sham condition (22.2 ± 2.3 vs. 13.0 ± 2.5 words, $P < 0.001$, paired t-test, Figure 4.5A). In both In-phase and Sham conditions, overnight retention of word pairs was positively correlated with the percentage of SWS during the stimulation period, which was significant for the Stimulation condition ($r = 0.68$, $P = 0.022$) but not for the Sham condition ($r = 0.50$, $P = 0.115$, Figure 4.5B). Overall, this pattern suggests that entraining slow-wave rhythms during SWS to the SO frequency through external stimulation is critical for the retention of the word-pair memories.

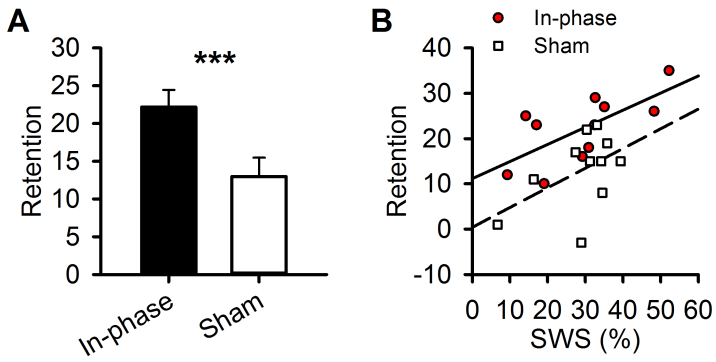

Figure 4.5 – Auditory In-phase Stimulation enhances Declarative Memory.
(*A*) Retention of word pairs across sleep for In-phase (black bar) and Sham conditions (empty bar); $^{***}P < 0.001$. (*B*) Correlation between retention of word pairs and SWS percentage during the stimulation period separately for In-phase stimulation (red circles, solid line, $r = 0.68$, $P = 0.022$) and Sham (empty squares, dashed line, $r = 0.50$, not significant) conditions.

4.3.2 In-Phase Stimulation Modulates SOs and Phase-Locked Spindle Activity

In a more fine-grained analysis of oscillatory activity, off-line SO events were identified during non-REM sleep (Table 4.1 summarizes SO characteristics). Subsequent averaging limited to the stimulation period revealed that In-phase stimulation increased both the amplitude of the hyperpolarization down-phase and that of the surrounding depolarization up-phases of the SO ($F_{1,10} = 23.1$, $P = 0.004$ and $F_{1,10} > 13.4$, $P < 0.004$, respectively, Figure 4.6*A*).

Event correlation histograms of SOs, with reference to the auditory stimulation, confirmed that the stimulation was indeed capable of inducing trains of SOs, as indicated by an increased probability that one or two SO cycles followed the endogenous SO used for triggering the stimulation ($P < 0.001$, compared with the Sham condition, paired *t*-test; Figure 4.6*B*). Also, probability for a third SO cycle was significantly elevated ($P = 0.021$, paired *t*-test), pointing out that the auditory stimulation elicits a damped oscillation. Interestingly, the increased occurrence of SO trains after stimulation did not translate into an overall increased number of identified SOs in SWS (Table 4.1). In fact, there were no significant differences in sleep architecture between the Stimulation and Sham conditions for the stimulation period (Table 4.2), underlining that the In-phase stimulation chiefly entrained SO activity, leaving the processes initializing SOs unaffected. Solely, increased S1 was observed towards the end of In-phase stimulation nights after stimulation had stopped ($P = 0.014$), possibly reflecting a greater reduction

Table 4.1 – Properties of Slow Oscillation Cycles during In-phase Auditory Stimulation

	In-phase	Sham	*P*-value
Number of SO cycles (Stimulation period)	781.3 ± 89.7	801.7 ± 89.8	0.839
Number of SO cycles (Entire night)	1070.9 ± 118.7	983.6 ± 106.1	0.333
SO amplitude (neg to pos. peak, μV)	133.6 ± 11.4	122.6 ± 10.2	0.009
Slope (μV/s)	351.0 ± 33.3	322.3 ± 28.9	0.008
Duration (s)	0.98 ± 0.01	0.97 ± 0.01	0.086

Number of SO cycles (off-line) identified in SWS epochs during the 210 min stimulation period and during the entire night, their negative-to-positive peak amplitude, slope, and duration (see Figure 2.4) during the stimulation period for the In-phase auditory stimulation and Sham condition. *P*-values are indicated for pairwise comparisons between conditions.

in sleep propensity in this condition.

In parallel, stimulation boosted phase-locked spindle activity during the SO cycle, i.e. the increase in fast-spindle activity ($12 - 15$ Hz) typically accompanying the SO up-phase, and the increase in slow-spindle activity ($9 - 12$ Hz) occurring at the up-to-down transition of the SO cycle (Figure 4.6*C*, see Figure 4.3*B* for the evolving of spindle activity across the induced SO train). Remarkably, this significant increase in fast-spindle activity (In-phase: 1.3 ± 0.2 μV, Sham: 1.0 ± 0.1 μV, $P = 0.013$, for the peak RMS amplitude derived during the up-state after the negative half-wave) revealed for both the Stimulation ($r = 0.79$, $P = 0.004$) and Sham ($r = 0.69$, $P = 0.018$) conditions a strong positive correlation with the overnight retention of word pairs (Figure 4.6*D*). As spindle power averaged across SWS of the entire stimulation period did not differ between conditions (Figure 4.4), these observations indicate that In-phase stimulation chiefly enhanced the synchronization of spindles to the SO and corroborates findings showing that the synchrony with the SO cycle rather than the amount of fast-spindle activity is critical for memory consolidation.

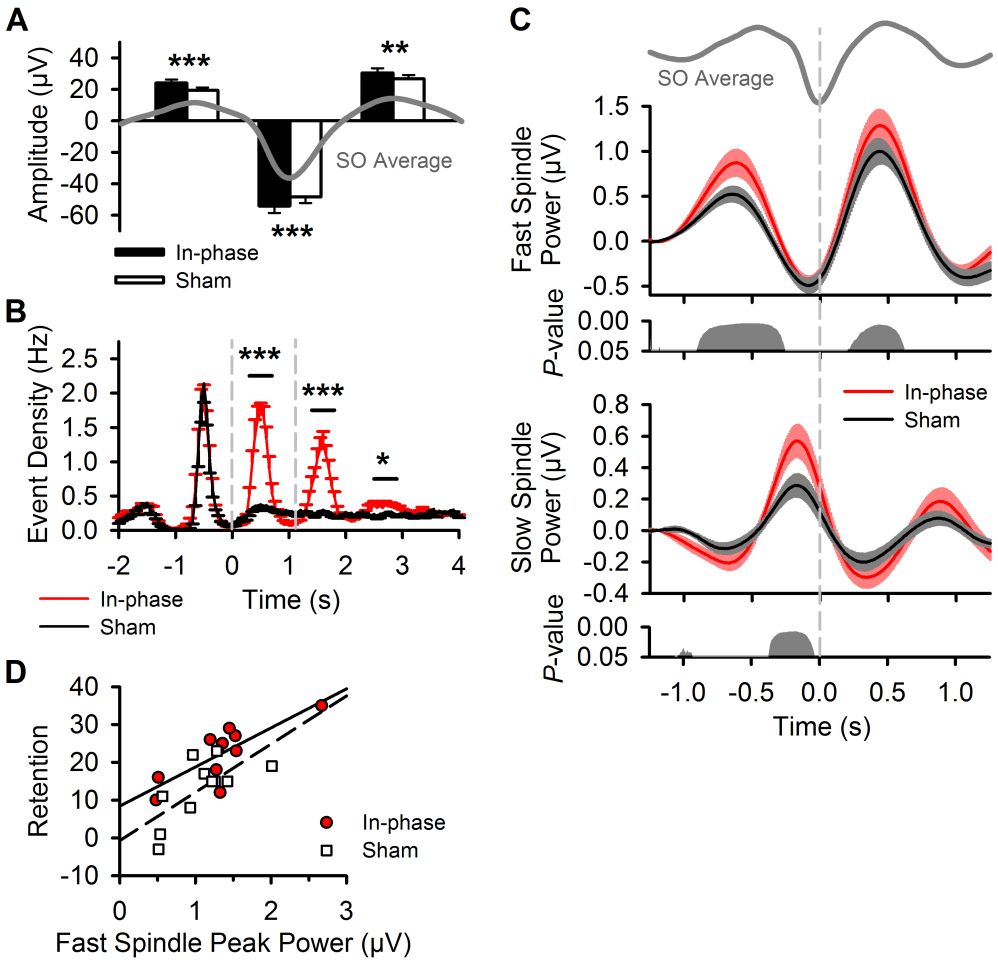

Figure 4.6 – In-phase Auditory Stimulation Enhances SOs and Spindle Activity. (*A*) Amplitude (across all EEG channels) determined for two succeeding up-states and the intermittent down-state of off-line detected SOs during SWS and the stimulation period for In-phase (black bars) and Sham (empty bars) conditions; the gray line illustrates an average potential curve. (*B*) Event-correlation of SO events during SWS for an interval between -2 s and +4 s around the first auditory stimulus ($t = 0$ s) for In-phase stimulation (red line) and Sham (black line) conditions. The vertical lines indicate auditory stimuli. (*C*) Fast- (*top*) and slow- (*bottom*) spindle band RMS activity at Cz averaged time-locked to the negative half-wave peak of all off-line detected SO cycles (indicated by the vertical dashed line) during SWS epochs of within the stimulation period for both Stimulation (red line) and Sham (black line) conditions. The grey waveform on top again illustrates an average potential curve. Black horizontal lines mark 250-ms intervals where spindle power differed significantly. (*D*) Correlation between retention of word pairs and fast-spindle RMS amplitude of the peak following the negative SO peak in (*C*) for the Stimulation (red circles, solid line, $r = 0.79$, $P = 0.004$) and Sham (empty squares, dashed line, $r = 0.69$, $P = 0.018$) conditions. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, for comparisons between Stimulation and Sham conditions.

Table 4.2 – Sleep Stage Distribution for Different Time Intervals and Conditions

		In-phase		Out-of-phase	
	Parameter	Stim	Sham	Stim	Sham
210-min Stimulation	W (%)	6.3 ± 1.6	7.3 ± 1.1	5.9 ± 1.5	8.4 ± 1.7
	S1 (%)	8.2 ± 1.0	7.5 ± 1.4	3.5 ± 0.8	5.7 ± 1.9
	S2 (%)	48.6 ± 2.7	47.3 ± 2.2	44.9 ± 5.8	46.8 ± 4.2
	SWS (%)	29.1 ± 4.1	28.7 ± 2.8	39.3 ± 7.0	30.8 ± 5.2
	REM (%)	7.7 ± 1.6	9.2 ± 1.8	6.4 ± 1.8	8.3 ± 2.2
	Arousal (%)	6.7 ± 0.4	6.7 ± 1.0	5.2 ± 0.7	5.3 ± 0.8
Entire night	W (%)	5.8 ± 1.2	5.4 ± 0.9	3.3 ± 0.8	4.7 ± 0.9
	S1 (%)	$\underline{9.6 \pm 1.0}$	$\underline{7.0 \pm 0.8}$	3.7 ± 0.8	4.6 ± 1.3
	S2 (%)	49.0 ± 2.6	50.3 ± 1.7	53.9 ± 4.0	53.3 ± 3.1
	SWS (%)	20.1 ± 2.4	19.0 ± 2.0	23.2 ± 3.6	21.3 ± 2.8
	REM (%)	15.5 ± 1.2	18.3 ± 1.8	15.9 ± 0.9	16.0 ± 1.2
	Arousal (%)	7.9 ± 0.6	6.7 ± 0.5	6.4 ± 0.9	5.6 ± 0.6
	TST (min)	413.5 ± 6.6	417.6 ± 2.8	420.4 ± 8.2	434.5 ± 7.3

Percentage of time spent in different sleep stages during the 210-min stimulation period and the entire night for auditory stimulation in phase with SO up-states and respective Sham condition (In-phase) and for the control study with auditory stimulation out of phase with predicted SOs and respective Sham condition (Out-of-phase). A significant difference ($P < 0.05$) for S1 during In-phase stimulation across the entire night is indicated by underlined values.
W, wake; S1 and S2, non-REM sleep stages 1 and 2; SWS, slow-wave sleep; REM, rapid eye movement sleep; TST, total sleep time.

4.3.3 Induced SOs Do Not Differ from Spontaneous Slow Oscillations

Does the induced SO represent a true SO? To answer this question, the topography, slope, and travelling of SOs during In-phase stimulation were more closely examined. For this purpose, an identification of SOs was additionally performed within each individual EEG channel, applying basically the same algorithm as described above to the filtered ($0.3-3.5$ Hz) EEG signal (rather than to a virtual channel). To compare the topography of SOs between the Stimulation and Sham conditions, we normalized negative SO half-wave peak amplitudes for each subject by dividing amplitude values in each channel by the absolute value of the mean across all channels (Landsness *et al.*, 2009). The slope of individual SO cycles was determined in the down-to-up state transition as the ratio between the absolute value of the negative half-wave peak and the time delay to the next zero crossing (Riedner *et al.*, 2007). For analyses of SO travelling, single SO cycles

were assessed using the timing of the negative half-wave peak in each EEG channel with respect to the channel revealing the earliest peak, with the latter considered to reflect the origin of the travelling SO (time $t = 0$). Topographical maps of SO origins and their delays were generated with regard to the SO negative half-wave peaks (subdivided into SOs emerging in frontal, central, and parietal areas) by third-order spherical spline interpolation (Massimini *et al.*, 2004). Finally, the path length of SO travelling was evaluated in an undirected network constructed by connecting neighboring electrode positions, i.e., for each SO cycle and beginning with the SO origin, the neighboring nodes with the smallest (forward) delay were iteratively selected until a break in the gradient occurred. The path length refers to the number of traversed nodes in each SO

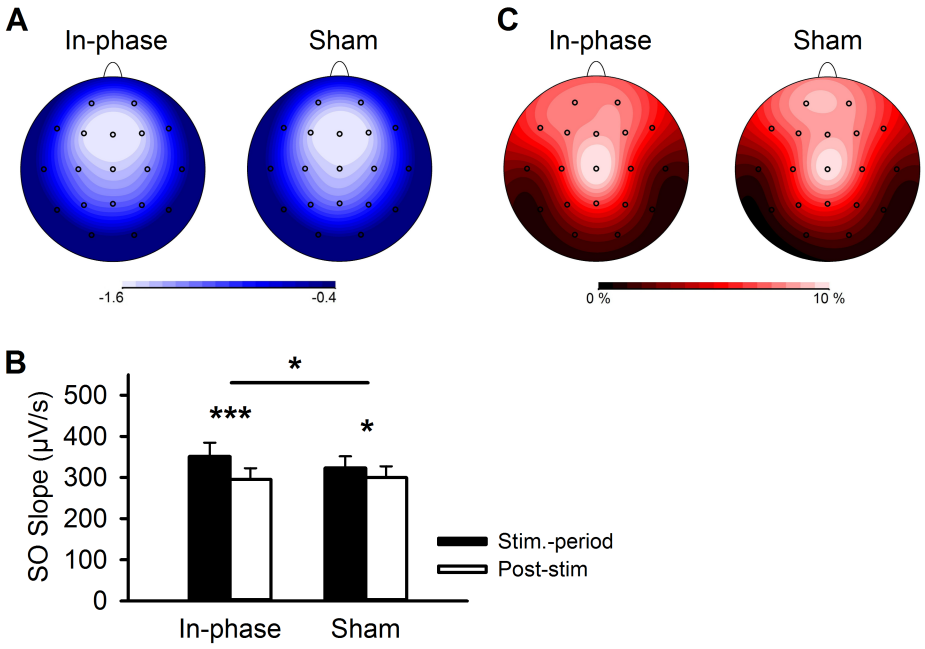

Figure 4.7 – Induced and Spontaneous SOs Exhibit Identical Topography, Downscaling, and Travelling Characteristics.

(*A*) Topography of off-line detected SOs for the In-phase stimulation (*left*) and Sham (*right*) conditions. The topographical distribution was determined with respect to the negative half-wave peak amplitude for all SOs detected during the stimulation period. Color code depicts the peak amplitude averaged across all subjects. Before averaging, individual amplitude values were normalized by the subject's absolute mean across all EEG channels for the respective condition. (*B*) Downscaling of SO slope of off-line detected SOs averaged across all EEG channels for the 210 min stimulation period (Stim.-period, black bars) and for the remaining sleep period during the later half of the night (Post-stim, empty bars) for the In-phase stimulation and Sham conditions. $n = 10$, data from one subject were discarded because he did not enter SWS during the late-night half. $^{*}P < 0.05$, $^{***}P < 0.001$, for pairwise comparisons. (*C*) Topographical distribution of SO origins for the In-phase stimulation (*left*) and Sham (*right*) conditions, expressed (color-coded) as percentage of all travelling SOs detected during the 210-min stimulation period. Maps indicate means across all subjects.

cycle. For statistical comparisons, SOs were categorized into 'local' SOs with a path length equal to zero and 'travelling' SOs showing a path length greater than zero.

In both Stimulation and Sham conditions, the SO showed the typical maximum over fronto-central cortical regions (Figure 4.7*A*). Analyses of variance indicated a significant effect of topography ($F_{1,18} = 193.6$, $P < 0.001$) but no difference between Stimulation and Sham conditions in the topographical distribution ($F_{18,180} = 1.294$, $P > 0.295$, for Condition × Topography interaction). Also, the decrease in SO slope across sleep time, which is considered to reflect a global downscaling of cortical synapses, reached comparable values in the late-night half (Stimulation: $F_{1,9} = 31.2$, $P < 0.001$, Sham: $F_{1,9} = 6.2$, $P = 0.034$, Figure 4.7*B*). However, In-phase auditory stimulation acutely increased the SO slope so that the overnight decrease was greater than in the Sham condition ($F_{1,9} = 6.8$, $P = 0.028$, for Condition × Night period interaction).

Analyses of SO travelling revealed in both conditions a preferential origin of SOs from anterior cortical sites (Figure 4.7*C*) and, depending on the point of origin, i.e. frontal, central, or parietal, a travelling mainly to anterior or posterior areas, respectively (Figure 4.8*A*). There were no differences in topographical or temporal features of travelling between In-phase and Sham conditions ($F_{18,180} < 1.3$, $P > 0.300$, for all Condition × Topography interactions). Interestingly, an analysis of travelled path lengths indicated a reduced number of SOs that stayed local but more SOs travelling longer paths in the Stimulation condition ($P = 0.011$ for solitary and travelling SOs, respectively, paired *t*-test; Figure 4.8*B*) (Nir *et al.*, 2011). Additional analyses revealed a significant negative correlation between travelling speed (assessed by the average delay in SO negative peak latency along the travelled path) and overnight retention of word pairs specifically for induced SO cycles originating at central sites and spreading towards anterior sites (Figure 4.8*C*). In combination, these analyses showed that In-phase auditory stimulation acutely amplifies SOs in terms of amplitude, slope, and spreading. However, the basic SO features of topography, morphology, and temporal dynamics remained essentially the same as under non-stimulated conditions.

4.3.4 Out-of-Phase Stimulation Disrupts SO Activity and Does Not Affect Memory Consolidation

Is phase locking of the stimulation to the rhythm of SO up-states crucial for the enhancing effect on SO activity? To test this, the stimulation protocol was modified. Not

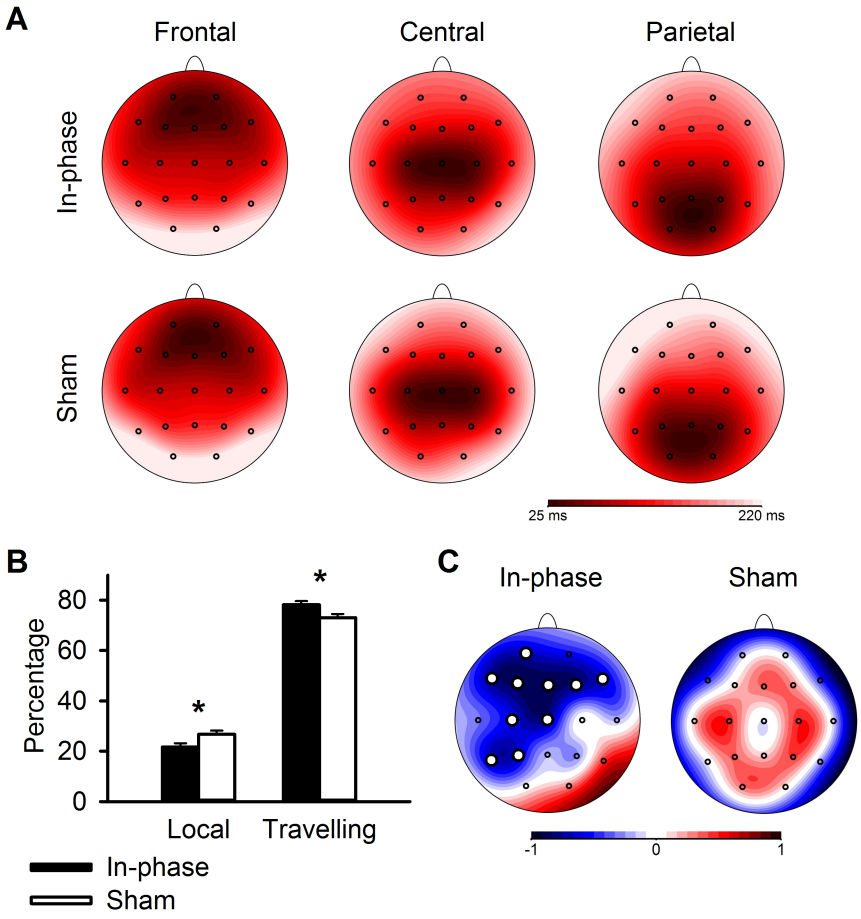

Figure 4.8 – In-phase stimulation prolongs travelling distance of SOs.
(*A*) Delay maps for In-phase stimulation (*top row*) and Sham conditions (*bottom*) depicting the propagation (in ms, colour coded) of the negative SO half-wave peak over the cortex. SO propagation is shown separately for SOs originating at frontal (*left*), central (*middle*) and parietal (*right*) sites. (*B*) Percentage (of total number) of detected SOs staying local or travelling to at least one neighboring recording site for the In-phase Stimulation (black bars) and Sham condition (empty bars). $^{**}P < 0.05$, for comparison between Stimulation and Sham condition). (*C*) Topographic distribution of color-coded Pearson coefficients for correlation between travelling speed of SO originating from central sites and overnight retention of word pairs. White circles indicate correlations significant at $P < 0.05$ (post-hoc t-Test, not corrected for multiple testing).

surprisingly, maintaining the ISI of 1,075 ms but merely shifting the auditory stimulation forward into the down state did not effectively disrupt endogenous SO activity. The first stimulus delayed occurrence of the succeeding SO cycle such that the second stimulus tended to fall into the depolarizing phase of an SO, thereby promoting rather than suppressing consecutive SOs (Figure A.3). However, additionally reducing the ISI to about half of the SO period effectively interfered with the development of SO trains (Figure 4.9*A*). With this protocol, the second stimulus tended to coincide with the hyperpolarization induced by the first stimulus. Indeed, spectral analysis confirmed

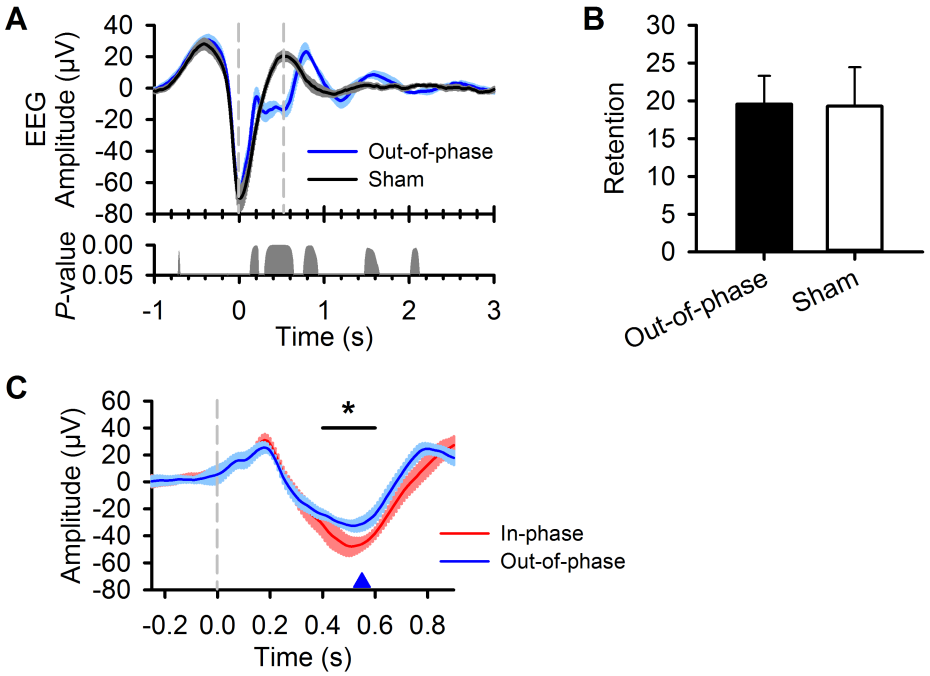

Figure 4.9 – Stimulation Out of Phase with SO Up-State Disrupts SO Activity and Does Not Improve Memory Consolidation.

(A) EEG signal (at Cz) averaged in a 4 s interval time-locked to the first auditory stimulus (t = 0 s) for Out-of-phase stimulation (blue line) and Sham (black line) conditions. Bottom panel indicates significant differences between conditions. (B) Overnight retention of word pairs for Out-of-phase stimulation and Sham conditions ($P = 0.93$). (C) Evoked potential response (at Cz) for In-phase (red, $n = 11$) and Out-of-phase (blue, $n = 7$) stimulation derived by subtracting the average response of the Sham condition from that of the Stimulation condition. Vertical dashed line, onset of the first stimulus. Note that occurrence of second stimulus during Out-of-phase stimulation at 550 ms (blue triangle) could confound the evoked response in this condition during later portions. $^*P < 0.05$ for difference in amplitude between In-phase and Out-of-phase stimulation.

a decrease in power for frequencies < 1 Hz during intervals of acute Out-of-phase stimulation ($F_{1,6} = 14.6$, $P = 0.009$, Figure 4.10A). However, power in this frequency band recovered normal values as soon as acute stimulation was discontinued ($F_{1,6} = 38.5$, $P < 0.001$, for Condition × Acute/Discont interaction, Figure 4.10B), reflecting the strong drive of cortical networks to oscillate in the natural SO frequency. Accordingly, power in the < 1 Hz band did not differ between conditions for the entire time in SWS during the stimulation period ($F_{1,6} = 3.0$, $P = 0.136$, Figure 4.10 A and B).

Importantly, in the absence of any SO enhancement, Out-of-phase stimulation, unlike In-phase stimulation, did not produce any improvement in retention of word pairs ($p = 0.925$, paired t-test; Figure 4.9C). These data underline that phase synchrony of the stimulation to the SO up-state is essential for the enhancement of memory consolidation.

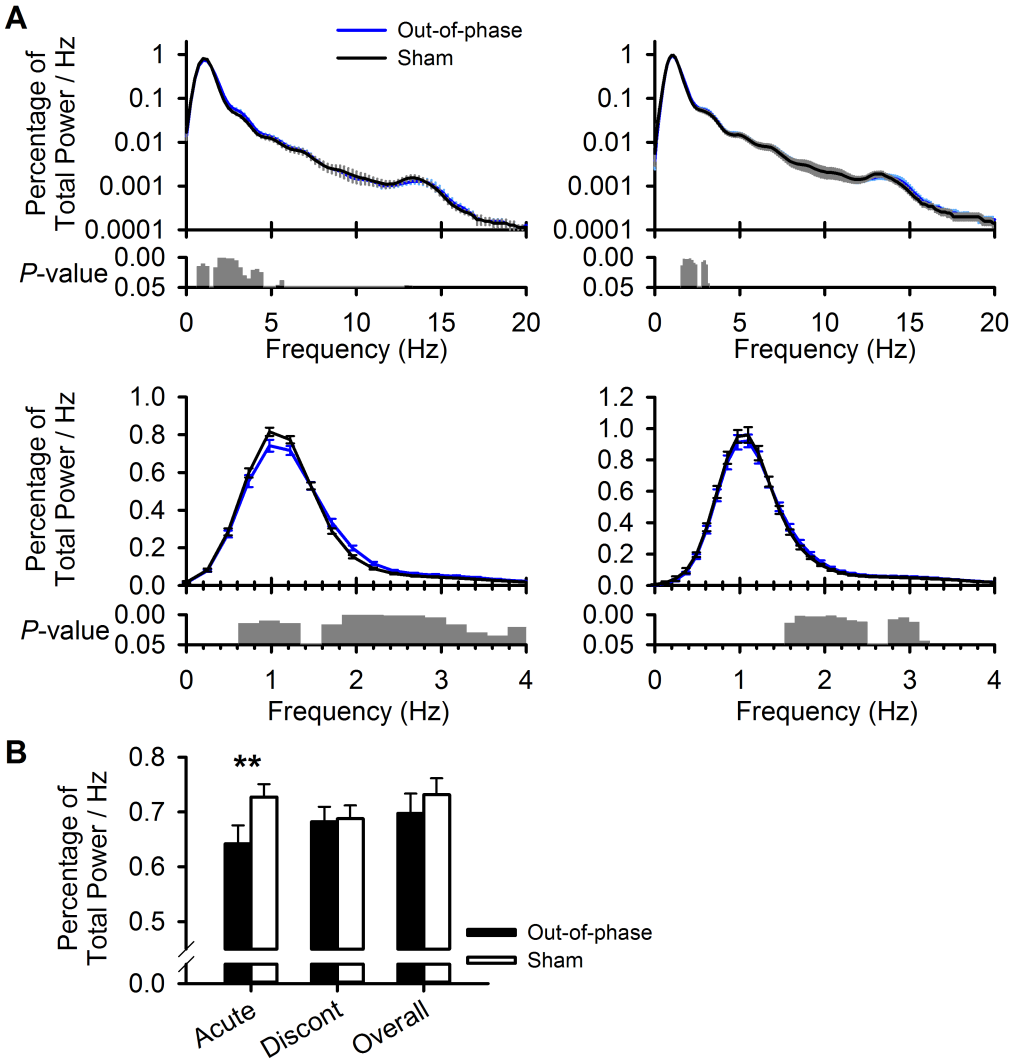

Figure 4.10 – Out-of-phase stimulation acutely disrupts slow oscillation activity.
(*A*) Spectral power during Out-of-phase stimulation (across all EEG-channels) determined for (left) acute epochs of stimulation and (right) for all SWS epochs of the 210-min stimulation period for the Stimulation (blue line) and Sham condition (black). Power is shown separately for the ranges up to 20 Hz (*top*, y-axis log-scaled) and up to 4 Hz (*bottom*). Bottom panels indicate significant differences between the effects of Stimulation and Sham condition. (*B*) Average power for the < 1 Hz SO frequency band (across all EEG-channels) during the stimulation period, separately for intervals of acute stimulation (*left*) and for succeeding intervals where stimulation was discontinued (*middle*, Discont) and across the entire 210-min stimulation period (*right*, Overall) for the Stimulation (black bars) and Sham condition s(empty bars). $^*P < 0.05$, for pairwise comparison between Stimulation and Sham conditions.

4.3.5 Separation of Auditory-Evoked Responses from Spontaneous Ongoing SO Activity

To separate the potential response evoked by the auditory stimulus during both In-phase stimulation and Out-of-phase stimulation from ongoing spontaneous SO activity, the EEG average time-locked to the onset of the first stimulus for the Sham condition was subtracted from that obtained for the Stimulation condition. The evoked potential responses revealed this way comprised an early positive component with two overlapping peaks at about 75 and 180 ms post-stimulus onset, followed by a pronounced negative component with a maximum at approximately 500 ms (Figure 4.9*D*). Early components did not differ between stimulation conditions, arguing against the view that SO down and up states are linked to a gating of ascending information, although the scalp-recorded EEG might not be sufficiently sensitive to reveal such effects (Rosanova & Timofeev, 2005). Amplitude of the late negative component was slightly smaller for the Out-of-phase than In-phase stimulation ($P = 0.019$ for comparison at Cz), which probably reflects reduced neocortical excitability when the stimulus is presented during the SO down-state (Bergmann *et al.*, 2012). Latency of these components did not differ between In-phase and Out-of-phase stimulation ($P > 0.347$). Note that whether and to what extent the auditory-evoked response revealed by the subtraction reflects potential activity elicited by the stimulus itself or changes to ongoing oscillations cannot be discriminated.

4.4 Summary

Altogether the findings of this experiment indicate that an enhancement of SO activity and overnight memory consolidation by auditory stimulation depends essentially on the timing within the SO cycle. Utilizing a closed-loop control, the stimulation was fine-tuned to the endogenous SO rhythm, which if applied in synchrony with SO up-phases induced a resonant slow oscillatory activity paralleled by an increase in phase-locked fast spindle activity and a benefit in memory consolidation. In contrast, stimulation out-of-phase targeting a disruption only revealed a brief impact on suppressing slow oscillatory activity and therefore resulted in no significant changes in memory performance.

Chapter 5

Experiment III: Overdriving of Sleep Slow Oscillations by Auditory Closed-loop Stimulation - A Self-limiting Process?

5.1 Introduction

The previous experiment demonstrated that closed-loop stimulation is particularly effective in producing selective increases in SO activity and promote its functional role in memory consolidation. Because the on-line detection of SOs paused for 2.5 seconds after the presentation of the two clicks and then restarted, the protocol stimulated only shorter trains of SOs and was not apt to explore possible limits of SO responsiveness to stimulation.

Intriguingly, the same neural networks that oscillate during sleep can generate aberrant activity associated with diseases, e.g. schizophrenia or Alzheimer's (Herrmann & Demiralp, 2005). Beyond benefiting cognitive brain function, studies have indicated that SO up-states represent phases of increased synchrony and reduced inhibitory signalling, which has been shown to favor epileptiform activity during SWS both in hippocampal and neocortical networks (McCormick & Contreras, 2001, Nazer & Dickson, 2009, de Guzman *et al.*, 2010). Conversely, these studies show that in the healthy brain mechanisms are at work during SO activity which counter network processes of hypersychronization and disinhibition and thereby prevent the emergence of seizure-like activity,

although the nature of such mechanisms needs to be clarified.

For this purpose, a final experiment was conducted to test the limits of the auditory closed-loop stimulation approach. With a new pressing stimulation, in which auditory stimuli were basically presented as long as an SO-train was on-going, the goal was to overdrive an closed-loop stimulation of SOs to unravel possible mechanism which counteract such an induced hypersynchrony and thereby limit the enhancing effects of such a stimulation.

In this chapter, I will present two complementary studies comparing on the one hand this Pressing stimulation to a non-stimulated Sham control (Study I, $n = 16$ (7 women), age: 24.0 ± 0.7 years), in order to examine the overall efficacy and validate the findings from the previous experiment. In a second study (Study II, $n = 14$ (6 women), age: 24.3 ± 0.7 years) on the other hand, participants were tested under both the Pressing and In-phase stimulation from Experiment II (in the following referred to as "2-Click") to allow a direct comparison of both stimulation approaches and reveal potential constraints for an optimization of a closed-loop approach.

5.2 Stimulation Protocol: The Pressing Stimulation

All stimulation protocols relied on the on-line detection of SO down-states as described in the previous chapter. In the new Pressing stimulation protocol after the initial SO detection and click presentation, further stimuli were applied whenever the EEG signal again met the threshold criterion within a 1-s time window starting with the last stimulus presentation. Furthermore, the threshold value was decreased by 20% (with respect to the preceding threshold value) with each stimulus cycle, to alleviate repetitive stimulation patterns (Figure 5.1). The 20% reduction rule was implemented based on observations indicating that, in the absence of stimulation, the negative peak of SOs gradually diminishes within trains of several succeeding SOs. In the 2-Click protocol, stimulus presentation remained identical to In-phase stimulation in Exp. II, i.e. the second click followed after a fixed interval of 1.075 s. In all stimulation protocols the detection algorithm paused for 2.5 s after the last click was applied. Exact timing of auditory stimuli was marked in the EEG for later off-line analysis. In the Sham control condition of Study I, detections of SOs and marking of the EEG were performed as in the Pressing stimulation condition of this study but with no stimuli delivery. Please note, in both studies one subject was discarded due to technical problems (missing stimulation

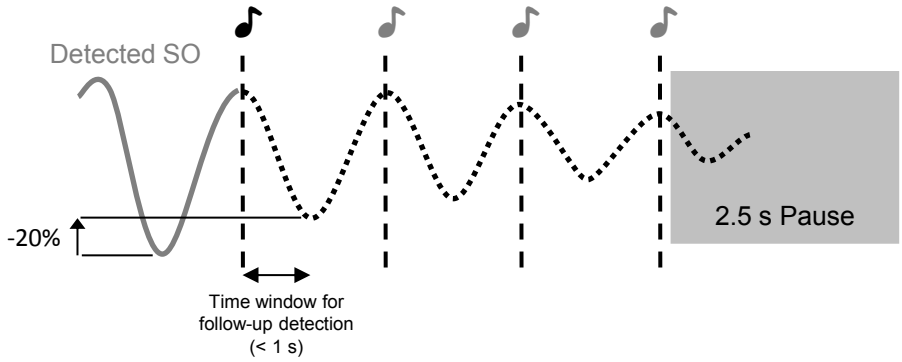

Figure 5.1 – Pressing Stimulation Protocol.
The procedure to detect SO during Pressing stimulation was identical to Experiment II, i.e. the detection was based on the negative half-wave and also included the individual delay adaptation to target the predicted up-state. However, instead of presenting two clicks with a fixed interval, further stimuli were delivered only whenever a follow-up detection occurred within a 1-s time window after the last stimulus. Furthermore, with every detection the threshold value was decreased by 20% based on empirical observations of a gradual decrease in SO negative peak amplitude within a train of several succeeding SO cycles. During Sham conditions of Study I, corresponding time points of stimulation were marked in the EEG without click presentations.

markers). Results obtained from electrophysiological data were therefore based on 15 and 13 participants, respectively.

5.3 Results

5.3.1 Immediate Effects of Closed-loop Stimulation on EEG Activity

In order to compare the EEG response to closed-loop stimulation during SWS between the conditions, stimulus trains in both conditions were first categorized according to the number of presented clicks presented in a train (corresponding to the number of successively identified SO negative half-wave peaks), i.e. whether stimulation occurred as a single click or in a train of 2, 3 or 4 stimuli (Figure 5.2A). Then, time intervals between clicks were normalized to enable comparisons of whole stimulation-trains between conditions. The resulting EEG signal was then averaged time-locked to the first click of a train.

Compared with spontaneously occurring SOs during Sham, Pressing stimulation prolonged SO trains, i.e. produced a greater number of SO trains with 2 or more succeeding SO events (Figure 5.2*A*). Thus, the number of SO trains with 2, 3 and 4 SO events in a row averaged 118.7±17.8 in the Pressing stimulation condition compared with 79.1±12.3 in the Sham condition ($P = 0.004$). Concurrently the number of singular SO events was

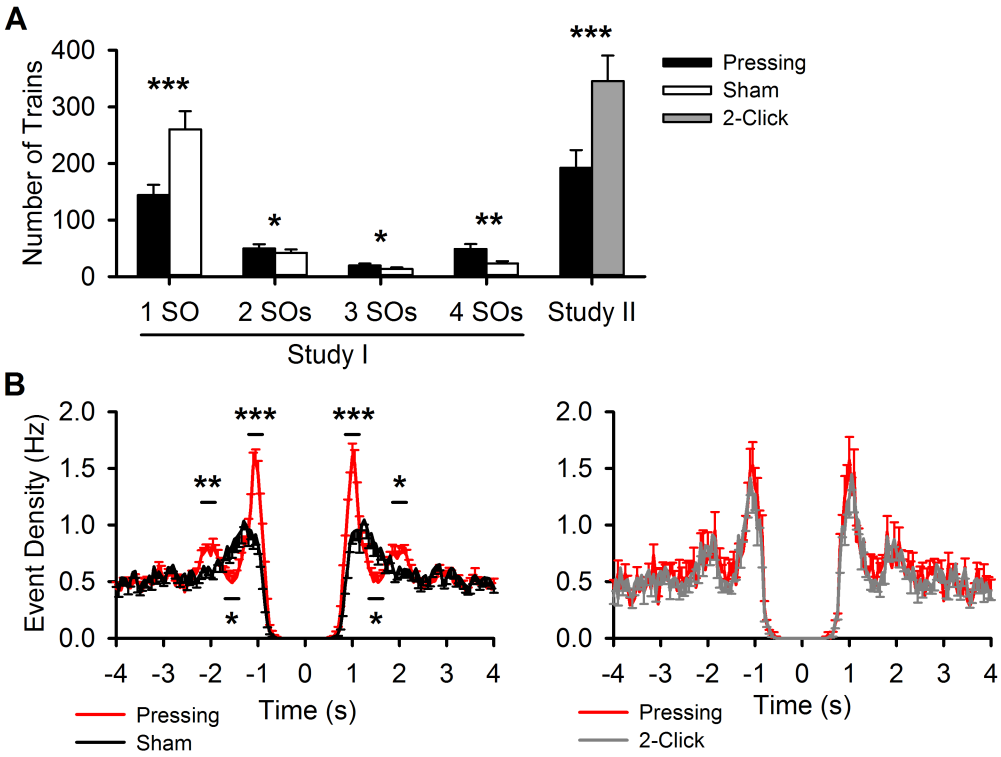

Figure 5.2 – Pressing and 2-Click Stimulation Evoke Sequences of SOs.
(**A**) Number of SO trains of 1, 2, 3 or 4 SO cycles and clicks presented during SWS within the 210-min stimulation interval of Study I (Pressing stimulation vs. Sham) and Study II (Pressing stimulation vs. 2-Click). Results for the 2-Click stimulation protocol are compared with Pressing stimulation for the train length of two SOs,always two clicks were were always presented. (*B*) Auto-event correlations determined from off-line detected SO events for Study I (*left*), Pressing (red line) vs. Sham stimulation (black line) and for Study II (*right*), Pressing (red) vs. 2-Click stimulation (grey). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, for comparisons between conditions.

decreased during Pressing stimulation (144.0 ± 18.5 vs. 260.4 ± 32.0, during Sham, $P < 0.001$). The total number of off-line identified SOs was comparable after Pressing stimulation as compared to the Sham condition (400.9±62.8 vs. 409.4±39.5, $P = 0.885$). The prolonging effect on SO trains of Pressing stimulation was likewise evident when analysing auto-correlations for off-line determined SO events (Figure. 5.2, *left*) which revealed a significant increase of SO events followed by one or two further SO events (i.e., trains of 2 and 3 SOs, $P < 0.021$, compared with Sham). By contrast, in Study II, comparing the effects of Pressing stimulation with the 2-Click protocol, no significant differences were observed for the respective SO event autocorrelations ($P > 0.329$, Figure 5.2, *right*), indicating that both protocols were similarly effective in inducing SO trains, despite the increased number of successively presented clicks (i.e. in a row) in the Pressing stimulation protocol. Accordingly, the overall number of off-line detected SO events was also comparable between the two stimulation conditions (451.5 ± 50.0

vs. 503.1 ± 55.4, $P = 0.372$). Although more stimuli were presented in a train, the total number of clicks presented was higher in the 2-Click protocol with the Pressing stimulation protocol (691.1 ± 90.0 vs. 494.8 ± 66.4, $P = 0.020$) which simply reflects the fact that with the latter protocol presentation of the 2nd click was unconditioned and did not require prior identification of a negatives SO half-wave.

Compared with Sham stimulation, Pressing stimulation also distinctly increased the SO amplitudes (determined by the post-click negative half-wave amplitude). The increase in amplitude was observed regardless of whether SOs occurred singularly or in trains of several succeeding SOs ($F_{1,14} > 6.176$, $P < 0.026$, for all comparisons), except for the last of the 3-clicks trains ($F_{1,14} = 1.81$, $P = 0.20$, Figure 5.3*A*). The pause after a stimulation sequence (until detection of the next spontaneous negative SO half-wave) was generally longer during Pressing stimulation than during Sham stimulation, regardless of the length of the preceding SO train ($F_{1,14} = 6.015$, $P = 0.028$), suggesting that Pressing stimulation goes along with increased refractoriness in the SO generating system.

Comparing the effects of Pressing stimulation specifically for trains of two clicks with 2-Click stimulation in Study II revealed that Pressing stimulation was associated with a higher SO amplitude in response to the first click (-106.2 ± 6.4 μV vs. -46.6 ± 5.4 μV, $F_{1,12} = 85.611$, $P < 0.001$), but with lower SO amplitude in response to the second click (-16.1 ± 2.7 μV vs. -32.1 ± 4.6 μV, $F_{1,12} = 10.563$, $P = 0.005$, Figure 5.3*B*). Also, pauses after stimulation with two clicks during Pressing stimulation were longer than during 2-Click stimulation (10.1 ± 0.4 s vs. 9.4 ± 0.3 s, $P = 0.038$), i.e. an overall pattern suggesting that conditions during Pressing stimulation favour the development of refractoriness in SO generating networks.

5.3.2 Induced Spindle Activity

Averaging root mean square fast spindle activity (12-15 Hz) time-locked to the first clicks of trains during Pressing stimulation revealed a pronounced increase in spindle activity which occurred in-phase with the succeeding SO up-state, and which was highly significant in comparison with the Sham condition (Figure 5.4*A*). Importantly, this phase-locked increase in spindle activity was clearly restricted to the first stimulus presentation independent of the number of clicks delivered in the train ($F_{1,14} > 10.269$, $P < 0.006$).

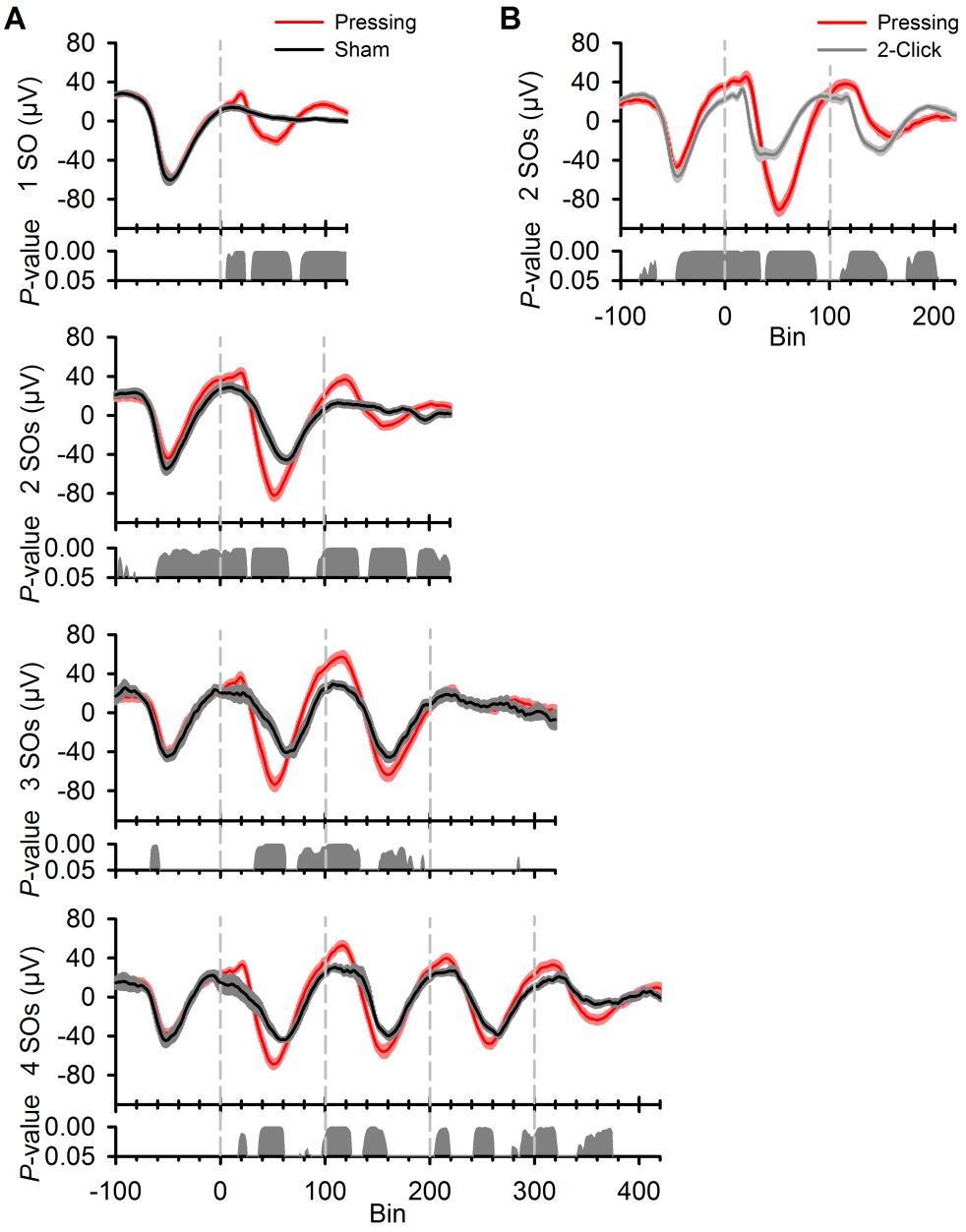

Figure 5.3 – Pressing Stimulation Evokes Trains of SOs.

(*A*) Average EEG signal at Cz for the Pressing stimulation (red) and Sham condition (black line) from Study I time-locked to the first stimulus presentation. Auditory stimulations were categorized by the number of successive detections/stimulations (1 SO, 2 SOs, 3 SOs and 4 SOs) and separately shown on different rows. Bottom panels below each graph indicate significant differences between conditions. Vertical grey dashed lines indicate stimulation time points. Note, intervals between successive auditory stimulation were standardized to 100 bins to account for the temporal jitter caused by the repetitive detection of SOs. (*B*) Corresponding average for Study II comparing Pressing stimulation with 2 successive stimulations (2 SOs, red) with the 2-Click stimulation (grey line).

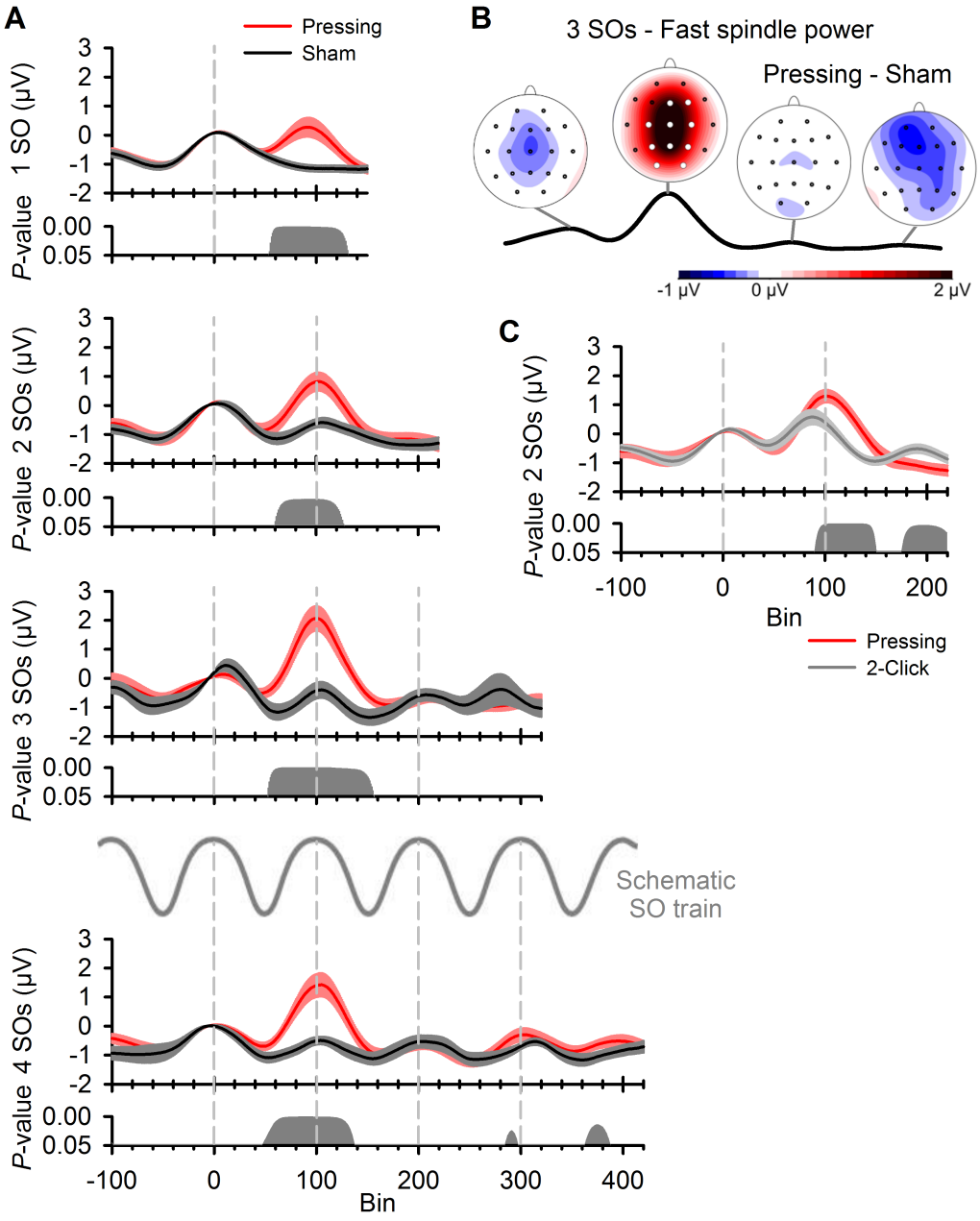

Figure 5.4 – Fading Fast Spindle Response.

(*A*) Fast spindle power (at Cz) corresponding to the EEG signal shown in Figure 5.3*A* time-locked to the first auditory stimulus ($t = 0$) for Study I with Pressing stimulation (red) vs. Sham condition (black). Bottom panels indicate pairwise comparison between conditions. The grey line between results for '3 SOs' and '4 SOs' depicts a schematic SO-train to illustrate stimulation time points (vertical grey dashed lines). (*B*) Topographical distribution of fast spindle power. Difference maps are shown between Pressing Stimulation and Sham condition, exemplified for the case of trains with 3 SO cycles. The black line indicates fast spindle activity for the corresponding train and the vertical lines mark time points of click presentation. Significant ($P < 0.05$, corrected for multiple comparisons) differences between Pressing stimulation and Sham condition at specific electrode locations are indicated by filled white circles. (*C*) Corresponding fast spindle activity for Study II comparing effects of the Pressing stimulation specifically for trains with 2 successive clicks (2 SOs, red line) with 2-Click stimulation (grey line).

Succeeding clicks, i.e., presentation of the 2nd, 3rd or 4th click, remained entirely ineffective ($F_{1,14} < 1.184$, $P > 0.295$). The increase in spindle activity to the first click showed a widespread centro-parietal topography ($F_{18,252} > 6.550$, $P < 0.001$, for Stimulation × Topography, Figure 5.4*B*).

In Study II, basically the same pattern of a strong increase in spindle activity only to the first but not second click was obtained with the 2-Click stimulation protocol (response to 1st click vs. 2nd click: 0.090 ± 0.109 μV vs. -0.338 ± 0.075 μV, $F_{1,18} = 36.010$, $P < 0.001$, Figure 5.4*C*), corroborating the view that induced spindle activity is highly prone to refractoriness. Comparing the effects of Pressing stimulation specifically for trains with 2 clicks and the 2-Click stimulation revealed that Pressing stimulation induced a stronger spindle response to the first click ($F_{1,12} = 16.420$, $P = 0.002$), but a distinctly reduced response to the second click ($F_{1,12} = 10.423$, $P = 0.007$, Figure 5.4*C*). Overall, induced spindle activity derived from both click stimuli was comparable between the conditions ($F_{1,12} = 1.177$, $P = 0.299$).

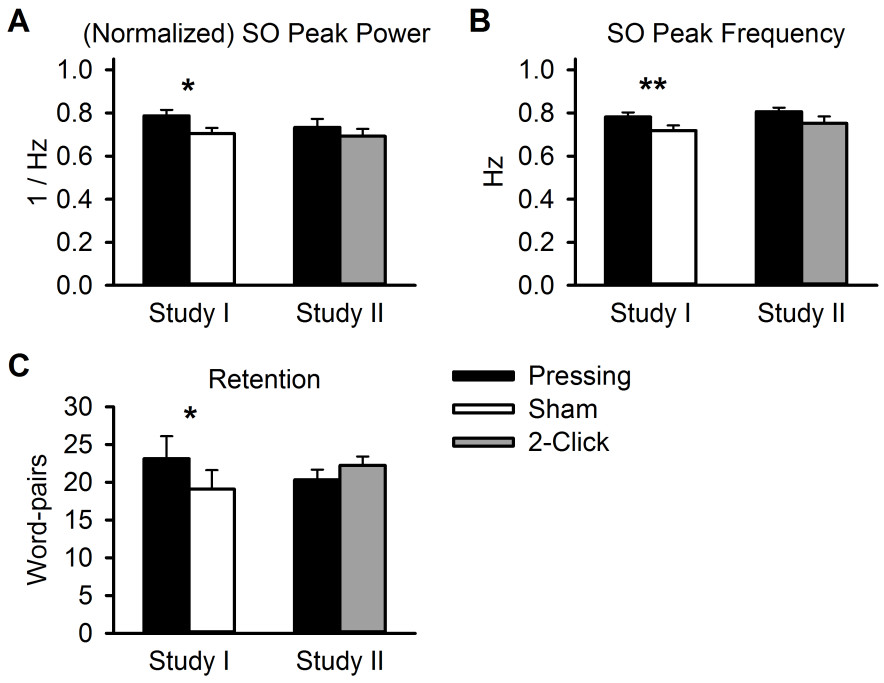

Figure 5.5 – Auditory Stimulation Enhances SO Power and Memory Retention.
(*A*) Spectral power of the individually determined SO peak and (*B*) SO peak frequency during SWS within the 210-min stimulation period averaged across all subjects and EEG. (*C*) Retention of word-pairs across the 7-h nocturnal sleep period. Data are presented separately for the Pressing stimulation (black bars) and Sham condition (empty bars) of Study I and the Pressing stimulation (black) and 2-Click stimulation condition (grey bars) of Study II. $^*P < 0.05$, $^{**}P < 0.01$ for pairwise comparisons between conditions.

5.3.3 Sleep and Spectral Power

In both Study I and Study II, there were no differences between stimulation conditions in mneter sleep architecture, total sleep time, sleep onset, percentages of time awake, Non-REM and REM sleep stages for the full nights (all $P > 0.1$), nor for the 210-min stimulation period (all $P > 0.288$, Table 5.1). In Study I, as expected, analysis of spectral power during SWS indicated that SO peak power was significantly increased during Pressing stimulation as compared to the Sham control (0.786 ± 0.028 Hz^{-1} vs. 0.705 ± 0.025 Hz^{-1}, $F_{1,14} = 16.424$, $P = 0.0012$, Figure 5.5*A*). In parallel, during Pressing stimulation the peak frequency in the SO range was slightly shifted towards faster frequencies (0.78 ± 0.02 Hz vs. 0.72 ± 0.02 Hz, averaged over all channels; $F_{1,14} = 14.090$, $P = 0.002$, Figure 5.5*B*). There was no difference in fast spindle power between the Pressing and Sham stimulation conditions ($F_{1,14} = 0.291$, $P = 0.598$). In Study II, effects of Pressing and 2-Click stimulation were quite comparable and there were no

Table 5.1 – Sleep Stage Distribution for the 210-min Stimulation and Full Retention Interval

		Study I		Study II	
	Parameter	Pressing	Sham	Pressing	2-Clicks
210-min Stimulation	W (%)	1.8 ± 0.6	2.1 ± 0.9	2.3 ± 0.9	1.1 ± 0.9
	S1 (%)	7.1 ± 1.4	6.0 ± 0.6	5.8 ± 0.8	5.0 ± 0.6
	S2 (%)	57.0 ± 2.3	57.0 ± 2.6	50.2 ± 2.1	51.8 ± 2.4
	SWS (%)	25.6 ± 2.9	27.4 ± 2.7	28.5 ± 2.3	31.0 ± 2.7
	REM (%)	8.5 ± 1.4	7.6 ± 1.2	13.2 ± 1.7	11.1 ± 1.2
	Arousal (%)	12.3 ± 1.2	13.1 ± 1.5	7.5 ± 0.9	7.0 ± 0.4
Entire night	W (%)	2.1 ± 0.7	4.2 ± 1.9	5.4 ± 1.9	2.8 ± 1.3
	S1 (%)	6.1 ± 1.0	5.4 ± 0.5	6.2 ± 0.7	5.8 ± 0.9
	S2 (%)	57.6 ± 1.8	56.3 ± 2.1	50.1 ± 2.0	53.0 ± 1.8
	SWS (%)	16.2 ± 1.4	16.6 ± 1.4	21.6 ± 1.0	21.2 ± 1.8
	REM (%)	17.9 ± 1.3	17.5 ± 1.2	16.7 ± 1.6	17.2 ± 1.2
	Arousal (%)	12.3 ± 1.2	12.5 ± 1.3	7.2 ± 0.7	7.6 ± 0.5
	TST (min)	410.0 ± 6.1	421.7 ± 4.4	417.8 ± 3.9	422.7 ± 2.5

Time spent in different sleep stages during the 210-min stimulation interval and the entire 7-h retention period for Study I (Pressing stimulation vs. Sham) and Study II (Pressing vs. 2-Click stimulation). There were no significant differences between conditions. W: Wake, S1-SWS: Sleep stages 1-SWS, REM: Rapid Eye Movement, TST: Total sleep time (in min).

significant differences in EEG power and frequency measures of interest between the conditions ($F_{1,12} < 2.27$, $P > 0.158$).

5.3.4 Memory Performance and Behavioural Control Measures

Study I indicated that Pressing stimulation distinctly enhanced the retention of word pairs across sleep, as compared to the Sham control where no clicks were presented (24.63 ± 1.74 vs. 20.25 ± 1.47 word-pairs, $P = 0.024$; Figure 5.5*C*). Overnight retention did not differ between the Pressing and 2-Click stimulation condition of Study II (19.43 ± 1.40 vs. 21.21 ± 1.20 word-pairs, $P = 0.180$). In both studies, learning performance before sleep was comparable between conditions ($P > 0.255$).

In both studies, no significant differences were found for performance on the Psychomotor Vigilance Test at learning before sleep (Pressing vs. Sham: 332.88 ± 6.65 ms vs. 324.45 ± 7.13 ms, Pressing vs. 2-Click: 277.37 ± 12.14 ms vs. 283.23 ± 12.16 ms) or at retrieval after sleep (Pressing vs. Sham: 333.62 ± 8.32 ms vs. 333.96 ± 11.07 ms, Pressing vs. 2-Click: 276.05 ± 8.34 ms vs. 276.93 ± 10.20 ms, for all $P > 0.201$). Stimulation did also not affect behavioural control measures of retrieval from long-term memory or working memory as measured by word fluency or digit span performance (for all $P > 0.88$). Likewise, measures of subjective sleepiness (Stanford Subjective Sleepiness) and mood (PANAS) did not reveal any differences between conditions (for all $P > 0.390$).

5.4 Summary

The findings presented in this chapter indicate that repetitive closed-loop auditory stimulation of SOs during SWS as established with the Pressing stimulation protocol, effectively prolongs trains of slow oscillatory activity, enhances SO amplitudes together with spindle activity phase-locked to the SO up-state, and distinctly improves the overnight retention of declarative word pair memories, when compared with a Sham control condition devoid of any stimulation. However, the Pressing stimulation protocol which was basically designed to drive trains of multiple succeeding SO cycles by presenting up to four clicks in succession, did not prove to be superior to a less driving "2-Click" stimulation procedure adopted from Experiment II, where just two clicks were given in succession. There was no difference between the stimulation protocols in the efficacy to prolong SO trains or enhance SO amplitudes, nor were there differences in induced spindle activity or memory performance. The failure to enhance efficacy of closed-loop

stimulation by enhancing the repetitions of stimulation in an SO train indicates the presence of mechanisms that prevent the overdriving of slow oscillatory activity.

Chapter 6

Conclusion

6.1 Discussion

The effect of auditory stimulation on the sleeping brain has been thoroughly investigated. However, the majority of studies in humans either focused on amplitudes and latencies of specific components of the AEP response to investigate information processing during sleep (Bastuji *et al.*, 2002, Campbell & Colrain, 2002, Dang-Vu *et al.*, 2011), or aimed at disturbing sleep by selectively suppressing specific sleep stages like SWS via auditory stimulation to assess respective consequences on, e.g. learning performance during subsequent wakefulness (Landsness *et al.*, 2009, van der Werf *et al.*, 2009) or insulin levels (Tasali *et al.*, 2008). In animals, namely anaesthetized guinea pigs, it has been shown on the other hand that repetitive sound stimulation produces a highly stable entrainment of SO activity in thalamic neurons (Gao *et al.*, 2009). To the best of my knowledge, Experiment I (Chapter 3) is the first study to examine whether rhythmic stimulation can be used to entrain sleep-associated brain EEG oscillatory phenomena like the SO in humans.

In line with other experimental studies, the results of Experiment I show that auditory stimulation during SWS evokes a specific electrophysiological response, consisting of a strong hyperpolarization after about 500 ms followed by a depolarization that is maximal at about 900 ms (Amzica & Steriade, 1998, Plihal *et al.*, 1996, Riedner *et al.*, 2011). Strong hyperpolarizations followed by depolarizations are characteristic features of SOs which are also identified by the respective detection algorithm. It is well established that this evoked response is associated with strong cortical synchronized activity and interacts with the thalamo-cortical system to generate the K-complex (Bastien &

Campbell, 1994, Contreras & Steriade, 1995). K-complexes bear striking similarities with the SO in morphology and generating mechanisms, although differences may exist between these phenomena (Amzica, 2010, Cash *et al.*, 2009, De Gennaro *et al.*, 2000, Rosanova & Timofeev, 2005). Thus, in light of the fact that the increase in SO power during the 0.8-Hz stimulation condition was not statistically different from that of Random stimulation, it could be argued that these effects on SO power were mainly driven by sound-evoked K-complexes rather than by an entrainment to the rhythmic 0.8-Hz stimulation. To clarify this issue, analyses of AEPs were performed which revealed that for stimuli presented during Random stimulation with short ('overlapping') ISIs, the late negative-to-positive component complex bearing great similarity with the SO was significantly smaller than in the AEP to the stimuli presented at 0.8 Hz, likely reflecting the refractoriness of the AEP response with short ISIs (Durrant & Boston, 2006). Also, AEPs averaged across all sounds of the Random stimulation condition revealed smaller component amplitudes than in the 0.8-Hz stimulation condition, especially during sleep stage 2. However, parallel analyses of evoked fast spindle activity revealed that the observed increase in SO activity during the 0.8-Hz stimulation condition cannot be entirely reduced to K-complexes (evoked at this specific ISI). K-complexes are typically associated with a transitory increase in fast spindle activity (Contreras & Steriade, 1995). In fact, such an increase was observed in response to the sounds (about 900 ms post-stimulus) during Random stimulation, with this increase significantly exceeding that during 0.8-Hz stimulation. By contrast, in the 0.8-Hz stimulation condition, the suppression of fast spindle activity during the preceding hyperpolarization of the AEP (300-600 ms post-stimulus) predominated (Figures 3.5 and A.2). Thus, whereas isolated random stimuli caused a steady increase in fast spindle activity over the entire 1.1-s post-stimulus interval, the sounds of the 0.8-Hz stimulation condition produced a phase-dependent modulation of fast spindle activity rather similar to that observed during spontaneous SOs (Mölle *et al.*, 2002, 2011). Moreover, similar to the temporal pattern during spontaneous SOs, also slow spindle activity during the 0.8-Hz stimulation condition (during stage 2 sleep) was significantly increased at the transition of the AEP into the negative phase ($\sim$ 300 ms post-stimulus; (Mölle *et al.*, 2011)). This differential pattern of fast and slow spindle activity, which was specifically observed during the 0.8-Hz stimulation condition and closely mimics the temporal relationships between slow and fast spindles during spontaneous SOs, strongly argues for the view that factors other than K-complexes significantly contribute to the entrainment of SO activity observed

during 0.8-Hz stimulation. The moderate effect on SO amplitude *per se* might reflect habituation concurrently developing with the periodic signal presentation. In demonstrating that rhythmic auditory stimulation can induce and entrain sleep SOs, the data suggest the use of this approach in the study of functions known to be promoted by SOs, such as the consolidation of memory contents and the post-sleep facilitation of the encoding of new memories (Antonenko *et al.*, 2013, Marshall *et al.*, 2006, van der Werf *et al.*, 2009). Such studies may reveal the induction of trains of SOs to be more critical for memory processes than mere changes in SO amplitude (Mölle *et al.*, 2011).

A main finding of Experiment I is that the efficacy of the 0.8-Hz stimulation is state-dependent. SO power was enhanced by rhythmic stimulation only after Non-REM stage 2 sleep had become manifest. No similar effects were obtained during waking before sleep, and the click stimulation also did not shorten sleep latency as hypothesized, which diverges from a previous study (Bohlin, 1971), which however used much longer ISIs (varying between 20 and 40 s). A comparable dependency of the effects of tone stimulation on the brain state has been shown for the AEP that changes its waveform (and frequency content) when the brain transits from wakefulness to light sleep and SWS (Campbell & Colrain, 2002, Cote, 2002). A dependence on brain state was revealed specifically in terms of the predominant EEG rhythm. The failure of 0.8-Hz stimulation to increase SO activity in the waking brain, when the EEG is dominated by faster frequencies, together with the significant delay of sleep onset resulting from the periodic 0.8-Hz stimulation implies that the brain's susceptibility to external drive is determined by its current state of vigilance. Wakefulness either does not allow an entrainment *per se*, or this specific brain state is characterized by a resonance frequency disjoint to the 0.8-Hz stimulation, which would explain the delayed transition into sleep stage 1 inasmuch as the system's dynamics are perturbed. The idea of a resonance effect induced by 0.8-Hz stimulation is in particular in accordance with the finding of a higher accumulation of SO power after SWS was reached. However, although suggesting a brain state-dependency of the stimulation effect, the findings do not entirely rule out that other factors, like circadian rhythm, add to the effects of 0.8-Hz stimulation on EEG activity being restricted to sleep.

State dependence of the effects of 0.8-Hz stimulation on SO activity has similarly been observed in recent studies using tDCS oscillating at a frequency of 0.75 Hz. The oscillating tDCS induced wide-spread endogenous SO activity when applied during Non-REM and SWS, whereas the increase in SO activity was marginal and restricted to the

prefrontal cortex when the stimulation was applied to the waking brain (Kirov *et al.*, 2009, Marshall *et al.*, 2006). However, the waking brain responded to 0.75-Hz tDCS with increased theta activity. The convergence of these findings tempts to speculate that distinct brain state-dependent resonance frequencies characterize the oscillatory EEG response to rhythmic stimulation. In this view, SWS is essentially characterized by a 0.8-Hz resonance frequency, whereas wakefulness as well as light sleep at the transition to deep sleep represent brain states resonating at frequencies different from the 0.8-Hz stimulation frequency applied here. The modulation of $12-15$-Hz spindle activity caused by Random stimulation suggests for the transitory period of light sleep a faster resonance frequency above the SO range, as Random stimulation contained a high proportion of shorter ISIs. Consistent with this view, in a previous study, instrumental conditioning of a sensorimotor rhythm in the $12-15$-Hz frequency band effectively decreased sleep onset latency (Hoedlmoser *et al.*, 2008). Yet, such assumptions are in need of experimental validation. To conclude, the results of Experiment I indicate that rhythmic acoustic stimulation can be used to induce sleep SO activity, which makes it indeed a promising and simple approach for the investigation of putative sleep functions linked to the SO rhythm.

The outcome of Experiment II indicates that the enhancement of SO activity and overnight memory consolidation by auditory stimulation essentially depends on the timing of stimulation in-phase with SO up-states. Utilizing closed-loop control, a novel approach aimed at fine-tuning auditory stimulation to the ongoing SO phase and thereby inducing resonant slow oscillatory activity. Although similar resonance in neocortical networks might be invoked by other kinds of stimulation, e.g. transcranial electrical or magnetic stimulation (Marshall *et al.*, 2006, Massimini *et al.*, 2007), auditory stimulation at low intensity is quite simple to apply and, more importantly, auditory stimuli during non-REM sleep, as we have seen in Experiment I, are known to evoke a K-complex-like response often characterized by spindles nesting in the transition toward depolarization. Since the K-complex originates from the same cortico-thalamic circuitry that underlies the generation of SOs, this suggests that this circuitry also mediates the resonant SO activity observed here after in-phase auditory stimulation (Amzica & Steriade, 2002, Rosanova & Timofeev, 2005). However, K-complexes occur as singular events, rather than in a rhythmic fashion.

Analyses aimed at the separation of evoked auditory activity from spontaneous ongoing SO activity corroborated that the observed enhancement in SO activity by In-phase stimulation cannot simply be reduced to a change in the response evoked by the auditory stimulus. In fact, responses to the (first) auditory stimulus during In-phase and Out-of-phase stimulation showed quite similar waveforms with a comparable temporal component structure, although the amplitude of the late component differed slightly. This finding is consistent with the view that the primary impact of the phase-dependent administration of the stimuli was on ongoing SO rhythm generation. Whether and to which extent the evoked response obtained by subtracting spontaneous ongoing oscillatory activity reflects potential activity elicited by the stimulus itself or evoked changes in ongoing oscillatory activity (including SO activity) cannot be answered (e.g. Makeig *et al.*, 2002).

Notably, In-phase stimulation did not produce an increase in SO events *per se* but rather caused an enhancement in SO amplitude, probably reflecting increased synchrony of up- and down-state transitions, and prolonged sequences of SO cycles together with an enhanced alignment of fast- and slow-spindle activity to the SO cycle. Specifically, fast-spindle activity accumulated in the up-phase and slow-spindle activity in the up-to-down transition within the SO cycle. Changes in slow oscillatory activity and nested fast-spindle activity have been consistently associated with a consolidating effect on memory traces (Huber *et al.*, 2004, Mölle & Born, 2011, Ruch *et al.*, 2012, Sejnowski & Destexhe, 2000, Timofeev *et al.*, 2002, Wilhelm *et al.*, 2011). In addition, spindle activity synchronized to the SO cycle might favor off-line memory processing by gating external sensory input to the neocortex (Dang-Vu *et al.*, 2011, Schabus *et al.*, 2012).

In sum, while closed-loop stimulation has previously been successfully used to suppress pathological EEG rhythms in rats (Bérenyi *et al.*, 2012), Experiment II demonstrates that auditory closed-loop stimulation can be administered in humans to enhance normal brain rhythms, specifically of sleep SOs, and their functional effects on memory consolidation. Indeed, closed-loop in-phase auditory stimulation at low intensity might be a promising tool to generally ameliorate the efficacy of sleep rhythms not only in healthy subjects but also in pathological conditions as discussed in the following outlook.

In accordance with the results obtained in Experiment II, the final experiment adds support to the notion that closed-loop stimulation in general is an effective tool to

specifically manipulate oscillatory EEG activity (Bérenyi *et al.*, 2012, Paz *et al.*, 2013). If applied time-locked to the ongoing SO up-phases during SWS, the presentation of clicks reliably induced further SO cycles with high amplitude, accompanied by surges of spindle activity that occur in-phase with the invoked SO up-state. At the behavioural level, the changes express themselves in an enhanced overnight retention of hippocampus-dependent declarative memories, altogether corroborating the view that the induced SOs are functionally effective in the same way as endogenous SOs (Chauvette *et al.*, 2012, Cox *et al.*, 2012, Phillips *et al.*, 2012, Wilhelm *et al.*, 2013). In fact, the changes following both closed-loop stimulation protocols used here are highly specific, as indicated by comparisons with the effects of a Sham control condition which were performed for the 2-Click protocol in Experiment II and for the Pressing stimulation protocol in Experiment III. Accordingly, both stimulation protocols left the gross sleep architecture, the amount of SWS, REM sleep, number of awakenings entirely unaffected and likewise the changes induced in the EEG power spectrum during SWS were restricted to the SO frequency band. Thus, general features of sleep remained untouched but closed-loop SO stimulation chiefly induced an acute temporal re-structuring of SO and nested spindle activity.

Indeed, a closer examination of the acute dynamics of induced SO and spindle activity after Pressing stimulation and 2-Click stimulation revealed surprising insights into the network conditions underlying the generation of SOs. First of all, Pressing stimulation was indeed capable of inducing SO trains of up to 3 and 4 successive cycles. Such trains of several successive SOs have been suspected to be particularly powerful in promoting sleep-dependent memory consolidation as their occurrence is increased after intense learning (Mölle *et al.*, 2011). However the occurrence of such SO trains is still relatively rare, and even more so under non-stimulated conditions (< 10 % of all SOs), indicating an overall low tendency for the spontaneous occurrence of longer groups of SOs, and that it is difficult to evoke a resonant response of this length. As for the generation of SO trains during SWS, network conditions appear to be inert during SWS. This is further supported by the effects of 2-Click stimulation which, as revealed by auto-event correlations (Figure 5.2B), exhibited the same capability to induce longer trains of SO events as the Pressing stimulation protocol. Also, overall SO numbers were comparable for the two stimulation protocols, although the 2-Click protocol lacked instances of 3 or 4 click presentations in succession. Altogether, this picture indicates that more than 2 click stimulations in succession, as they occurred only during Pressing stimulation,

remain basically ineffective. This conclusion is ultimately also supported by the fact that the memory enhancing effect of both stimulation protocols was equivalent.

The comparison of acute effects of 2-Click stimulation with those of Pressing stimulation resulting in trains of 2 clicks in succession suggested that Pressing stimulation even enhanced the network's inertia to produce SOs, as the decrease in SO amplitude as well as the parallel decrease in induced phase-locked spindle activity across the two induced SO cycles was much greater for the Pressing stimulation protocol than for the 2-Click stimulation protocol. However, the comparison of these two conditions, although apparently alike, may be misleading given that the SO amplitude induced by the first click in the Pressing stimulation condition was significantly greater than that of the first click in the 2-Click stimulation protocol. This finding must be ascribed to the fact that unlike the 2-Click protocol where the first click was always followed by a second click, the Pressing stimulation protocol provided a second click only when the first click induced a supra-threshold negative SO half-wave. Nevertheless, the view that Pressing stimulation aggravated refractoriness in SO-generating networks is indeed supported by the fact that with this protocol the pauses until stimulation was resumed were significantly enhanced compared with the Sham condition, and also in comparison with the 2-Click stimulation protocol.

A key finding of Experiment III is that independent of the stimulation protocol, only the first click induced a robust increase of fast $(12-15)$ Hz spindle activity phase-locked to the SO up-state, whereas increases in spindle activity accompanying the up-states of succeeding SO cycles were comparable to those seen during sham conditions. This finding indicates that spindle generating networks in thalamic circuitry build up an immediate resistance to stimulation (von Krosigk *et al.*, 1993) which most likely reflects refractoriness of spindle generation. Thalamic spindle generation is well-known to underlie refractoriness periods (between 5-20 s, in the ferret) due to the persistent activation of the hyperpolarization-activated cation current I_h in thalamo-cortical cells (Lüthi & McCormick, 1998, Destexhe *et al.*, 1998). The dynamical up-regulation of I_h in these networks, that appears to be also partly controlled by descending cortico-thalamic projections, is considered the critical determinant of the time course of the refractory period. Importantly, the thalamo-cortical cells confer relative refractoriness to the entire thalamo-cortical network, including cortical networks generating regular SO activity as well as pathological spike-and wave seizure activity (Destexhe *et al.*, 1998).

Interestingly,in spontaneous SO trains spindle activity associated with the first SO cycle is also of particular importance (Mölle *et al.*, 2011). Fast 12-15 Hz spindle activity is highest during the up-state of first SO and lowest during the last cycle of a train. Moreover, intense declarative learning prior to sleep does not only enhance the number of SOs occurring in a train but also enhanced spindle activity, most profoundly during up-states of SOs initiating an SO train, as well as shortly before an identifiable SO. The present findings in conjunction with those previous analyses speak for a loop-like scenario where spindles, by promoting excitability changes in local cortical pyramidal networks (Destexhe *et al.*, 2007, Ayoub *et al.*, 2013), enhance the likelihood and amplitude of succeeding SO cycles. Emergent depolarization in the succeeding SO cycle, in turn, exerts a driving influence on thalamic spindle generation (Contreras & Steriade, 1995). However, due to refractoriness in thalamo-cortical neurons, the resulting increase in spindle activity is profoundly decreased (Mölle *et al.*, 2011). Basically, this view assumes an initiating role of spindles for trains of SO, which is also in accord with the occurrence of spindles in the absence of strong SO activity during human Non-REM sleep stage 2, typically preceding SWS periods with consolidated SO activity (see also (Kim *et al.*, 2012)). Indeed, results from the Pressing stimulation protocol of the present study indicate that closed-loop click stimulation can produce robust increases in spindle activity even when concomitantly induced SO activity is marginal, i.e. remains below the criterion threshold used here for identifying SOs. This actually happened in all instances when the on-line detection of a negative SO half-wave peak was followed by the presentation of only one isolated click (Figure 5.3A). Considering also that such single-click events represent a substantial portion of SO events during Pressing stimulation, it is tempting to speculate that the closed-loop auditory stimulation of SOs is at least partly effective via a primary influence on spindle generating networks. If so, this raises the question, to be answered in future studies, whether the closed-loop presentation of single isolated auditory stimuli already provides the maximum effect with regard to the stimulation of SO trains and accompanying memory consolidation.

To conclude, Experiment III confirms the efficacy of closed-loop auditory stimulation presented in-phase with SO up-states, to prolong SO trains, to enhance SO amplitude and SO up-state associated spindle activity, and to eventually improve retention of declarative word-pair memories. The Results indicate, however, that the repetitive stimulation of more than two SO cycles in succession does not increase efficacy of stimulation. Presentation of two stimuli in succession and in-phase with the up-state of ongoing SO

activity already provides maximum stimulation efficacy. The insensitivity of the network to the enhancing effects of Pressing stimulation comprising the stimulation of more than two successive SO cycles appears to be linked to the refractoriness of SO up-state associated spindle activity, because this was enhanced only after the initial click stimulus. The spindle generating network, hence, may not only be the system primarily conveying the memory enhancing effects of closed-loop SO stimulation (Schabus *et al.*, 2004, Ruch *et al.*, 2012, Mednick *et al.*, 2013). Its refractoriness might also provide a mechanism protecting the thalamo-cortical system from hypersynchronisation and the occurrence of seizure activity in conditions of overdriving SO activity (Beenhakker & Huguenard, 2009).

6.2 Outlook

The results presented in this thesis highlight auditory stimulation as a very simple, straightforward and yet reliable approach to induce slow oscillations and, moreover, promote their essential function in the consolidation of declarative memories during sleep. In particular, with the utilization in a closed-loop manner this work for the first time demonstrates the importance of the timing of auditory stimuli with respect to ongoing brain activity. Such targeted stimulus presentation represents a unique and direct way to explore distinct brain rhythms. In the following I will outline some ideas that have emerged during my work on this thesis and which are addressed in ongoing studies or might be scrutinized in future projects.

6.2.1 Exploring the Interplay between Sleep Oscillations and Memory Consolidation

The following projects investigate brain mechanisms of sleep-related memory consolidation beyond SOs. They focus on (i) sleep spindles and (ii) memory reactivation, and aim at a concept that integrates sleep-associated brain oscillations and theories of memory consolidation.

(i) The Role of Sleep Spindles in Memory Consolidation

Recent studies and the present work have demonstrated that sleep spindles do not solely serve to conserve sleep, gating any incoming sensory information (Dang-Vu *et al.*, 2010), but also play a crucial role in the consolidation of memory and neural plasticity

(Steriade, 2003, Rosanova & Timofeev, 2005, Mölle *et al.*, 2011) based upon a unique temporal phase relation between SOs and fast spindles (Cox *et al.*, 2012). Importantly, as demonstrated in the previous chapters, auditory stimulation by means of brief clicks evokes a general response which resembles a SO and is most likely followed by spindle activity nested within its positive component (Figure 3.5). This pattern renders the current approach inapt to directly investigate sleep spindles as a distinct brain rhythm and to scrutinize how they contribute to memory consolidation. Animal models, in contrast, are very valuable to overcome limits of human studies because they can implement invasive and hence more direct stimulation techniques. Above all, the novel optogenetic stimulation in mice represents a very intriguing possibility to induce spindle activity directly via modified light-sensitive neurons within thalamic networks (Halassa *et al.*, 2011, Kim *et al.*, 2012). Combining optogenetic stimulation with a closed-loop control would allow a direct comparison of induced spindle activity in-phase and out-of-phase with slow oscillations and hence enlighten the causal relation between fast spindles and slow oscillations during processes of memory formation. This idea is currently under investigation in a newly established cooperation with Dr. Charles Latchoumane and Dr. Hee-Sup Shin from the Institute of Basic Science in Seoul, South Korea.

Nevertheless, in a recently launched project we have also implemented a new detection algorithm to identify fast spindles on-line in humans. Adapted from an off-line procedure to identify spindles as discrete events (Mölle *et al.*, 2002), it utilizes the envelope of fast spindle activity obtained from a centro-parietal location, where fast spindles are most dominant. A spindle event is identified and triggers a stimulation whenever the envelope exceeds an individually determined threshold. This new stimulation protocol has been integrated into a study design identical to Experiment II and, for the time being, utilizes cathodal transcranial DC stimulation. The overarching goal is to suppress on-going spindle activity based on the hypothesis that such an inhibitory stimulation will disrupt the functional role of fast spindles and consequently lead to an impairment of declarative memories. If successful, this experiment would be the first to examine the causal contribution to memory function of fast spindles in humans by means of a direct manipulation and disentangle their relation to the superior slow oscillations. Furthermore, experiments applying transcranial magnetic or sensory, e.g. auditory or visual, stimulation are envisaged to compare different modalities and explore the most efficient approach to manipulate spindles.

(ii) Is memory reactivation dependent on the SO-phase?

As reviewed in the previous chapters, it is generally assumed that overnight memory consolidation takes place by reactivation and that this process can be manipulated by olfactory stimulation during SWS (Rasch *et al.*, 2007). This finding has been replicated with auditory stimuli (Rudoy *et al.*, 2009, Bendor & Wilson, 2012, Schreiner & Rasch, 2014) in both humans and animals and therefore has proven to be a very robust approach to boost reactivation and strengthen memories during sleep.

To examine the actual role of slow oscillations in memory reactivation, we have recently designed a new learning task in which visual stimuli are paired with an auditory cue. This allows us to implement targeted auditory reactivation of the previously encoded associations in a closed-loop fashion. In a current pilot study, we are comparing a reactivation in-phase with SO up-states with random presentations and a sham control condition. We hypothesize that in comparison to the other experimental conditions, in-phase reactivation leads to the strongest enhancement in memory performance, as assessed by correctly drawing the encoded visual stimulus upon presentation of the corresponding auditory cue. If this attempt turns out to be successful, it would corroborate the notion of SO up-states representing the critical time window when the information transfer from hippocampal to neocortical sites takes place.

6.2.2 Modelling the Thalamo-cortical System during Sleep

To understand how exactly the strengthening of memories takes place, it is crucial to unravel the exact interaction between the involved brain rhythms. Likewise, detailed knowledge on how different stimulation modalities can improve these brain rhythms and how their efficacy depends on specific parameters would greatly benefit both scientific and clinical advances.

To achieve these goals, the use of mathematical models is inevitable, as previous computational work in particular in conjunction with experimental data has led to meaningful insights that could advance theoretical frameworks and predictions for future experiments (e.g. Mayer *et al.*, 2007, Fröhlich & McCormick, 2010). However, the complexity of the brain on the structural as well as the neuronal level has always been a challenge for modelling approaches. Against this backdrop, neural mass models that were pioneered by the work of Wilson & Cowan (1973) and Lopes Da Silva *et al.* (1974) and have been further developed since (Jansen *et al.*, 1993, Wendling *et al.*, 2002, David

& Friston, 2003), have so far shown great success in the reproduction of rhythms seen in human wake EEG. The success of neural mass models stems from a vast simplification of the underlying structures. By describing complex networks composed of numerous single cells in terms of a population and still allowing an integration of physiologically motivated cell dynamics, neural mass models represent a compromise between a very detailed and abstract modelling approach. They are therefore ideal to examine dynamics and phenomena observed in the EEG (Coombes, 2005, Deco *et al.*, 2008).

In collaboration with Arne Weigenand and Michael Schellenberger Costa (Institute of Neuro- and Bioinformatics, University of Lübeck), a thalamo-cortical neural mass model was developed to simulate the EEG of a sleeping human, including slow oscillations and sleep spindles. In the following I will outline the model and some main results (Weigenand *et al.*, 2014, Schellenberger Costa *et al.*, under review).

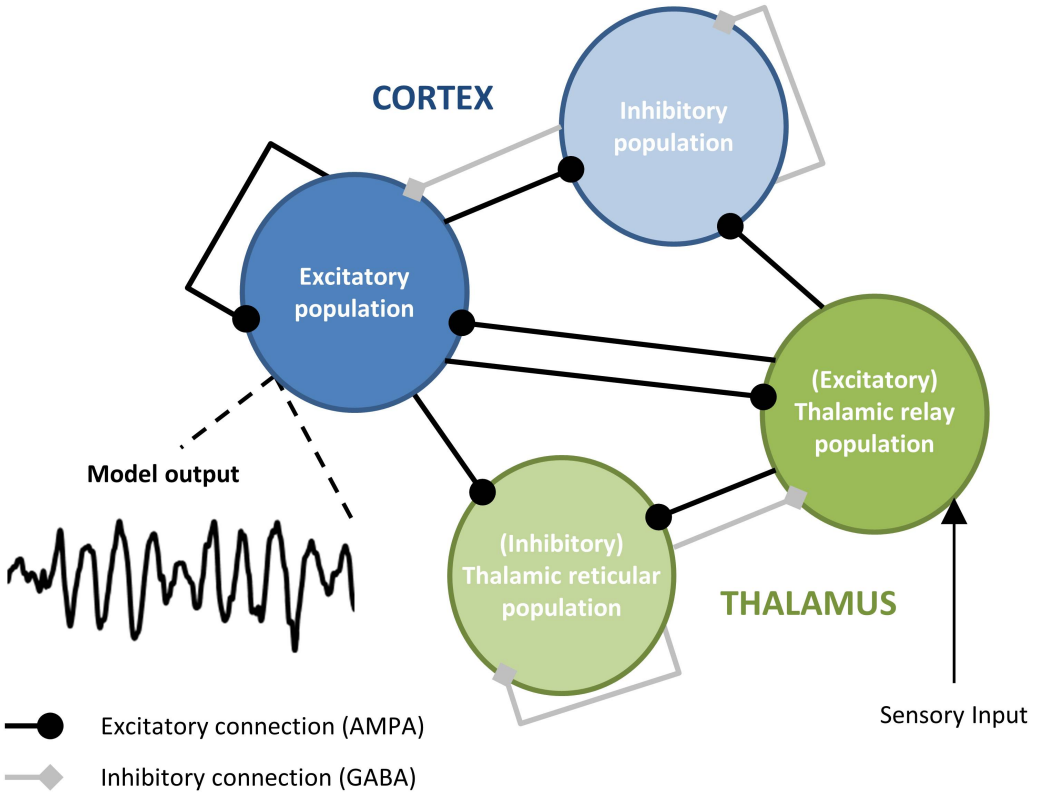

Figure 6.1 – The Thalamo-cortical Neural Mass Model.
The cortical and thalamic modules are each comprised of an excitatory and an inhibitory population. The dynamic of the cortical excitatory population provides the output of interests as it reflects neural activity resembling a recording of a surface EEG. To simulate sensory stimulation, a corresponding input is fed to the thalamic reticular population. Excitatory AMPAergic connections are depicted as black lines and circles, whereas inhibitory GABAergic connections are shown in light grey with diamond symbols.

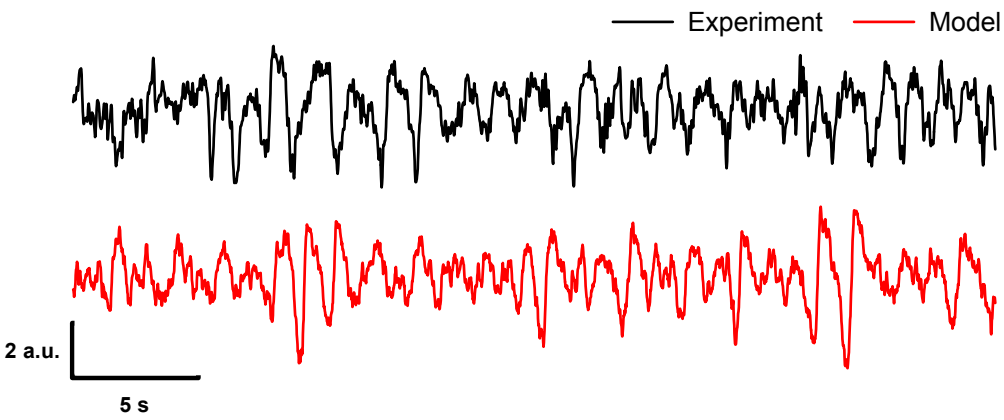

Figure 6.2 – Thalamo-cortical Model Reproduces SWS.
30-s excerpts of an EEG obtained from a human subject during SWS (black line) and a time series generated by the thalamo-cortical neural mass model (red line). Both time series have been z-scored and are therefore depicted in arbitrary units.

A schematic representation of the thalamo-cortical model is given in Figure 6.1. Incorporating a cortical and thalamic structure, this model permits the generation of cortical activity displaying both slow oscillations and sleep spindles in strong agreement with EEG data obtained from an actual experiment (Figure 6.2). Remarkably, identification of discrete SOs followed by averaging with respect to the identified negative peaks of both the EEG and fast spindle RMS signal revealed that the model is also capable of reproducing the experimentally observed phase-relation between SO and fast spindles (Figure 6.3). Furthermore an integration of an external input to the thalamic population mimicking in this case a sensory stimulus yielded results (data not shown) that showed a high level of conformity with the auditory evoked potentials obtained in Experiment I (Figures 3.5 and A.2).

These findings demonstrate that the thalamo-cortical model can serve as a basis for predictions of the stimulation of brain rhythms, e.g. "What is the response when a stimulus is presented within phases of a SO other than the up- and down-state?", and optimizations of parameters for existing stimulation protocols. Furthermore, since neural mass models feature a rather low mathematical complexity, they are ideal for analyses drawn from the repertoire of non-linear dynamics, like the bifurcation analysis, for a better understanding of the underlying dynamics on a more theoretical level.

Based on these promising findings and with a continuing interaction between theorists and experimentalists, we have already set our minds on a future goal, i.e. integrating a hippocampal structure into the model - the next essential step to understanding the

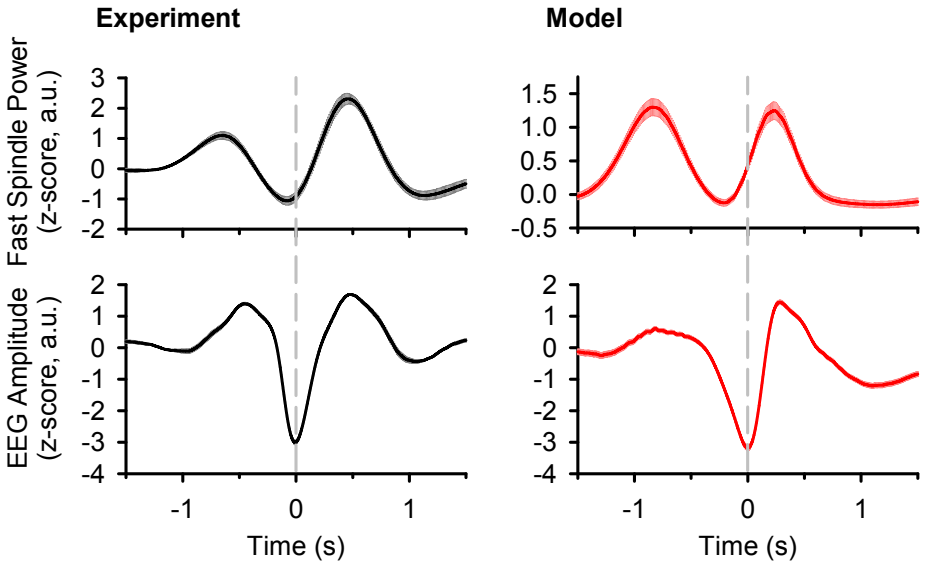

Figure 6.3 – Thalamo-Cortical NeuralMass Model Generates SO and Groups (Fast) Spindles.

Averaged EEG amplitude (*bottom row*) and fast spindle power (*top*) time-locked to the negative peak of detected SO ($t = 0$, vertical grey line) in data from an experiment and a time series generated by the thalamo-cortical model, shown in black and red, respectively. Identical to Figure 6.2, all time series were z-scored. Please note the different scaling of the y-axis for fast spindle power between the experiment and simulation.

contributions of all relevant brain rhythms to memory consolidation.

6.2.3 Application in Clinical Settings

All the experiments presented in the previous chapters were conducted in healthy young adults. However, the high degree of practicability in conjunction with the absence of side effects when compared to e.g. transcranial electrical stimulation or pharmacological interventions, underlines the great potential of auditory closed-loop stimulation in the treatment of patients with sleep disorders, symptoms related to dysfunctional sleep or distinct pathologic brain activity. Against this backdrop, we are currently starting two projects in (i) children with attention deficit hyperactivity disorder and (ii) children with benign epilepsy with sharp-wave activation, while a third project is planned which will focus on (iii) insomnia in elderly people.

(i) Normalization of declarative memory function in children with ADHD

Attention deficit hyperactivity disorder (ADHD) is the most diagnosed psychological disorder among children. It is characterized by signs of insufficient concentration or restlessness (American Psychiatric Association, 2000). More recently, it has also been

related to an impairment of sleep and declarative memory function, attributed to a delay of brain maturation (Shaw *et al.*, 2007) and a diminished expression of (frontal) slow oscillations (Ringli *et al.*, 2013). Accordingly, increasing slow oscillatory activity by means of transcranial DC stimulation led to an improvement of declarative memory performance in ADHD patients (Wilhelm *et al.*, 2012). However, the application of sub-threshold electrical stimulation, especially in children, will always raise ethical concerns despite the need for further investigations along this promising line. Sensory stimulation offers itself as a more harmless alternative to promote slow oscillations. Therefore, we are currently exploring the efficacy of auditory closed-loop stimulation on children with ADHD and healthy controls in collaboration with the Department of Child and Adolescent Psychiatry and Psychotherapy at the University of Kiel.

(ii) Disrupting epileptic spike-waves in children with benign epilepsy

Benign epilepsy with sharp-wave activation or continuous spikes and waves during slow wave sleep (CSWS) belongs to one of the most diagnosed syndromes of epilepsy in children. It is usually first diagnosed in both boys and girls at the age of 3 to 13 and is characterized by a substantial occurrence of epileptic discharges immediately upon onset of sleep stage S2 that continues throughout Non-REM sleep (Wirrell, 1998). Although due to proceeding brain maturation, the symptoms disappear when the children reach puberty between 14 to 18 years and most of the children with mild CSWS cope very well, however, in more severe cases this pathologic brain activity leads to a progressive deterioration of cerebral functioning which impairs cognitive and motor functions, e.g. speech, temporal and visuo-spatial orientation and memory (Loddenkemper *et al.*, 2011, Tassinari *et al.*, 2009). In principle, these children can be treated with anti-epileptic drugs, but currently there is no consensus on adequate pharmacological interventions (Hughes, 2010). E.g. steroids, which are indicated to have an impact on Non-REM sleep itself (Holsboer *et al.*, 1988), may suppress seizure activity but nevertheless confound a recovery of cognitive and motor skills.

In contrast to the previous project in ADHD children, we propose an alternative treatment for children with CSWS based on the results of the out-of-phase stimulation in the control group in Experiment II and following an approach described by (Bérenyi *et al.*, 2012), who applied intracranial electrical stimulation in a closed-loop fashion in an animal model. This approach targeting epileptic spike discharges efficiently suppressed emerging epileptic activity in cortical networks. Since intracranial stimulation is not

feasible in children with CSWS, auditory closed-loop stimulation aiming at a disruption, i.e. being out-of-phase with the epileptic seizure activity, might have the same impact. This idea is pursued in collaboration with the Department of Neuropaediatrics, Developmental Neurology and Social Paediatrics at the University Children's Hospital in Tübingen.

(iii) A Proposal to treat insomnia in elderly people

Insomnia, a lack of restorative sleep caused by difficulties to fall asleep or maintain sleep, is a frequent sleep disorder in the elderly and has become common in our modern society (Riemann *et al.*, 2011, Buysse, 2013). Insomnia in elderly is characterized by a severe change in sleep architecture, i.e. a significantly reduced level of slow wave sleep in conjunction with severe fragmentation of sleep, i.e. an increased number of arousals and awakenings over the course of the night. Insomnia is primarily treated with pharmacological interventions, but also new behavioural therapeutic interventions have been proposed (Altena *et al.*, 2008). While pharmaceuticals entail side effects like addiction or a gradual resistance to the drug, behavioural therapy is a very costly option. Hence, we believe that closed-loop feedback stimulation might offer a promising non-pharmacological and comparatively cost-effective alternative treatment. Potential interventions might work in two different ways: The first would be to apply closed-loop stimulation to latch onto any slow oscillations occurring during Non-REM sleep and thereby facilitate the slow oscillatory activity. On the other hand, studies have shown that sleep onset is marked by a distinct increase in theta activity (Marshall *et al.*, 2003). A modification of the stimulation protocol, in terms of both detection and stimulation of the theta rhythm might lead to an acceleration of sleep onset. This approach seems more promising than our rhythmic 0.8-Hz stimulation (Experiment I) because it is more customized to the resonant frequency dominant during the transition from wakefulness to light sleep. If and to what extent this approach is successful remains to be seen.

The ideas presented above are only some examples of possible applications of auditory closed-loop stimulation in clinical settings. Previous studies have demonstrated an influence on glucose homeostasis of impaired sleep (Buxton *et al.*, 2012) and in particular decreased SWS (Tasali *et al.*, 2008), suggesting that sufficient and healthy sleep might be a factor that reduces the risk to develop diabetes (Schmid *et al.*, 2015). Moreover, a beneficial effect of sleep on the generation of antibodies after vaccination has been found (Lange *et al.*, 2011, Besedovsky *et al.*, 2012), which likewise clearly indicates that the

function of sleep goes beyond the neurobehavioural level and comprises the metabolic and immunological domain.

In sum, I believe that with this set of outlined experiments, we will contribute to the endeavour of unravelling the causal role of sleep rhythms and inspire new scientific ideas to gain exciting new insights into the psychological functions of brain rhythms. In the meantime, our group has received several inquiries to develop an everyday device that combines all the advantages of our approach. This endeavour is not as far-fetched as it sounds, since current obstacles are not impossible to overcome and in the end depend on technological advances. Practical EEG headbands and affordable EEG amplifiers become more and more available and are already raising public awareness. On-going efforts to implement sleep scoring algorithms for real-time use based on techniques derived from machine learning (Längkvist *et al.*, 2012, Phan *et al.*, 2013, personal communication with Matthew Roos, John Hopkins University, Baltimore) might allow a fully automated and therefore easy-to-use implementation of auditory closed-loop stimulation. So perhaps we might indeed be able to purchase a small device called the ”sleep-memory-enhancer” in the near future.

Appendix A

Supplementary Figures

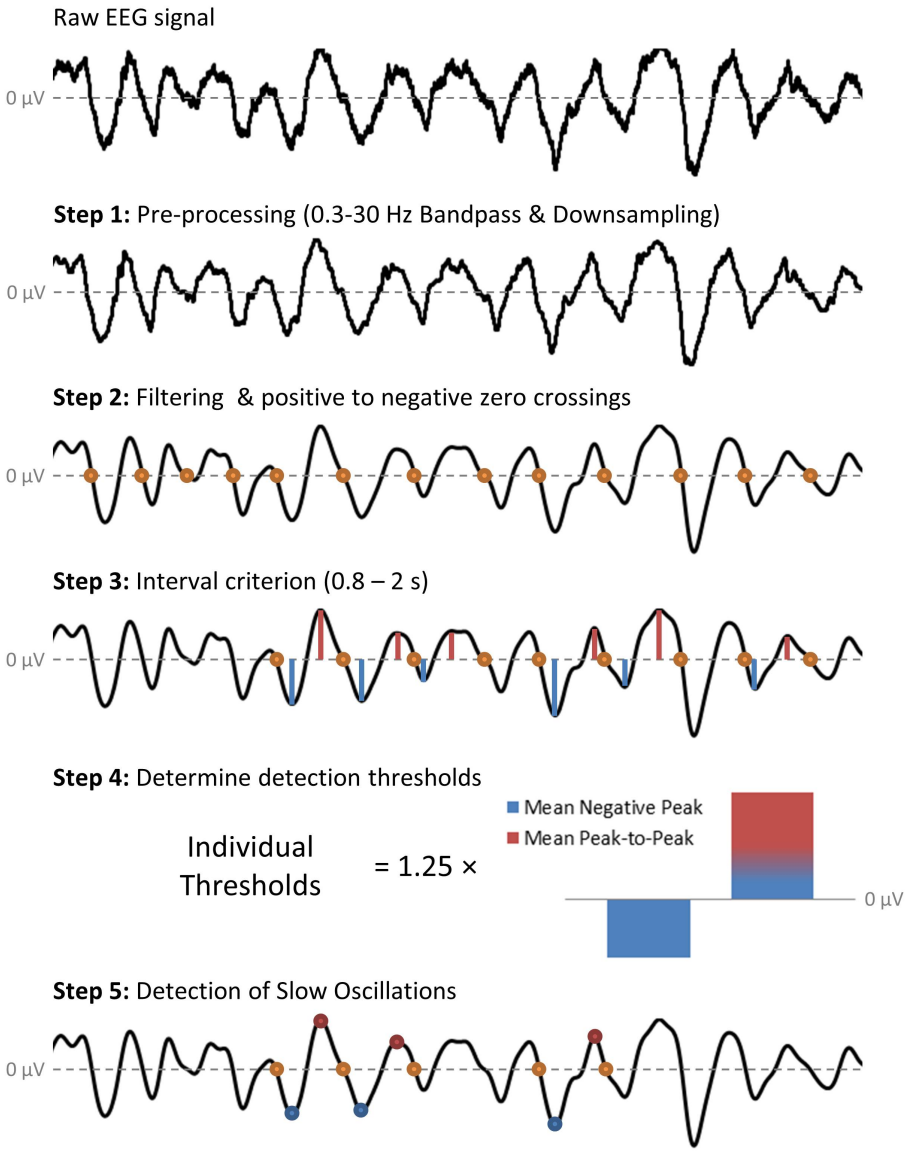

Figure A.1 – Algorithm for Off-line Detection of SO Events.
To identify discrete SOs in a given EEG channel, a pre-processing consisting of band-pass between 0.3 to 30 Hz and a down-sampling to 100 Hz is performed first (Step 1). The signal is then low-pass filtered at 4 Hz and all positive to negative zero crossings (ZC) are marked (Step 2). The filtering covers the full slow wave range to account for the sharp waveform of the negative half-wave. Next, each pair of subsequent ZCs is checked if they are between 0.8 and 2 s apart, i.e. a period corresponding to oscillations of 0.5 to 1.2 Hz (Step 3). For intervals which fulfil this criterion the average negative peak and negative to positive peak amplitude are calculated, representing two individualized thresholds. In this case, the resulting values were further scaled by a factor of 1.25, since global SO were of interest. (Step 4). In a final step, all previously determined ZC intervals that have a negative peak and peak-to-peak amplitude larger than the corresponding thresholds are identified as a slow oscillation. Detected SOs were then characterised by their negative peak position.

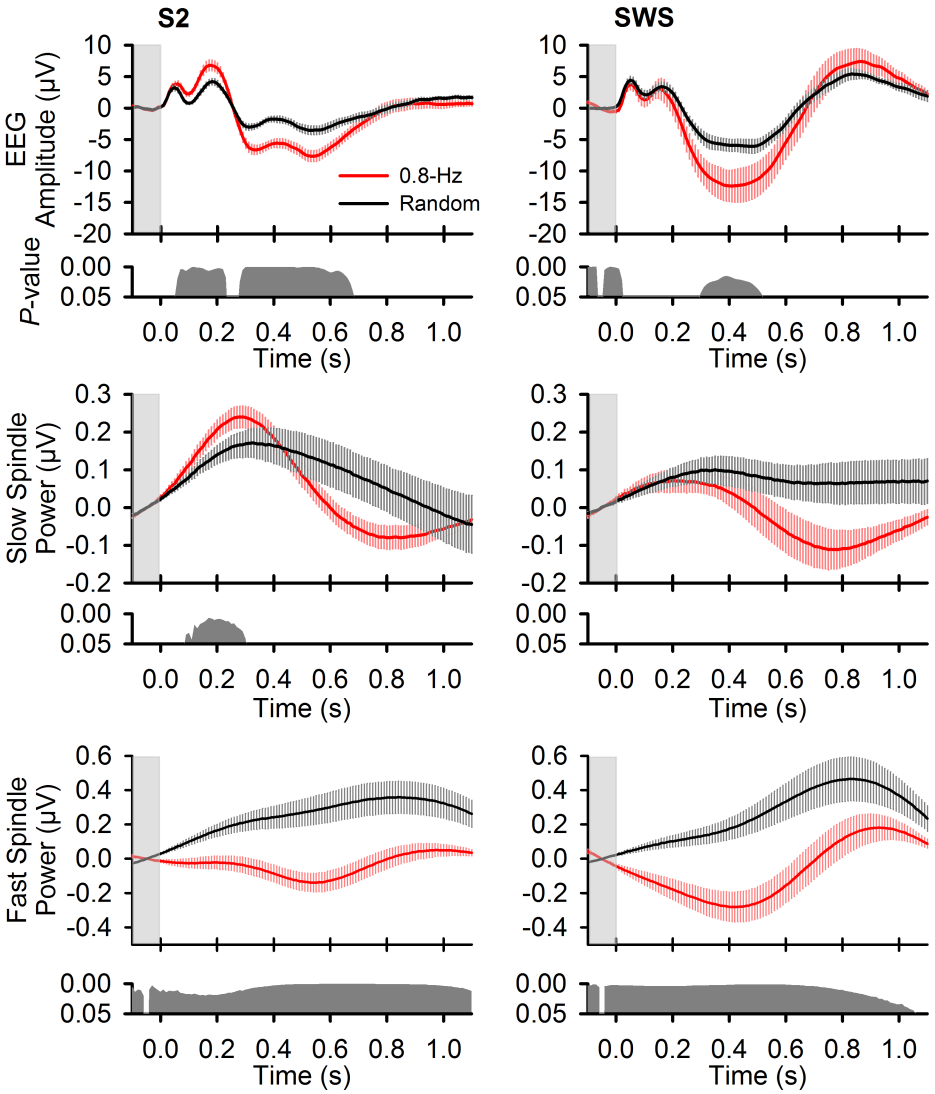

Figure A.2 – Auditory Evoked Potentials Over All Stimuli (Experiment I).
AEPs derived from all auditory stimuli during 0.8-Hz stimulation (red line) and random stimulation conditions (black line), and categorized by sleep stage S2 (*left column*) and SWS (*right column*), as well as conventional EEG band (0.3-30 Hz, *top row*), slow spindle band (9-12 Hz, *middle row*) and fast spindle band (12-15 Hz, *bottom row*). Additionally, point-wise statistical comparisons are indicated below each AEP. Vertical grey bars indicate intervals for baseline normalization. AEPs during S2 for 0.8-Hz stimulation: 2001.5 ± 254.5 and Random stimulation: 1978.4 ± 130.0 ($P = 0.734$); and during SWS for 0.8-Hz stimulation: 1685.0 ± 261.4 and Random stimulation: 1730.0 ± 138.7 ($P = 0.736$).

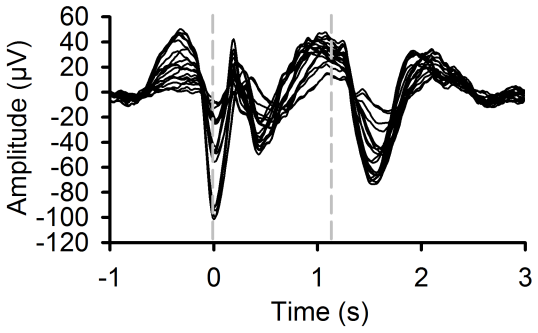

Figure A.3 – Experiment II - 1st Attempt of an Out-of-Phase Stimulation.
Averaged EEG signal (time locked to the first auditory stimulus, $n = 116$) from a representative subject stimulated in-phase with the SO down-state, i.e. the first auditory stimulus was presented immediately upon detection of a SO negative half-wave and the second 1,075 ms later, corresponding with the SO period length. Waveforms from all 19 recording channels are shown. Dashed vertical lines indicate presentation of auditory stimuli. Note, the second stimulus already falls into an emerging depolarizing up-phase, thus stimulating rather than disrupting a subsequent SO.

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Overview of Contributions

All the experiments presented in this Ph.D. thesis were conducted under the supervision of Prof. Dr. Thomas Martinetz (Institute of Neuro- and Bioinformatics, University of Lübeck), Prof. Dr. Jan Born (Institute of Medical Psychology and Behavioural Neurobiology, University of Tübingen) and mentor ship of Dr. Matthias Mölle (Department of Neuroendocrinology, University of Lübeck).

The scientific ideas underlining this line of research originated from discussions with Jan Born, Thomas Martinetz and Matthias Mölle. Together we developed the concepts and created the study designs for this set of experiments.

In general, Matthias Mölle and myself were responsible for the technical realisation and conducting of the experiments. Data assessment for the second and third experiment were performed with assistance of the medical students Jonathan Beyer, Simon Janz and Isabel Faude as part of their medical doctoral degree and Arjan Miedema, M.Sc., who stayed for an internship under my supervision at the Institute of Medical Psychology and Behavioural Neurobiology in Tübingen.

Data and statistical analyses of the acquired behavioural and electrophysiological data were performed together with Matthias Mölle, who at first instructed me and later only assisted on specific aspects dedicating the responsibility more and more to me. The results were afterwards interpreted by Jan Born, Thomas Martinetz, Matthias Mölle and me.

For all the publications I drafted the first manuscript, which were then edited by Matthias Mölle, Jan Born and Thomas Martinetz.

List of Publications

- Schellenberger Costa, M., Weigenand, A., **Ngo, H.-V.V.**, Marshall, L., Martinetz, T. & Claussen, J.C. (under review). A thalamo-cortical neural mass model of the EEG during NREM sleep and its response to auditory stimulation.
- **Ngo, H.-V.V.**, Miedema, A., Faude, I., Martinetz, T., Mölle, M. & Born, J. (2015). Driving sleep slow oscillations by auditory closed-loop stimulation - a self-limiting process? *Journal of Neuroscience* 35, 6630–6638.
- Weigenand, A., Schellenberger Costa, M., **Ngo, H.-V.V.**, Claussen, J.C., Martinetz, T. (2014). Characterization of K-complexes and slow wave activity in a neural mass model. *PLoS Computational Biology* 10, e1003923.
- **Ngo, H.-V.V.**, Martinetz, T., Born, J. &, Mölle, M. (2013). Auditory Closed-Loop Stimulation of the Sleep Slow Oscillation Enhances Memory. *Neuron* 78, 545–553.
- **Ngo, H.-V.V.**, Claussen, J.C., Born, J. & Mölle M. (2013). Induction of slow oscillations by rhythmic acoustic stimulation. *Journal of Sleep Research* 22, 22–31.
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- Mayer, J., **Ngo, H.-V.V.** & Schuster, H.G. (2012). Dynamical mean-field equations for a neural network with spike timing dependent plasticity. *Journal of Statistical Physics* 148, 676–685.
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- **Ngo, H.-V.V.**, Köhler, J., Mayer, J., Claussen, J.C. & Schuster, H.G. (2010). Triggering up states in all-to-all coupled neurons. *Europhysics Letters* 89, 68002.