Aus dem Institut für Medizinische Psychologie der Universität Tübingen

Targeted memory reactivation during sleep with closed-loop auditory stimuli: Comparing the effects of slow oscillatory up-phase and down-phase cueing on sleep-dependent declarative memory consolidation

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To my parents

Li Fang-Heck and *Dr. Alfred Heck*

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List of Abbreviations

ACLS	Auditory closed-loop feedback system
AEP	Auditory evoked potential
DS	Digit Span
EEG	Electroencephalogram
EMG	Electromyography
EOG	Electrooculography
ERP	Event-related potential
fMRI	Functional magnetic resonance imaging
GABA	Gamma-Aminobutyric acid
LLR	Late latency response
LTM	Long-term memory
MLR	Middle latency response
MMN	Mismatch negativity
MT	Movement time
Non-REM	Non-rapid eye movement
PAL	Paired associate learning
PANAS	Positive and Negative Affect Schedule
PTSD	Post-traumatic stress disorder
PVT	Psychomotor Vigilance Task
REM	Rapid eye movement
RWT	Regensburg Word Fluency Test
SEM	Standard error of mean
SF-A-R	Sleeping Quality Questionnaire (Schlaffragebogen A)
SO	Slow oscillation
SPL	Sound pressure level
SPSS	Statistical Package for the Social Sciences
SSS	Stanford Sleepiness Scale
STM	Short-term memory
SWA	Slow-wave activity

SWR	Sharp wave-ripple
SWS	Slow-wave sleep
WASO	Wake after sleep onset

1 Introduction

1.1 Memory

‘Memory’ is a single term that encompasses a wide range of abilities and crucial implications for human development. By making new memories, storing them and then later retrieving them when needed, we are able not only to remember the past but also to use that knowledge to adjust our current and future behavior according to past experiences. In other words, the capability to form memories is a fundamental component of adapting behavior to changing environmental needs during human development, making it one of the most important functions of the human brain. (Rasch and Born, 2013)

1.1.1 Basic memory functions

The concept of memory consolidation and the term ‘consolidation’ itself were first introduced by Müller and Pilzecker in their seminal monograph ‘Experimentelle Beiträge zur Lehre vom Gedächtnis’ (Experimental Contributions to the Science of Memory) published in 1900. Their work proposed that memories are not induced instantaneously but that the fixing (or consolidation) of permanent memories takes time, concluding that memories continue to be vulnerable to disruption for a period of time after learning (Lechner et al., 1999).

Encoding, consolidation and retrieval

In modern day neuropsychology, three fundamentally different subprocesses for the formation of memory are distinguished: encoding, consolidation, and retrieval. (Rasch and Born, 2013)

The term *encoding* corresponds to a learning process in which sensory information from the environment is perceived by our sensory organs and subsequently coded into a preliminary neuronal trace in the brain. The encoding process is still vulnerable to interferences which lead to the forgetting of the newly acquired information.

During *consolidation*, the labile newly encoded memory trace is strengthened and integrated into the pre-existing network of long-term memories without overriding the already stored memories.

Retrieval describes the process of bringing information back into conscious awareness from memory storage.

Whereas the encoding of new information and memory retrieval are processes mainly occurring during wakefulness, the "sleeping brain provides optimal conditions for consolidation processes that integrate newly encoded memory into a long-term store". (Rasch and Born, 2013; p. 683)

The modal model of memory

The multistore model of memory, also known as 'the modal model of memory', was first described by Richard Atkinson and Richard Shiffrin in 1968. It divides memory processing into three stages along a temporal dimension of duration: sensory memory, short-term memory, and long-term memory (see Figure 1). (Atkinson and Shiffrin, 1968)

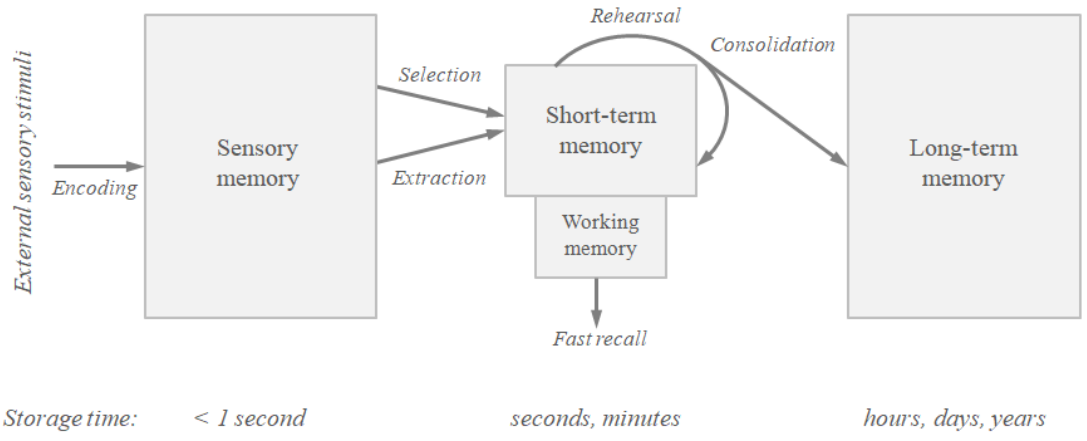


Figure 1: Stages of memory processing

Figure 1 depicts the three stages of memory processing and each stage's respective storage time. Information is encoded into sensory memory, selectively transferred to short-term memory, and can be further consolidated into long-term memory through repeated rehearsal of certain memory content. Working memory represents a special form of short-term memory, which allows fast recall of information, especially explicit memory content. Sensory and long-term memory stores have a rather large memory capacity compared to the limited short-term memory capacity. (derived from Pape et al., 2018)

Once a stimulus is perceived by sensory organs, the information is encoded into a labile neural trace which is subsequently stored in *sensory memory*, the first of the three stages. While a lot of information can be perceived simultaneously at this stage, the information can only be stored for less than one second after perception before being overwritten by

new incoming data. The sensory memory system includes iconic memory for processing visual stimuli (Sperling, 1960), echoic memory for processing auditory stimuli (Neisser, 1967), and haptic memory for tactile sensory stimuli (Bliss et al., 1966). While most memories are lost through distraction or passage of time during this stage of memory processing, a small selected part of data remains and is saved into *short-term memory (STM),* the second memory stage. STM stores information for a period of several seconds to minutes, with storage times growing with repetitious mental rehearsing of the information. George A. Miller was the first to introduce the idea that STM capacity is relatively limited, only being able to store a maximum of 7±2 'chunks' of information simultaneously (Miller, 1956). In psychology, 'chunking' represents the organization of information into groups or units, facilitating recall of more information (with more information being stored in one informational unit than before). STM capacity can for instance be tested using the Digit Span Task, by asking subjects to repeat a maximum of 9 numbers in the same order as presented shortly before (see Chapter 2.2.2 Psychometric assessment and additional cognitive tests). A special type of STM is *working memory*, which is essential for forming and accessing information particularly from declarative (explicit) memory. It allows fast access, holding information in consciousness for immediate use. Only when facilitated through repetition and conscious processing can information be further consolidated and stored into *long-term memory (LTM),* the third stage of memory. The LTM can store information over long periods of time, e.g., for hours, days, and years. Some memories, for instance childhood ones, even last until death. (Pape et al., 2018)

1.1.1.1 Long-term memory classification systems

LTM can be divided into two essentially qualitatively different memory systems: declarative (explicit) memory and non-declarative (implicit) memory (Squire, 1987; 2004). These two forms of memory can be further classified into various subcategories (see Figure 2).

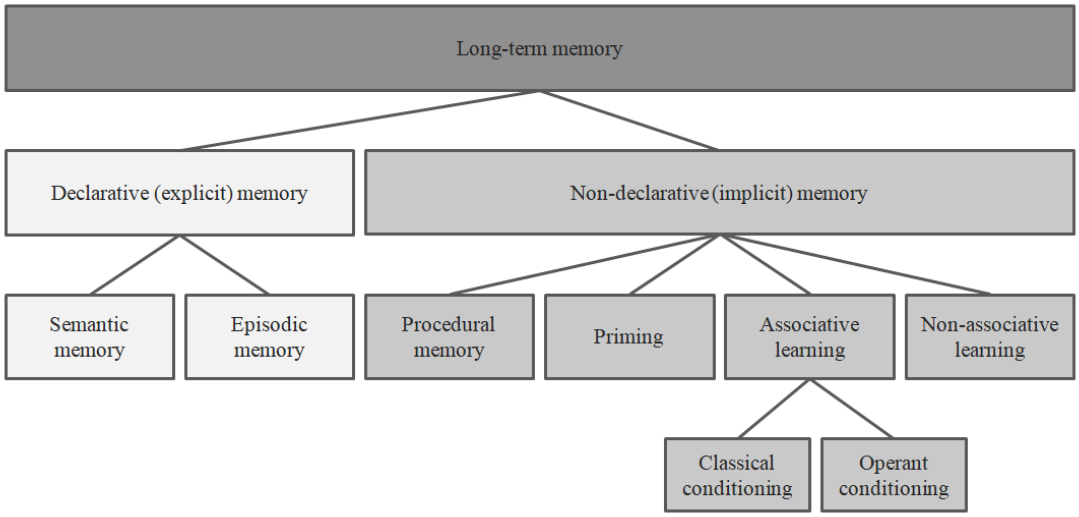


Figure 2: Long-term memory classification systems

Figure 2 depicts the classification of the declarative (explicit) and non-declarative (implicit) memory systems and their respective subcategories. (derived from Pape et al., 2018)

The *declarative memory* system is subdivided into semantic memory and episodic memory. *Semantic memory* stores factual knowledge, such as the knowledge that 6+2=8 or the knowledge that Bern is the capital of Switzerland. This thesis primarily focuses on this type of memory, since it also includes the conscious recollection of previously learned vocabularies or word pairs. Recall for this type of memory generally occurs consciously. *Episodic memory* contains the knowledge of personal experiences and past events, for instance, the recollection of one's last visit to Bern. An intact hippocampal function is imperative for encoding and short-term retrieval of declarative memories.

Non-declarative memory further includes four memory categories: procedural memory, priming, non-associative learning, and associative learning. This type of memory is generally less accessible to conscious awareness and is supported by widely varying brain regions, depending on which sensory mode is involved in a given task and whether or not performance of the task involves higher associative functions. The most important brain regions include the striatum, cerebellum, and the cortical association areas (Cohen and Squire, 1980). *Procedural memory* contains memory for motor skill acquisitions, such as tying a shoelace or riding a bike, and enables gradual learning of habits and skills. *Priming* is characterized by a change in performance associated with the repeated

processing of a stimulus. As an example, a person who has seen the word ‘mountain’ before might identify the word more quickly after being presented with the same word again (semantic priming). During *non-associative learning*, another category of implicit or non-declarative memory, the sensory perception or behavioral response to a sensory stimulus is altered upon repeated or continual presentation of the stimulus. It is either systematically attenuated (*Habituation*) or augmented (*Sensitization*). As opposed to non-associative learning, which depends upon the presentation of a single stimulus, *associative learning* requires the temporal pairing between two different sensory stimuli. One example of associative learning is *classical conditioning*. This concept was introduced for the first time by behavioral scientist Ivan Pavlov in 1927. He demonstrated that an initially neutral stimulus, such as a tone, when repeatedly paired with a biologically significant stimulus, such as food, could elicit a response showing the anticipation of the food (Pavlov, 1960/1927). Another form of associative learning is known as *operant (i.e., instrumental) conditioning*. During this form of conditioning, a certain behavior is either rewarded or punished, leading to positive or negative reinforcement of the behavior.
(Brem et al., 2013; Pape et al., 2018)

1.1.2 Hippocampus and memory consolidation

In 1957, the seminal case study H.M. first provided crucial facts that led to the understanding of the underlying anatomic and physiologic mechanisms that form the basis of memory consolidation known today. After the bilateral surgical removal of the hippocampus and nearby structures of the entorhinal cortex, patient H.M. displayed the inability to form new memories and a partial retrograde amnesia of declarative memories, but retaining remote memories from his childhood (Scoville and Milner, 1957).
This discovery led to a vast increase in interest in the hippocampal brain region and its seemingly essential role in the formation of declarative, especially episodic memory. Today, the hippocampus is one of the most extensively researched brain regions. It lies deep within the medial temporal lobe and receives and outputs neuronal information from and to other brain regions mainly through the entorhinal cortex. Based on the two-stage model of memory consolidation, especially the bidirectional information flow between hippocampus and neocortex seems to enable the formation of declarative memories by forming

the neuronal basis for active memory consolidation during slow-wave sleep. (see Chapter 1.3.2.1 Active system consolidation hypothesis) (Knierim, 2015)

1.1.2.1 The two-stage model of memory consolidation

"A fundamental question in memory research is how our brains can form enduring memories" (Frankland and Bontempi, 2005; p. 119). This key issue has also been referred to as the 'stability-plasticity dilemma' and, more precisely, deals with the question as to how the brain integrates new knowledge into pre-existing neural networks, which requires plasticity, without forgetting older memories in the process, which calls for stability. The *two-stage model of memory consolidation* is currently the most influential model of human memory consolidation and provides a widely accepted solution to this fundamental dilemma. The model is based on two separate memory stores, a temporary and a long-term store. In theory, newly acquired data is concurrently encoded into both stores. The temporary memory store, allows encoding and processing of newly acquired memory traces at a fast rate, but stores information only temporary. At this stage, the memory trace still remains unstable and prone to interference from more incoming data. The model assumes that the newly encoded memory traces are repeatedly reactivated in the temporary store, which drives simultaneous reactivation processes in the long-term store. This way, the new memory trace is gradually transferred to the long-term memory store, which processes information more slowly and over time strengthens and integrates the data into pre-existing neural networks without overriding old ones. This reactivation and redistribution process of newly acquired memories is assumed to take place during sleep, a time period without interferences from new incoming data. For declarative memory, the hippocampus is assumed to take the role of the fast learning, temporary memory store, whereas the neocortex seems to represent the slow learning long-term memory store (see Figure 3). (Frankland and Bontempi, 2005; Diekelmann and Born, 2010)

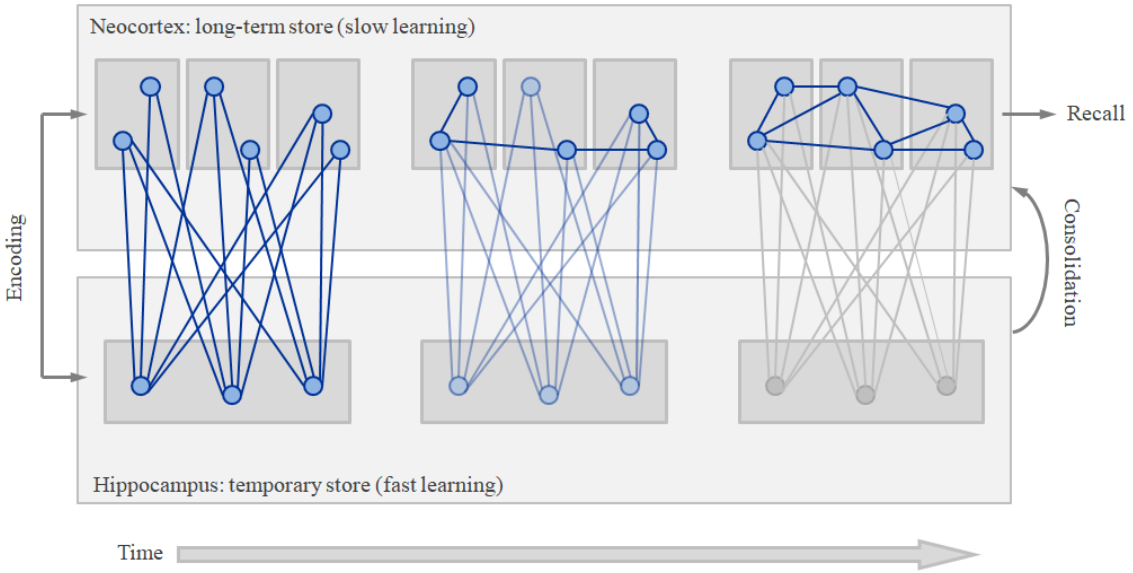


Figure 3: Two-stage model of memory consolidation for declarative memory

Repeated reactivation of memory content in the temporary memory store (Hippocampus) drives slow repeated reactivation processes in the long-term memory store (Neocortex). This eventually allows long-lasting neuronal changes in neocortical sites which gradually grow independent of the hippocampus. This mechanism enables slow and progressive transfer and integration of memories to hippocampus independent long-term memory stores for later recall. (derived from Frankland and Bontempi, 2005; Diekelmann and Born, 2010)

Nonetheless, the two-stage model not only seems to apply to declarative memory consolidation but might also explain how procedural memories are consolidation over time., with e.g. the cerebellar cortex representing the initial temporary store (Krakauer, 2006). This discovery entails the assumption that the two-stage model of memory might be generally applicable to all types of memory. (Rasch and Born, 2013)

1.2 Sleep

"It seems as if the world did not wholly possess us adults, it has only two-thirds of our life, we are still one-third unborn. Each awakening in the morning is then like a new birth." [Translated from German]

Sigmund Freud, A General Introduction to Psychoanalysis, 1920

The mysterious condition of body and mind known as sleep has fascinated people of different eras and cultures for centuries. Sleep is an essential part of our life; about one-third of our lifetime is spent sleeping. While various functions and underlying mechanisms of sleep remain unknown to this day, extensive sleep research during the last decades has led to new scientific methods and enabled the discovery of various components and physiological characteristics of sleep that form the basis of modern sleep research today.

1.2.1 Polysomnography

Polysomnography is an objective method used to continuously register and record multiple physiologic measurands during sleep, generally including inter alia brain activity, cardiac and respiratory parameters, muscle tone of extremities, and body positioning.
In this study, polysomnography was performed comprising only three main measurands: electroencephalography (EEG) to record brain activity, electrooculography (EOG) to register eye movements, and electromyography (EMG) to measure facial muscle tone during sleep.

1.2.1.1 Electroencephalography

In 1875, British physiologist Richard Caton was the first to record electrical brain activity (Caton, 1875). His initial finding on animals laid the foundation for German psychiatrist Hans Berger, who was the first to ever measure brain 'waves' on humans during the first half of the twentieth century. (Berger, 1929)
Since then, electroencephalography (EEG) has become firmly established as an important, non-invasive measurement method used to analyze sleep by detecting, amplifying and recording bioelectric activity in the living human brain. More precisely, the bioelectric activity originates from the summation of synaptic electrical potentials generated by

groups of cortical neurons, the pyramidal cells. Neurons communicate with each other through electrical impulses. Due to signal attenuation caused by the human skull, only the cumulative synchronous electrical activity from large populations, i.e., thousands of neurons, create detectable cortical potentials. Detection is then accomplished by electrodes attached to the surface of a subject's scalp. The recorded data is subsequently sent to a computer where the potential difference is amplified and displayed as distinct EEG waveform patterns. (Tatum, 2014; Pape et al., 2018)

EEG waveform patterns

On a cellular level, the amplitude of EEG waveform patterns depends largely on the synchrony of the neuronal activity. Groups of neurons that are simultaneously activated result in a high amplitude brain wave recording. Unsynchronized activity leads to reciprocal erasure of single neuron signals and results in irregular brain waveform patterns of smaller amplitude. In addition, brain wave frequencies are highly susceptible to interferences and inter alia dependent on the activity of the cerebral cortex, often correlating with certain behavioral states, for example attention, sleep, and wakefulness. An increase in information processing leads to higher basic frequencies, whilst a decrease in sensory input during sleep causes the recorded brain waves to lower in frequency. Nonetheless, a series of distinct wave patterns is recognized to exist in healthy humans. Each individual pattern is named with a letter from the Greek alphabet. These EEG signals are generally classified according to amplitude, frequency, shape, and topography. (Tatum, 2014)
An example of typical EEG waveform patterns detected in healthy humans during EEG monitoring is depicted in Figure 4.

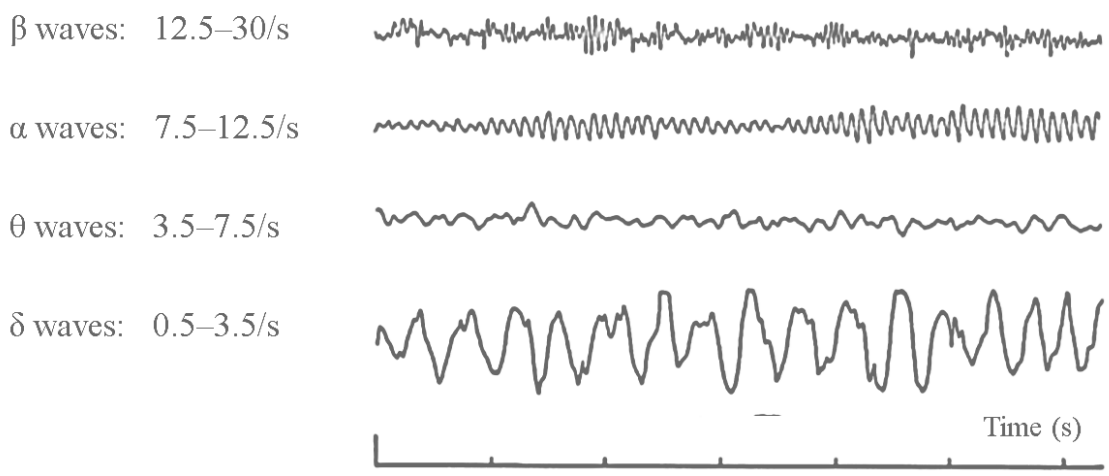


Figure 4: EEG waveform patterns

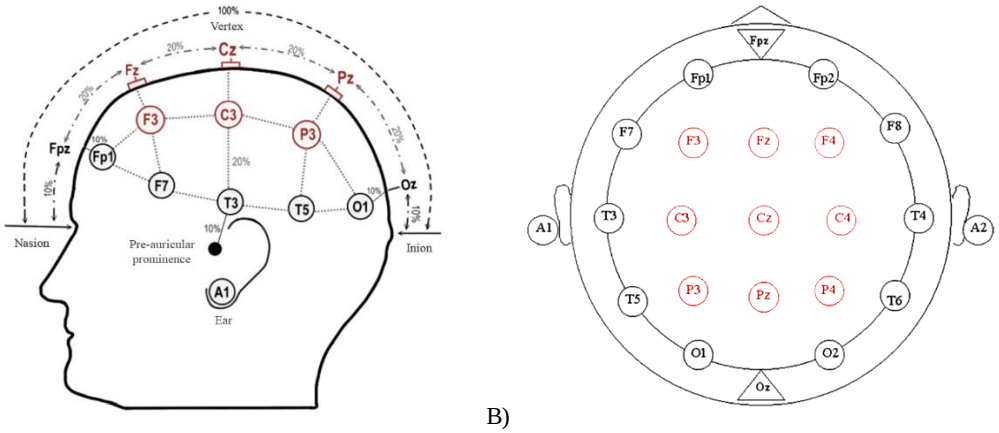
Figure 4 depicts the typical EEG waveform patterns and frequency ranges in use today. Γ (Gamma) waves with a frequency range above 30/s are not depicted because they are not typically discernible in the routine-EEG. They are usually examined using specially configured amplifiers and by means of spectral analysis. B: beta; α: alpha; θ: theta; δ: delta; s: seconds. (derived from Pape et al., 2018)

Delta rhythm (δ) with the lowest average frequency of 0.5–3.5 Hz and the largest amplitude of all waveforms, can be observed characteristically during deep sleep phases.
Theta rhythm (θ) waveforms are predominantly found during sleep onset with an average frequency of 3.5–7.5 Hz and varying amplitude and morphologies.
Alpha rhythm (α) waveforms are predominantly observed during relaxed wakefulness, when the subject's eyes are closed. Alpha waves are composed of 7.5–12.5 Hz frequencies (alpha frequency), distributed predominantly over occipital head regions and shifting anterior during drowsiness. Within the alpha frequency band, *Mu rhythm (μ)* and *Kappa rhythm* (κ) waveforms can be differentiated. Whereas Mu waves are higher in amplitude over motor-related and somatosensory cortical areas, Kappa rhythms primarily predominate over auditory cortical areas.
Beta rhythm (β) waveforms typically show frequencies of 12.5–30 Hz, spreading to temporal regions. Their appearance blocks the basic alpha rhythm when subjects open their eyes, causing the so called 'Berger Effect'.
Gamma rhythms (γ) of 30–90 Hz frequency occur during focused attention e.g., when subjects perform on a demanding cognitive task. (Bear et al., 2018; Pape et al., 2018)

1.2.1.2 The international 10-20 system

Appropriate placement of electrodes on the scalp is essential for accurate assessment of the electrical fields produced by subcortical neurons. Consequently, an international guideline for electrode placement, the so-called '10-20 system', has been developed to ensure a standardized testing method based on the relation between electrode locations and underlying cortical areas of the brain.

Specific anatomical landmarks, i.e., the nasion (= top of the nose), the inion (= external occipital protuberance) and the tragus (= pre-auricular prominence) are used to measure the scalp to create a system of transversal and longitudinal rows, with electrode placement sites along those rows. The distance between these sites is subdivided by intervals of 10% to 20%, allowing the recording of defined brain areas in accordance with a variety of head sizes and shapes. The sites are named after the underlying brain regions they are reading from (i.e., pre-frontal (Fp), frontal (F), central I, temporal (T), parietal (P), and occipital (O). The electrodes following the central line of the scalp are assigned an extra 'z' as a second letter (e.g., Cz = central midline). The other electrode positions are marked with additional numbers: odd numbers (1,3,5,7) on the left, and even numbers (2,4,6,8) on the right hemisphere (see Figure 5). (Masuhr et al., 2013)



A) B)

Figure 5: General placement of EEG electrodes following the international 10-20 system

View from the side (A); View from above (B).
Figure 5 depicts the standardized placement of EEG electrodes on the scalp according to the international 10-20 system. In the present study, EEG electrodes were attached at positions F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4 (highlighted in red). (derived from Milnik, 2009)

1.2.1.3 Electrooculography and electromyography

Electrooculography (EOG) is based on the corticoretinal potential in the eyeball, which derives from the potential difference between the cornea (relatively electropositive) and the retina. Eye movements change the orientation of the dipole, causing an electric field potential difference to occur. This change in potential is recorded by attached electrodes. One EOG electrode is typically placed lateral to and above the outer canthus of one eye, whereas a second EOG electrode is attached lateral to and below the outer canthus of the other eye.

Electromyography (EMG) is based on the change in membrane potential in muscle cells. Electrodes placed on the surface of the skin above the corresponding muscle are able to record this electrical change. Two EMG electrodes are typically attached to the chin of a subject for sleep scoring purposes, as they are particularly important when differentiating REM-sleep from wakefulness (see Chapter 1.2.2 Sleep cycle and sleep stages). (Tatum, 2014; Pape et al., 2018)

There are two different possibilities for interconnecting electrodes in order to display the potential difference between two measuring points: a bipolar or a unipolar interconnection. A unipolar interconnection measures the input of an electrode referenced to a reference electrode; a bipolar interconnection references two different electrodes against each other. (Pape et al., 2018)

1.2.2 Sleep cycle and sleep stages

The classification of sleep EEG signals into sleep stages is called sleep scoring and is performed following the standard rules of the ‘Association for the Psychophysiological Study of Sleep’, published by Rechtschaffen and Kales in 1968 (Rechtschaffen and Kales, 1968). EEGs, in combination with recordings of muscular and ocular electrical activity, are used to define five standardized stages of sleep. They include rapid eye movement (REM) sleep and non-REM sleep, the latter being divided into stages 1 (light sleep) to 4 (very deep sleep). The characteristics of the individual sleep stages are described in the following paragraph and are depicted in Figure 6.

Sleep stages

Sleep stage 1 is characterized by slow rolling eye movements and a very low arousal threshold. In this stage, predominant waveforms consist of theta and very small amounts of alpha sleep activity (<50%). Sleep stage 1 lasts on average only a few minutes.
Sleep stage 2 is associated with the occurrence of K complexes and sleep spindles. K complexes are single high amplitude sharp waves, described as 'the largest event in healthy human EEG' (Cash et al., 2009). They typically have an amplitude of > 75 μV and a frequency of about 1–2 Hz. A sleep spindle appears as a group of EEG waves of increasing and decreasing amplitude with an average frequency of 10–15 Hz, which generally lasts 0.5–3 seconds. Sleep spindles mainly occur during sleep stage 2 but also appear during stages 3 and 4, although less frequently. *Sleep stages 3 and 4* are collectively known as slow-wave sleep (SWS), a sleep form with sleep wave patterns of high amplitude and low frequency, predominantly delta waves. Delta waves have a frequency of 0.5–4 Hz, an amplitude of > 75 μV and a duration of > 0.5 seconds. They begin to arise in sleep stage 2 but mark the onset of sleep stage 3 when > 20 % of delta activity predominates. They appear even more regularly in sleep stage 4 where they amount to > 50 % of all EEG signals. Sleep stage 4 shows the highest arousal threshold of all stages. It lasts for approximately 20–40 minutes during the first sleep cycle and is characterized by the appearance of so-called slow oscillations of <1 Hz. (Pape et al., 2018)

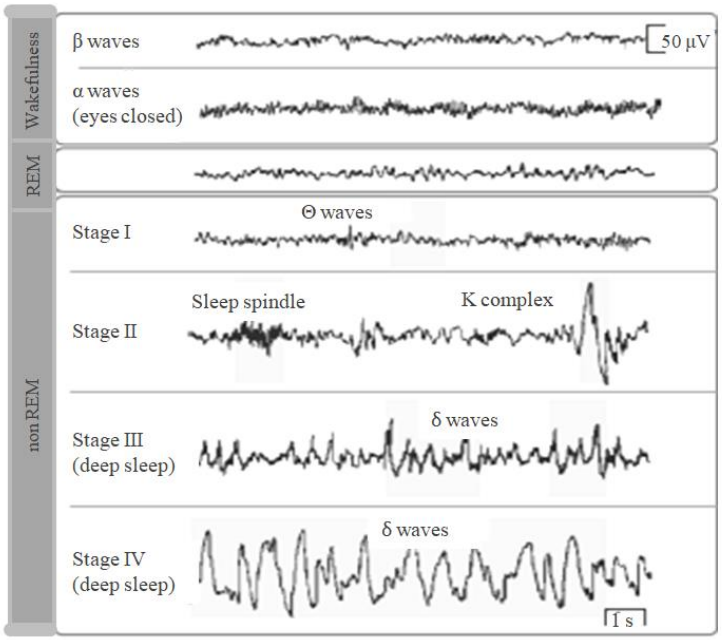


Figure 6: EEG rhythms during sleep

Figure 6 depicts the EEG waveform patterns characterizing the individual sleep stages. REM: Rapid eye movement; nonREM: sleep stages 1-4; β: beta; α: alpha; θ: theta; δ; delta; µV: microvolts; s: second (derived from Bear et al., 2018)

With the progression of these 4 sleep stages of *non-REM sleep*, EEG waveform patterns increase in amplitude and decrease in frequency, indicating an increasing underlying synchronized neuronal activity. Therefore, these sleep stages are collectively described as *synchronized* or *orthodox* sleep.

Non-REM sleep seems to represent a phase of rest as is characterized by an activation of the parasympathetic nervous system with, for example, reduced muscular tension, body temperature, and energy consumption.

In contrast, *REM sleep* is predominantly characterized by periodic occurrences of rapid eye movements (hence the name REM) and muscle atony in most of the body. First described and analyzed in 1953 by Aserinsky and Kleitman, REM sleep was shown to have a low fast frequency EEG similar to sleep stage 2, with short occurrences of beta waves which otherwise form during wakefulness (Aserinsky and Kleitman, 1953). It is therefore also known as *desynchronized* or *paradox* sleep. Although the arousal threshold for external stimuli remains high during REM sleep, spontaneous awakening typically occurs during this sleep phase. (Tatum, 2014)

Sleep cycle

Human nocturnal sleep typically follows a pattern of gradual cyclic changes of sleep depth and specific patterns of electrical field potential oscillations (see Figure 7).
It comprises of two fundamentally different sleep phases: non-REM sleep and REM sleep. Progression from light sleep (stage 1) to deep sleep stages 3 and 4 in non-REM sleep is followed by a period of REM sleep, before the cycle reverses and then repeats itself. These cycles of alternating sleep stages last for approximately 90-100 minutes each and ordinarily repeat three to five times during one night. SWS predominates during the first half of the night, decreasing in intensity and duration with each cycle. The amount of REM sleep increases during the second half of the night, from 10 to a maximum of 30 to 50 minutes per cycle, resulting in little or no SWS at the end of the sleeping period. (Bear et al., 2018; Pape et al., 2018)

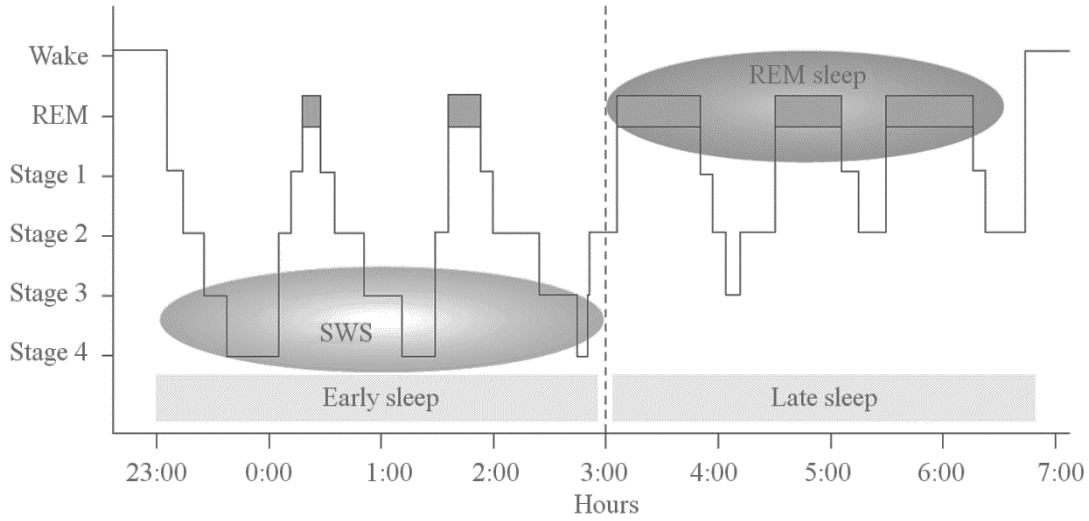


Figure 7: Hypnogram of an average sleep cycle

Figure 7 schematically depicts the generalized sleep architecture during the course of one night. Wake: wakefulness; REM: rapid eye movement; SWS: slow-wave sleep (i.e., sleep stages 3 and 4). (derived from Diekelmann and Born, 2010)

1.2.3 Spindles, ripples and slow oscillations

Sleep and individual sleep stages are associated with specific EEG field potential oscillations. Neocortical slow oscillations, thalamocortical spindles and hippocampal ripples are characteristic field potential oscillations associated with SWS (see Figure 8) and are

proposed to play a causal role in declarative memory consolidation during this sleep stage. (Rasch and Born, 2013)

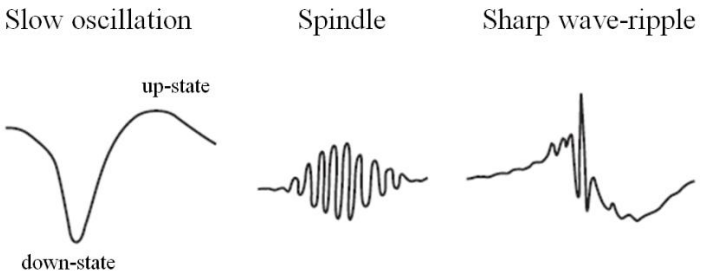


Figure 8: Field potential oscillations during slow-wave sleep

Figure 8 illustrates the characteristic EEG field potential oscillations associated with SWS; a slow oscillation, a spindle and a sharp wave-ripple. (derived from Diekelmann and Born, 2010)

Slow oscillations (SOs) primarily originate in the prefrontal neocortex during SWS. They consist of abrupt fluctuations in the membrane potential of neocortical neurons with a frequency of <1 Hz. Neuronal membrane hyperpolarization (neuronal silence) representing 'down states' transitions to neuronal depolarization ('up states'), which is caused by strong simultaneous firing of large groups of neocortical neurons (~10–20 mV difference) (Steriade, 2006). By globally inducing synchronized neuronal activity not only in the neocortex but also via efferent pathways in other brain regions, SOs have the important ability to temporally coordinate other cortical patterns, such as sleep spindles and hippocampal sharp wave-ripples. This seems to, inter alia, temporally coordinate the bidirectional information flow between neocortex and hippocampus, thereby enabling consolidation of hippocampus-dependent memories (Sirota and Buzsaki, 2005).

A Sleep spindle can be described as a group of EEG waves of increasing and decreasing amplitude with an average frequency of 10 to 15 Hz, which generally lasts 0.5–3 seconds. Sleep spindles mainly occur during sleep stage 2 but also appear during SWS, although less frequently. Spindle activity is predominantly generated in the thalamus, a structure in the diencephalon. GABAergic neurons of the thalamic reticular nucleus set the pace by generating repetitive inhibitory postsynaptic potentials which consequently lead to rebounding spike-burst activity in glutamatergic neurons of corticothalamic projections.

This rebounding excitatory activity can then be viewed as sleep spindles on the EEG. (Gennaro and Ferrara, 2003).

Two types of spindles are distinguished depending on frequency, functionality, and topography. (Mölle et al., 2011)

Slow spindles (<12 Hz) predominantly arise over frontal cortical areas. They seem to play a role in the transition of SOs from up state into down state and occur shortly (approx. 125 ms) before the negative SO peak.

Fast spindles mainly occur over the central and parietal cortex and precede slow spindles by approximately 500 ms. In contrast to slow spindles, fast spindles (>12 Hz) seem to show synchronization with SO up states and suppression during SO down states, which suggests that especially fast spindles play an important role in neocortical and hippocampus-dependent memory processing during sleep.

Supporting this theory are findings demonstrating an increase in fast spindle (12–15 Hz) density, activity and number in the night succeeding subjects' performance on a declarative memory task (Gais et al., 2002).

Using EEG-informed functional magnetic resonance imaging (fMRI), Bergmann et al. were additionally able to demonstrate reactivations of neocortical and hippocampal brain regions occurring temporally synchronized with fast spindle events during post-learning sleep of declarative information. The regions reactivated were the same ones that were previously activated during pre-sleep encoding processes. Reactivation strength also correlated with spindle amplitudes. (Bergmann et al., 2012)

Sharp wave-ripples (SWRs) are depolarizing events superimposed by high-frequency (100–300Hz) field potential oscillations generated in the hippocampus. During SWS and quiet wakefulness, ripple events seem to represent a temporally compressed reactivation of previous activity of hippocampal pyramid cells. Numerous studies have also proposed that SWRs facilitate synaptic potentiation in hippocampal and neocortical networks, which supports the current scientific view that SWRs are involved in off-line memory consolidation. (Girardeau and Zugaro, 2011)

The precisely timed interplay between SOs, SWRs and fast spindles with regard to their role in memory consolidation is further discussed in Chapter 1.3.2.1 Active system consolidation hypothesis.

1.2.4 Auditory evoked potentials

Presentation of external auditory stimuli elicit auditory evoked potentials (AEPs; or event-related potentials (ERPs)) in the EEG, which is a type of EEG signal that occurs in response to the stimulus and comprises of characteristic components (Joos et al., 2014). Compared to the general EEG, these evoked potentials show relatively small amplitudes (10 µV range), hence averaging the EEG activity time-locked to the presentation of the stimulus is required for analysis. Averaging is the process of separating the signal from the noise of unrelated EEG activity. AEPs correlate with synchronized neural activity in multiple cortical areas, auditory and non-auditory, reflecting the path of the sound's processing through the different brain structures. First proposed by Hallowell Davis in 1976, AEPs are to this day classified according to latency, which represents the time period between the onset of the stimulus and the onset of the response. Responses are consequently divided into early, middle and late responses (Davis, 1976). The exact latencies vary from subject to subject and vary from stimulus to stimulus but they are standardized to generally represent the average value in a young adult for a moderately intense stimulus (Picton, 2011). A schematic overview of these standardized AEPs is depicted in Figure 9.
Early responses, also called auditory brainstem responses, arise in the first 10 ms subsequent to the presentation of a stimulus and typically encompass five to six successive waves of the same polarity, numbered consecutively I-VI. These waves represent the sound's path through the brainstem and they seem able to be measured regardless of attention (Näätänen and Teder, 1991).
Auditory *middle latency responses* (MLRs) form 10-50 ms post-stimulus and comprise five peaks: P0, Na, Pa, Nb and Pb. They are named according to their polarity and in alphabetical order with 'P' standing for 'positive' and 'N' representing negative peaks. The path of the sound continues on through the higher nuclei of the brainstem (P0) and ends right below the auditory cortex (Na-Pb) where MLRs merge into *late latency responses* (LLRs). MLRs can be affected by attentional influences which generally causes high variability in waveform (Hansen and Woldorff, 1991).
LLRs are significantly larger in amplitudes than previous potentials which is due to the fact that they are generated in cortical areas that are closer to recording electrodes on the scalp. They comprise of waveforms between 100 ms and 300 ms post-stimulus: N100, P200, N200, Mismatch Negativity (MMN) and P300. The numbers behind the letters

represent the latency of each wave's peak in ms. MMN is a preattentive ERP that occurs when a regular sequence of auditory stimuli is disrupted by a deviant stimulus. It peaks at 100–200 ms (Näätänen et al., 1978).

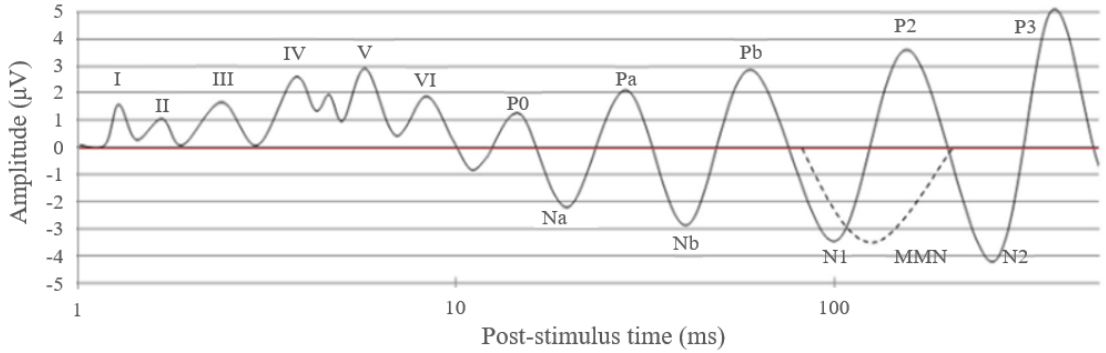


Figure 9: Schematic overview of auditory evoked potentials

Early auditory responses (waves I-VI), middle-latency responses (MLR; P0-Pb) and late-latency responses (LLR; N100-P300 ,i.e., N1-P3) are schematically plotted on a logarithmic axis. MMN: mismatch negativity; µV: microvolts; ms: milliseconds. (derived from Joos et al., 2014)

The different components of LLRs are determined not only by the multiple cortical areas contributing to its generation but are also affected by the composition of the acoustic stimulus, effects of selective attention and the anticipation of a known stimulus. LLRs are therefore also known as 'mesogenous', describing LLRs two main components, the earlier exogenous responses and later endogenous components. (Picton, 2011)

It has been suggested that earlier exogenous LLR components comprise of P50, N1–P2 and MMN and are mainly affected by physical properties of auditory stimuli and rather than by interference of higher order neural networks. P300 on the other hand, represents later endogenous responses which are predominantly associated with psychologic processes and the perception of a stimulus (Bekinschtein et al., 2009). The P300 response is therefore essentially influenced by higher order neural networks, the level of attention and awareness, as well as the cognitive context of the presentation of the stimulus (Polich and Kok, 1995).

Auditory evoked responses during sleep

Since sleep represents a state of reduced behavioral responsiveness, assessment of AEPs can function as a unique method to evaluate the extent of information processing of stimuli during sleep.

Analysis of averaged AEPs during sleep characteristically show the occurrence of a SO- or K complex like potential of high amplitude and low frequency. These evoked SOs showed theta/ slow spindle bursts nestled in the SO trough and increased fast spindle activity in the following SO peak. (Cairney et al., 2018)

Whereas various studies have additionally shown that even changes in intensity, frequency, timing and probability of occurrence of external sounds evoke changes in evoked potentials during sleep, it is not clear whether these changes merely reflect a physical reaction to changing stimulus characteristics or whether distinction of stimulus meaning is truly possible during sleep. Only the use of complex acoustic stimuli like e.g. words, which hold semantic information to some extent independently from their physical attributes, allow that differentiation. (Bastuji et al., 2002)

1.3 Memory consolidation during sleep

Upon observing a sleeping person, one would be tempted to describe sleep as a vulnerable state of decreased responsiveness to external stimuli, reduced motor activity, and most distinctively loss of behavioral control and consciousness. From an evolutionary point of view, particularly the loss of consciousness implies a significant survival disadvantage, contradicting the promotion of procreation in species. Yet, while many behavioral, physiological and neurological aspects of sleep vary broadly among species, sleep or sleep-like dormant states like rest, hibernation and torpor, have been researched and found to occur in various living organisms, even ones without nervous systems. These findings led to the hypothesis that sleep has a universal physiological function across all species, thus justifying sleep's existence and persistence in the evolution of species, but no consensus has been reached as to what that function might be. (Siegel, 2009)

One possible explanation for the loss of consciousness is the memory function of sleep. The processing of new information seems to exclude the possibility of simultaneous long-term memory storage of information because the same limited network capacities are used for both processes (Diekelmann et al., 2009).

1.3.1 Specific circumstances of sleep and memory consolidation

Sleep's role in memory consolidation has been widely researched for over a century and behavioral studies performed during the beginning of the 20th century, albeit not without conceptual flaws, established a basis for research on this subject today.

In 1914, Rosa Heine was the first to publish a systematic study, indicating a positive effect of sleep on memory. She tasked 6 subjects with the learning of word lists and nonsense syllables, and demonstrated that when subjects' learning phase was followed by a nighttime retention interval of sleep instead of a daytime retention interval of wakefulness, the amount of information forgotten 24 hours later was reduced significantly (Heine, 1914).

Building on Heine's experiment, multiple studies not only confirmed the beneficial effects of sleep on memory consolidation, but also specified the circumstances of the effect. For instance, sleep's effect on memory formation seems to be time-dependent, as the beneficial effect of sleep is greater the more directly after learning sleep occurs (Gais et al.,

2006). This can be explained by an idea first proposed by Müller and Pilzecker in 1900, which states that the consolidation process of new memories itself is time-dependent, with memories needing time to slowly strengthen against interference and decay (Müller and Pilzecker, 1990; Lechner et al., 1999). Furthermore, sleep appears to even strengthen memory traces against future interference (Ellenbogen et al., 2009). Sleep in general provides an optimal time window for the consolidation of memories, since it represents a state of reduced retroactive interference, i.e., less encoding of external stimuli and information processing than during wakefulness. Based on compelling evidence from research over the past two decades, the generally accepted belief today is that sleep not only passively protects the formation of newly encoded memories but also actively benefits memory consolidation (see Chapter 1.3.2.1 Active system consolidation hypothesis).

When subjects perform on a memory task, the depth of encoding can be modified, for example, by increasing the number of learning trials. Examining the encoding depth of declarative memories with a verbal paired associate learning task (similar to the one used in this study, see Chapter 2.2.1 Memory task), Drosopoulos and colleagues demonstrated that the beneficial effect of subsequent sleep was greater for word-pair lists learned to a criterion of 60% correct responses as opposed to 90% (Drosopoulos et al., 2007). This data suggests that the beneficial effect of sleep on memory consolidation changes with the strength of the new memory trace. A widely recognized assumption today is that initial memory strength and sleep-dependent memory enhancement correlate following an inverted U-shaped curve, in which memory traces that are initially encoded on an intermediate level benefit more from subsequent sleep than very weak and very strong memory traces. (Stickgold, 2009)

In addition, subjects' awareness of the learning process, i.e., explicit encoding, seems to increase the consolidation effect of sleep (Robertson and Pascual-Leone, 2004). As explicit encoding is associated with activation of the hippocampus, this implies a beneficial effect of sleep on hippocampus-dependent encoded memories (Greene, 2007). Furthermore, it has been demonstrated that memories show greater beneficial sleep-dependent memory consolidation effects when they are expected to be of future relevance (Wilhelm et al., 2011).

Whereas for a long time, REM sleep was assumed to play an essential role in memory consolidation due to its wake-like EEG activity, recent research suggests that memory

consolidation during sleep is influenced by the composition of sleep, demonstrating that different sleep stages influence the consolidation of different types of memory (Smith, 2001; Rauchs et al., 2005) and stressing the importance of the intact cyclic organization of sleep (Giuditta et al., 1995; Ficca et al., 2000).

1.3.1.1 Sleep and declarative and procedural memory

There is consistent evidence that sleep subsequent to performance on a declarative memory task can enhance declarative memory consolidation (Plihal and Born, 1997; Tucker et al., 2006). In most studies thus far, the tasks most commonly utilized to measure declarative memory retention during post-learning sleep are tasks which assess associative learning, e.g., verbal *paired associate learning (PAL)* (Plihal and Born, 1997; Marshall et al., 2004). Performance on a PAL task typically involves learning and immediate recall of a list of associated word pairs, which is followed by a period of subsequent sleep, and lastly cued recall. The difference in performance results between immediate recall before sleep and delayed recall after sleep is then evaluated to assess the benefits of overnight memory consolidation. Although not as common as PAL, other tasks have been utilized to suggest a functional significance of sleep on declarative memory, such as nonsense syllables (Benson and Feinberg, 1975), object locations (Rasch et al., 2007), and word lists (Lahl et al., 2008). Since declarative memory is generally highly susceptible to interference, the evaluation of sleep's effect on this type of memory is often characterized by the amount of less forgetting, or more specifically, memory retention. (Diekelmann et al., 2009)

Sleep has also been shown to benefit non-declarative memory, particularly procedural memory consolidation for motor skills (Fischer et al., 2002). Various tasks have been utilized to demonstrate and evaluate procedural motor memory consolidation during sleep, such as finger tapping (Walker et al, 2003), serial reaction time (Maquet et al., 2000), mirror tracing (Plihal and Born, 1997), and visual texture discrimination (Gais et al., 2000). Finger tapping tasks are motor sequence learning tasks that require subjects to repeatedly replicate sequences of numbers as rapidly and accurately as possible by tapping appropriate response buttons on a keyboard. Subjects are usually told the sequence during performance on the task. Serial reaction time tasks are tasks in which subjects are typically asked to react as quickly as possible to cues appearing at any one of four

positions arranged horizontally on a monitor by pushing spatially corresponding response buttons. The visual cues each appear according to underlying rules which are unknown to subjects during performance on the task. Results are primarily measured by subjects' individual response times to the appearing cues.

Sleep stages and memory

In the past, two main hypotheses have been introduced aiming to constitute the complex relation between sleep stages on memory consolidation.

The dual process hypothesis suggests that the first half of the night, dominated by SWS, particularly benefits the consolidation of declarative, hippocampus dependent memories, whereas REM sleep-rich retention sleep during the second half of the night selectively benefits procedural memories (Plihal and Born, 1997). This theory has mainly only been supported by studies in the past which performed selective sleep deprivation or compared retention performance across retention intervals that were filled with either early SWS rich sleep or late REM sleep-rich sleep (Yaroush et al., 1971; Plihal and Born, 1997). Additionally, the theory has been criticized and contrasted as results have not been consistent across the growing body of experimental studies which followed in recent years (Gais et al., 2000; Fogel et al., 2007; Schönauer et al., 2013). Also, possible contributions of sleep stage 2 to memory and potential confounds of induced stress by sleep deprivation were predominantly disregarded in studies supporting the hypothesis (Born et al., 2000). Multiple more recent studies, however, further support the central role of SWS in declarative memory consolidation. They implicate SWS in processes of synaptic downscaling (Tononi and Cirelli, 2006), reactivation, stabilization, and integration of declarative memories (Rasch et al., 2007) and – in contrast to previous findings – even of non-declarative memories (Schönauer et al., 2013). Whereas REM sleep may also benefit procedural memory consolidation, the specific underlying conditions and molecular mechanisms are still unknown, making future research necessary to define or redefine the role of REM sleep in memory processing (Ackermann and Rasch, 2014).

The *sequential hypothesis*, on the other hand, particularly highlights the importance of the undisturbed natural cyclic occurrence of non-REM and REM sleep stages during the night for an optimal effect of sleep on memory consolidation (Giuditta et al., 1995; Ficca et al., 2000).

Nonetheless, merely categorizing sleep into different sleep stages seems too simple when aiming to appropriately assess the complex effects of sleep on memory consolidation. More recent studies have therefore aimed to more precisely analyze the functional significance of distinctly prominent EEG features, such as sleep spindles and SOs, on sleep-dependent memory consolidation. Increases in sleep spindle and slow-wave activity (SWA) have been shown to correlate with and hence benefit sleep-dependent memory consolidation (Piosczyk et al., 2013, Ruch et al., 2012). Especially sleep spindles synchronized to the cycle of SOs seem to hereby benefit memory processing during sleep (Ngo et al., 2013). Sleep spindle activity and density have been shown to correlate with sleep-dependent declarative (Ruch et al., 2012) and procedural memory consolidation (Walker et al., 2002; Nishida and Walker, 2007).

1.3.1.2 Sleep and emotional memory

Every one of us knows from personal experience that emotional memories are better remembered than neutral ones. Accordingly, it has been demonstrated that sleep has a particularly beneficial effect on emotional memories, especially when filled with high amounts of REM sleep (Wagner et al., 2001). Even four years later the enhancing effect was still measurable, emphasizing the importance of REM sleep for the establishment of long-lasting emotional memories (Wagner et al., 2006). Furthermore, a significant correlation between the amount of REM sleep and emotional memory consolidation was demonstrated (Nishida, 2009). Furthermore, it has been suggested that the amygdala has the capability to enhance the hippocampo-neocortical dialog during sleep, consequently aiding in the long-term consolidation of episodic aspects of a memory (Sterpenich et al., 2007). Nonetheless, it is still unclear whether emotional stimuli during encoding processes simply enhance memory consolidation or if emotional memories are actually different in quality from neutral declarative memories.

To summarize, based on findings in current research it has been suggested that SWS, REM sleep and sleep stage 2 all aid memory consolidation, although a consensus has not yet been reached as to each sleep stage's specific role. It is important to note that performance on a specific learning task does not imply the activation of only one isolated memory system, but that memory systems have been shown to overlap or interact (Poldrack et al., 2001).

Furthermore, it has been demonstrated that procedural and declarative memory processes can be further subdivided into individual subprocesses, each benefitting from another sleep stage or a combination of these stages (Rauchs et al., 2005). Contradictory findings remain despite considerable advances in the last 10 years, demonstrating a need for future research to more clearly determine which memory process benefits from which sleep stage. Of particular note is the need for further research on the effects of sleep spindles and SOs, which represent more distinct EEG features and therefore a more precise way to analyze sleep-dependent memory consolidation than merely sleep stages.

1.3.2 Underlying mechanisms

To explain the underlying mechanisms of the beneficial effect of sleep on memory consolidation, two main theories have been proposed.

1.3.2.1 Active system consolidation hypothesis

Although this hypothesis is based on the standard two-stage model of declarative memory consolidation (see Chapter 1.1.2.1 The two-stage model of memory consolidation), it may also be valid for other types of memory processes.

The hypothesis suggests that sleep's active role in declarative memory consolidation relies on an active system consolidation process involving neocortical networks and the hippocampus. In this process, real life events are encoded in parallel into hippocampal and neocortical networks during wakefulness. During subsequent sleep, especially during SWS, newly encoded memory representations in the hippocampus are repeatedly reactivated and reorganized. During the reactivation process, these representations are selectively strengthened over time, and gradually transferred from the hippocampus to neocortical sites for long-term storage. There they are gradually integrated into the pre-existing networks in the neocortex, becoming increasingly independent from the hippocampus over time (Frankland and Bontempi, 2005). SOs, which are generated in neocortical circuits during SWS, play a central role in this dialogue between hippocampus and neocortex. They repeatedly initiate the memory reactivation process by synchronizing hippocampal SWRs together with thalamocortical spindle activity. Thalamocortical spindles are involved in the concurrent reorganization of the newly consolidated memory

representations by inducing enduring plastic changes in cortical areas and thus enabling long-term memory formation. (Born and Wilhelm, 2012)

The fine-tuned temporal interplay between SOs, spindles and ripples presumably mediates the redistribution of new memories from the hippocampus, serving as a temporary store, to neocortical regions for longer-term storage. The precisely timed interplay is regulated by neocortical SOs as depicted in Figure 10.

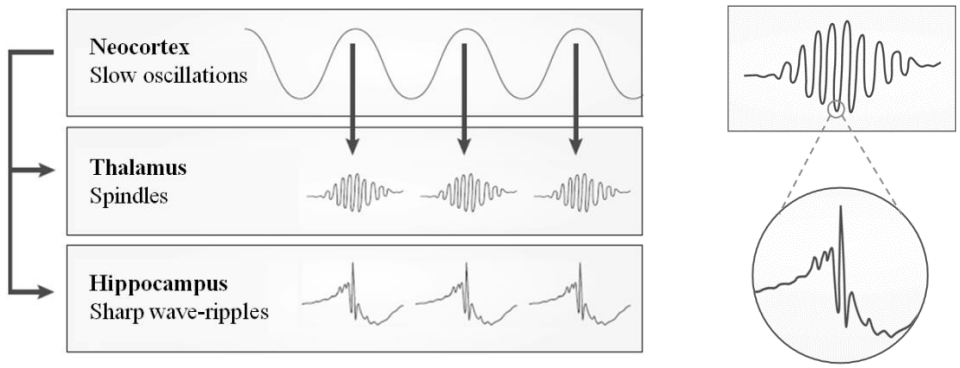


Figure 10: The hippocampal-neocortical dialogue

Neocortical slow oscillations temporarily regulate the dialogue between hippocampus and neocortex by driving repeated reactivation processes of hippocampal memory representations during SWS. During this process, SO up states occur temporally coupled to thalamocortical fast spindles, with hippocampal sharp wave-ripples nested in single spindle troughs (so called spindle-ripple events). (derived from Diekelmann and Born, 2010)

1.3.2.2 Synaptic homeostasis hypothesis

This widely accepted hypothesis proposes that the essential function of sleep is the restoration of synaptic homeostasis (Tononi and Cirelli, 2003, 2014). It is based on the following four main assumptions (see Figure 11):

First, during wakefulness, with the high intake of stimuli from real-life surroundings, synapses are potentiated in several cortical circuits in the course of subsequent encoding of information.

Second, encoding during wakefulness causes an increase in SWA during subsequent sleep, as the amount of synaptic potentiation correlates with SWA.

Third, SOs during SWS are associated with downscaling. By globally downscaling synaptic strength that was potentiated during wakefulness, SOs cause, for example, restoration of cellular energy and regulation of cellular homeostasis.

Fourth, this process of proportional downscaling during sleep enhances memory indirectly. Global downscaling of synaptic connections leads to erasure of previously weakly encoded memory representations, consequently improving the signal-to-noise ratio for the synapses of selected strongly encoded memories. (Tononi an Cirelli, 2003, 2014)

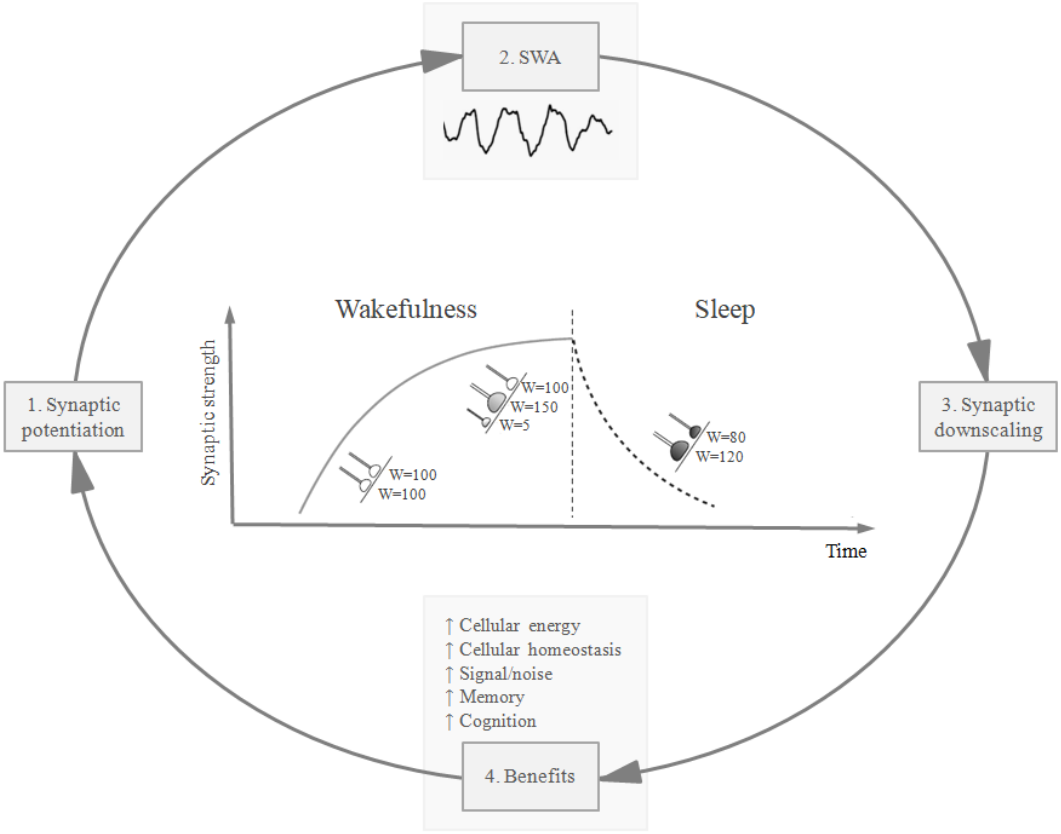


Figure 11: Synaptic homeostasis

Figure 11 depicts the four main assumptions of the synaptic homeostasis hypothesis. Encoding of new information during wakefulness leads to selective synaptic potentiation of neuronal representations, which is followed by a subsequent increase of slow-wave activity (SWA). During SWA, neuronal synapses are then globally downscaled which allows for, inter alia, restoration of cellular energy, regulation of cellular homeostasis, and selective enhancement of memory and cognition by preferably improving the signal-to-noise ratio for synapses of strongly encoded memories (derived from Tononi and Cirelli, 2006; Diekelmann and Born, 2010)

Both theories are well supported by several studies and do not mutually exclude one another (Diekelmann et al., 2009). For example, it has been suggested that the synapses of newly encoded memory representations, which are repeatedly reactivated during sleep,

might first be selectively potentiated and afterwards globally downscaled (Lewis and Durrant, 2011)

1.4 Memory reactivation during sleep

1.4.1 Endogenous memory reactivation during sleep

The widely accepted two-stage model of memory consolidation is based on the idea that repeated neuronal reactivations (or "replays") of newly encoded memory traces during sleep enable their gradual redistribution to a long-term memory store (see Chapter 1.1.2.1 The two-stage model of memory consolidation). These repeated reactivation processes of newly encoded memories during subsequent sleep have been strongly indicated to play a central role in the consolidation of these memories by various studies in the last decades. For example, an animal study conducted by Wilson and McNaughton first demonstrated that neuronal firing sequences in hippocampal cell ensembles during spatial behavioral tasks in rats had a tendency to repeat in the same order during subsequent SWS sleep (Wilson and McNaughton, 1994). Further animal studies found these post-learning repetitions of neuronal firing patterns during sleep not only in the hippocampus but also in other brain regions, such as the striatum (Pennartz et al., 2004; Lansink et al., 2008) and the medial prefrontal cortex (Euston et al., 2007). Neuronal reactivations of awake experiences during subsequent SWS have even been shown to occur temporally coordinated between different brain areas in rats, with hippocampal replays preceding cortical (Ji and Wilson, 2007) and striatal ones (Lansink et al., 2009). Another neuroimaging study on human subjects produced evidence of the processing of newly acquired spatial memories during post-learning non-REM sleep (Peigneux et al., 2004). The researchers demonstrated that the level of activity of hippocampal reactivations during sleep subsequent to performance on a declarative spatial memory task correlated with memory enhancement the next day. Another neuroimaging study on human subjects by Maquet and colleagues demonstrated that neuronal activity during performance on a serial reaction time task was replayed in the same brain areas significantly more during REM sleep in subjects who had previously performed on the task than in subjects who had not (Maquet et al., 2000). This finding supported the theory that newly encoded memories are processed during subsequent REM sleep in humans.

1.4.2 Cued memory reactivation during sleep

Building on findings of these various studies, Rasch and colleagues were the first to present evidence of the causal role of memory reactivations during sleep on subsequent memory consolidation (Rasch et al., 2007). In their landmark study in 2007, the researchers used an at that time new technique called *cued memory reactivation during sleep* (i.e., targeted memory reactivation) in order to directly modulate memory in humans. The researchers showed that presenting an odor (i.e., the smell of roses) to subjects first during their performance on a hippocampus-dependent object-location task, and then again during subsequent SWS, improved overnight declarative task related memory retention. Odor cueing proved to be ineffective when performed during REM sleep, during wakefulness, or when omitted during the previous learning task. Using fMRI, Rasch and colleagues additionally demonstrated that reapplication of the odor associated with prior learning reactivated the hippocampus during following SWS. Through these findings, the researchers concluded that the odor served as a cue which caused reactivation of the newly encoded object-location task memories in the hippocampus, thereby enhancing overnight memory retention. This evidence, demonstrating that externally applied cues during sleep could modulate and improve overnight memory consolidation, caused a sudden rise in interest in the new technique of cued memory reactivation within the scientific community. Inter alia, a following animal study on rats by Bendor and Wilson tested and reinforced Rasch and colleagues' conclusions by demonstrating that "a task-related auditory cue biased reactivation events toward replaying the spatial memory associated with that cue" (Bendor and Wilson, 2012; p. 1439). Their results were further evidence for the enhancing effect of externally applied cues on hippocampal neuronal reactivations and consequently memory consolidation, broadening the applicability of the cued memory reactivation technique even to animals.

Most recently, an increasing number of studies have focused on this evolving field of memory modulation during sleep and further demonstrated the effectiveness and beneficial effects of the cued memory reactivation technique for almost all types of memory, including declarative memory (Rudoy et al., 2009), procedural memory (Schönauer et al., 2014), and emotional memory (Lehmann et al., 2016).

General cueing procedure

In experimental set-ups, sensory cues, e.g., odors and sounds, are typically presented repeatedly to subjects while performing on a memory task, thus allowing an association between cues and learned memory content. During subsequent sleep, the same cues are presented again in order to trigger reactivation of the memories previously learned during encoding. As a consequence, memory recall performance the next morning is typically enhanced compared to a control condition without the presentation of cues during sleep. The type of cue (olfactory, auditory, etc.), the cueing protocol (frequency, intensity, duration, etc.), and the sleep stage during which the cues are presented largely affect performance results. (Schouten et al., 2017)

Types of cues

Studies to date have applied different types of sensory cues for the purpose of memory reactivation during sleep; in particular olfactory, auditory, and tactile cues. Each type of cue possesses unique properties and is processed differently in the brain, which makes each one suitable for different experimental set-ups and learning tasks.

Olfactory cues used in past studies mainly include pleasant odors such as the scent of roses (Rasch et al., 2007), violets, lavender and citrus (Cox et al., 2014), and occasionally also unpleasant chemical odors such as isobutyraldehyde (Diekelmann et al., 2012). Although the different olfactory cues were never directly compared, consistent results from previous studies suggest equal effectiveness on memory reactivation during sleep. When applying olfactory cues, they seem to become associated with the entire learning experience including not only the learning content but also external and internal aspects of the learning situation, for example the environment and subjects' current mood at the time of learning. One important advantage of olfactory cues is their inability to reliably disrupt ongoing sleep processes and sleep architecture (Carskadon and Herz, 2004), as opposed to auditory cues (Diekelmann, 2014). So far, olfactory cueing has elicited enhanced behavioral results mainly in declarative and emotional memory tasks. This may be due to the fact that odors enter the brain through the olfactory bulb and are directly transmitted to the hippocampus and amygdala, brain structures which are mainly associated with declarative and emotional memory processing (Zelano and Sobel, 2005).

Applied *auditory cues* vary in many aspects as they entail real-life sounds such as ‘the whistle of a kettle’ or ‘the meowing of a cat’ (Rudoy et al., 2009), simple tones (Simon et al., 2018) or even melodies (Schönauer et al., 2014). Additionally, some studies applied verbally spoken words from a subject’s native language (Cairney et al., 2017) or from a foreign language (Schreiner and Rasch, 2015a). Whereas most auditory cues elicit memory enhancing effects, their different properties and thus effectiveness are not yet clear and still subject to present research. The advantage of auditory cues seems to lie in their precise temporal controllability, which enables the reactivation of specific learning contents instead of an entire learning task or experience. As an auditory cue can express meaning, its content can be linked to a specific newly encoded memory trace; for example, the sound of the first syllable of a word can be linked to the picture of the word. Most importantly, this enables more accurate analysis of the effects of cueing since it is possible to compare recall of cue-associated memory contents with uncued contents within the same subject. In addition, they cause stronger and more consistent formation of evoked potentials in the EEG than olfactory cues, allowing accurate time-locked analysis of elicited neuronal responses. Auditory cueing has shown beneficial behavioral effects for all types of memory including declarative, procedural, and emotional memory. (Schouten et al., 2017)

Only one study so far has attempted the application of *tactile cues* in the form of mechanical fingertip stimulation (more specifically, imitation of the light pressure subjects felt when pressing keys while performing on a learning task). However, no behavioral effect was shown. (Pereira et al., 2017)

1.4.3 Auditory closed-loop stimulation

Enhancement of slow oscillatory activity

As illustrated in Chapter 1.3.2.1 Active system consolidation hypothesis, it is a widely supported assumption that SOs play a significant role in endogenous memory consolidation by repeatedly synchronizing hippocampal SWRs and thalamocortical spindle activity, thereby driving memory reactivation processes during SWS. However, the causal role of endogenous SOs on memory consolidation was first directly researched and indicated by Marshall and colleagues in 2006 (Marshall et al., 2006). The researchers externally

applied transcranial electrical currents with an equivalent frequency to endogenous SOs (0.75 Hz) during early non-REM sleep, not only enhancing endogenous cortical SOs and slow spindle activity, but also improving hippocampus-dependent declarative memory retention in human subjects. Due to these findings implicating SOs to be of functional significance for memory consolidation, further studies attempting and succeeding to induce SWA in humans through external application of stimuli followed, using inter alia transcranial magnetic (Massimini et al., 2007) and auditory stimulation (Tononi et al., 2010). Nonetheless, generally inconsistent results regarding memory retention were observed, which might be explained by the researchers not considering the phase of ongoing endogenous slow waves when performing stimulation.

Conclusively, since slow oscillatory activity in humans has been demonstrated to be modulated and enhanced by externally applied stimuli, and since this activity seems to play an essential and causal role in overnight memory consolidation, SOs are also indicated to represent an essential target time window for cued memory reactivation. However, there seems to be a need for precise SO phase-dependent cueing for optimal overnight memory enhancement.

The auditory closed-loop stimulation system

The precise presentation of external auditory stimuli in synchrony with recurring endogenous SOs was enabled by the development of a so-called *auditory closed-loop stimulation (ACLS)* system (Ngo et al., 2013). ACLS is triggered automatically after online detection of SOs via an EEG amplitude threshold detection algorithm. This stimulation method utilizes auditory cues that are particularly suitable because their precise temporal applicability enables stimulation during very specific time windows within a sleep stage, as opposed to olfactory cues. Ngo and colleagues demonstrated for the first time that closed-loop auditory stimulation in-phase with SO up states boosted SWA phase-coupled spindle activity, and improved overnight memory consolidation whereas out of phase stimulation remained ineffective. (Ngo et al., 2013).

1.5 Hypothesis

The main focus of this study lies in the identification of the most effective timing for auditory cued reactivation during non-REM sleep.

Following the widely accepted assumption that slow oscillations drive memory reactivation processes during SWS, this study compares the effectiveness of auditory cueing during SO up states with SO down states. Effectiveness is measured through post-sleep declarative memory recall performance during both conditions.

As SO up states are assumed to represent a state of increased neuronal firing, the hypothesis of this study proposes that auditory cueing in-phase with online detected SO up states leads to better declarative memory consolidation than cueing during SO down states.

2 Methods

2.1 Subjects and procedure

2.1.1 Subjects

Sixteen healthy, male, native German subjects (mean age 24.4 ± 0.76 years, range 18 – 30 years) participated in this randomized, within-subject cross-over study. They stated that they were nonsmokers, free of medication, had average hearing abilities, and did not have any history of sleep disturbances and depression. Additionally, subjects declared that they followed a regular sleep-wake cycle for 6 weeks prior to the experiments meaning they did not carry out night or shift work and did not nap during the day. Furthermore, all participants stated that they were not subjected to any extreme psychological or physical stress during the time of the experiments.
On the day of each session, all participants were instructed to abstain from drinking any caffeinated drinks after 2:00 p.m. Alcohol as well as any extreme sport was not permitted 24 hours prior to any session. If existing, participants were required to shave off their beard for optimal electrode placement on the chin.
Each subject's information was discussed verbally and documented in writing.

All participants gave written informed consent before participation. Participation was voluntary. A monetary reward was paid to each subject.
The study was approved by the Ethics Committee of the Eberhard Karls University (project no.: 32/2014BO2).

2.1.2 Experimental set-up/ Design

Adaptation and experimental nights took place in the sleep laboratories of the Institute for Medical Psychology and Behavioural Neurobiology of the University of Tübingen. Subjects spent experimental nights in the same room in which the adaptation night took place.
Signal processing of acquired signals, continuous monitoring of sleep quality and stimulation were conducted on-line by the examiner in the connecting monitoring room.
In this study, continuous closed-loop auditory stimulation of SO-signals was compared in two experimental conditions, an 'up-phase' stimulation condition and a 'down-phase'

stimulation condition. In the ‘up-phase’ stimulation condition, closed-loop auditory stimuli were delivered simultaneously with detected up-phases of SOs during non-REM sleep. In the ‘down-phase’ stimulation condition, closed-loop auditory stimuli were delivered likewise but simultaneously with detected down-phases of SOs. An interval of 14 days (±1 day) was set between both conditions. The order of conditions was balanced randomly across subjects with subjects not knowing which condition was being performed on which night.

2.1.3 Procedure

Adaptation nights

Prior to the experimental sessions, subjects were adapted to sleeping under laboratory conditions during one adaptation night, in which in-ear headphones were attached, as well as electrodes for polysomnographic recordings. To ensure auditory stimulation during subsequent experimental conditions, each subject’s personal hearing threshold level was tested and recorded. Additionally, an adaptation night allowed verification of the existence of sufficient non-REM sleep necessary during experimental conditions. Subjects were instructed to arrive 1.5 hours prior to their usual sleeping time (around 9:30 pm) during adaptation nights. After attachment of electrodes and assessment of the personal hearing threshold level, subjects went to bed at their usual sleeping time (around 11:00 pm) and were awakened 8 hours later. During SWS, each subject’s individual SO waveform average was measured to ensure auditory cueing in-phase with SO up states during subsequent experimental nights for up-phase stimulation conditions (see Chapter 2.2.4 Detection algorithm and in-phase auditory stimulation). The German original protocol used during adaptation nights is supplied in the appendix in the original language (see Appendix, A1). The outline of proceedings of adaptation nights is illustrated in Figure 12. An interval of at least one night was set between the adaptation night and the first experimental night.

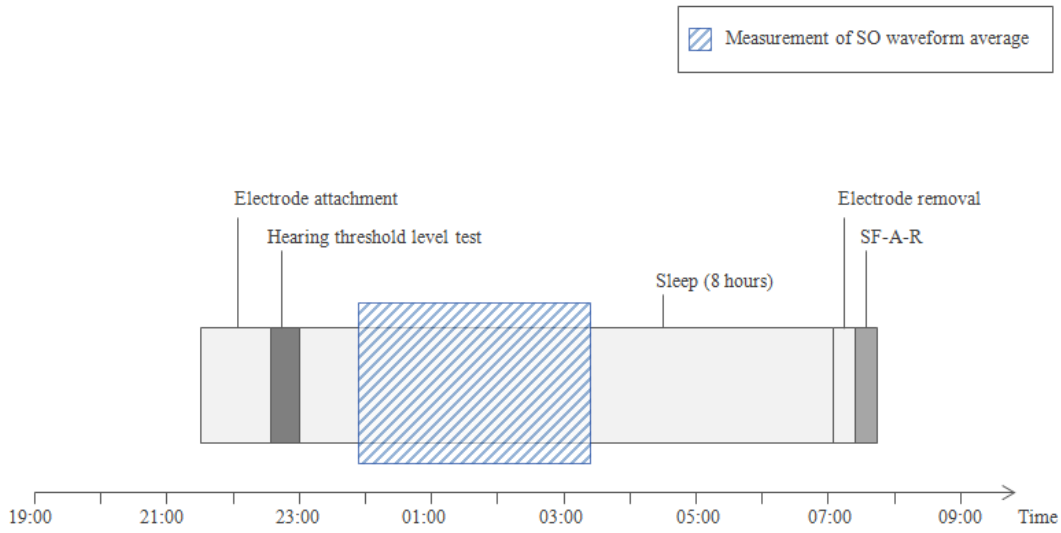


Figure 12: Outline of proceedings of adaptation nights

Subjects arrived on average at 21:30. Electrode attachment was followed by assessment of personal hearing threshold levels. Subjects went to bed at around 23:00 and awoke after approximately 8 hours of sleep at 7:00. Subsequently, electrodes were removed and subjects subjectively rated sleep quality in a questionnaire (SF-A-R). Each subjects' individual SO waveform average was measured during slow-wave sleep. SO: slow oscillation; SF-A-R: Sleeping Quality Questionnaire.

Experimental nights

Subjects were instructed to arrive 3.5 hours prior to their usual sleeping time (approximately 7:30 pm). Upon arrival, attachment of EEG electrodes for polysomnographic recordings followed. Subjects then rated their current emotional state on the Positive and Negative Affect Schedule (PANAS), and their sleepiness on the Stanford Sleepiness Scale (SSS). Following this, subjects performed on the Psychomotor Vigilance Task (PVT), on the Digit Span Task, and on the Word Fluency Test (RWT) (see Chapter 2.2.2 Psychometric assessment and additional cognitive tests).

Subsequently, subjects performed on a declarative memory task consisting of word pair associations (see Chapter 2.2.1 Memory task), including a learning phase and an immediate recall phase. In-ear headphones were attached and subjects went to bed at their usual sleeping time, which marked the beginning of polysomnographic and EEG recordings. 'Lights off' was marked in the EEG recordings as the starting time point for later sleep EEG analysis. Auditory stimulation began manually approximately 5 minutes after each subject entered stable SWS for the first time after sleep onset (i.e., after 30 seconds of

sleep stage 3 or 4 were confirmed on-line). Stimulation was paused whenever the monitored EEG recordings showed movement artifacts, signs of awakening, lighter sleep or REM sleep, and resumed after stable SWS was detected again. 180-210 minutes later, auditory stimulation was terminated. To allow better comparison between both conditions, stimulation during the second experimental night was terminated once the amount of stimuli presented during the first condition had been reached. Subjects were awakened after 8 hours of sleep, once no SWS or REM sleep phase was detected. A 'lights on' marker was placed in the EEG recordings. After electrode signal check and subsequent removal of the electrodes (roughly 30 minutes after awakening), subjects rated their sleep quality in a questionnaire (SF-A-R), and rated on the SSS and the PANAS once more. Afterward, PVT, Digit Span, and RWT were retested before recall of memories was reexamined. Experimental nights ended at approximately 9:00 am.

The protocol used during experimental nights is supplied in the appendix in the original language (see Appendix, A2). The outline of proceedings of experimental nights is illustrated in Figure 13.

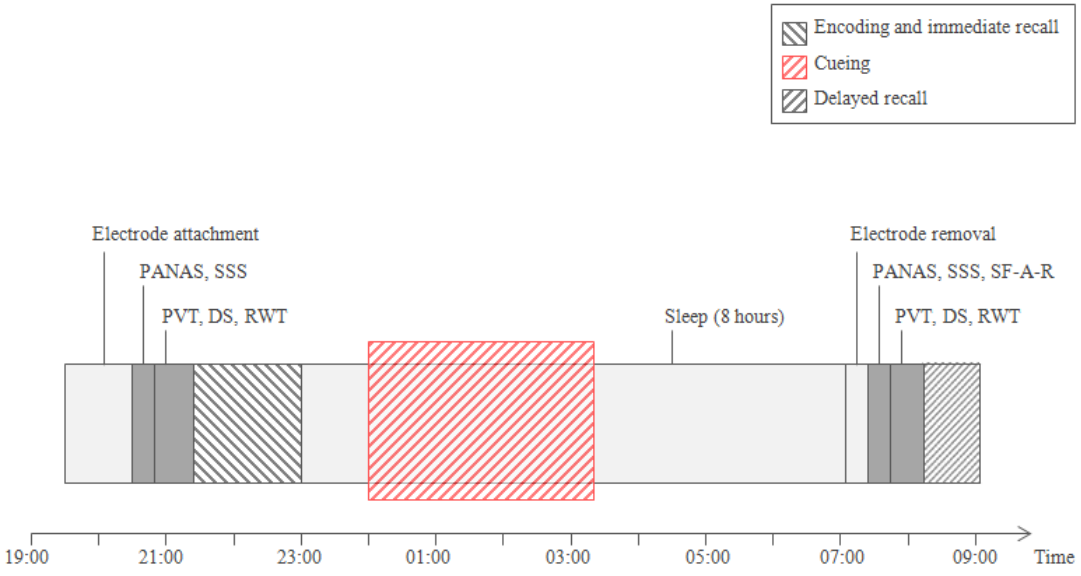


Figure 13: Outline of proceedings of experimental nights

Subjects arrived on average at 19:30. Electrode attachment was followed by self-rating on the PANAS and the SSS, and control tasks of PVT, DS, and RWT. Subsequently, subjects performed on a declarative memory task which included a learning phase and immediate recall before going to bed at approximately 23:00. Cueing was performed during the first 180-210 minutes of slow-wave sleep. Subjects awoke after approximately 8 hours of sleep at 7:00 the next morning. Electrodes were removed and subjects subjectively rated sleep quality in a questionnaire (SF-A-R). Self-rating was then once again performed on the PANAS

and the SSS, followed by control tasks of PVT, DS, and RWT. Sleep-dependent declarative memory retention was then tested during a delayed recall session. PANAS: Positive and Negative Affect Schedule; SSS: Stanford Sleepiness Scale; PVT: Psychomotor Vigilance Task; DS: Digit Span task; RWT: Regensburg Word Fluency Test; SF-A-R: Sleeping Quality Questionnaire.

2.2 Data acquisition

2.2.1 Memory task

Subjects were tested on a learning task for declarative memory called 'paired-associate learning task' (PAL), similar to PAL tasks used in previous studies (Plihal and Born, 1997; Marshall et al., 2004). In total, two different word pair lists containing 80 moderately semantically connected German word pairs, e.g., 'Winter – Unfall', 'Vulkan – Explosion' (winter – accident, volcano – explosion), were presented successively in a randomized order on a monitor. Simultaneously with the presentation of each word pair, the first syllable of the first word (i.e., the cue word displayed on the left) was played to the subjects four times over in-ear headphones, pronounced slowly and clearly by a neutral female voice. For instance, with appearance of the German word pair 'Vulkan – Explosion' (volcano – explosion) on the monitor, the syllable 'Vul' (vol) was played four times over headphones (see Figure 14). Subjects performed on one list of 40 word pairs during each experimental condition with the order of the lists randomized across subjects and conditions. To prevent serial learning, the sequence of word pair presentations within the lists was randomized for each round.

During the learning phase, the task consisted of memorizing single word pairs upon hearing the first syllable of the cue word in each pair. During the subsequent recall task, subjects were instructed to recall and verbally name single word pairs when presented with the first syllable of the cue word only (immediate recall). Thinking time was unlimited. After the subject gave a response, immediate feedback followed as the correct answer was displayed on the monitor while the corresponding syllable was played four times over the headphones once again. If, after the primary learning session, subjects failed to reach a minimum of 60% correct responses (i.e., 24 out of 40 correctly named word pairs), word pairs were presented again in a new randomized order, and cued recall was repeated. After four failed attempts at reaching the 60% criterion, exclusion from the study ensued.

As long as subjects reached the criterion within four attempts, they were tested again without any feedback presented on the screen. This immediate recall performance served as the pre-sleep performance. Subsequent to immediate recall, subjects went to sleep and auditory stimuli were presented during sleep. In the morning, delayed recall started approximately 1 hour after awakening. Subjects were tested on the same 40 word pairs in a different randomized order without feedback, presented in the same manner as during the last round of immediate recall.

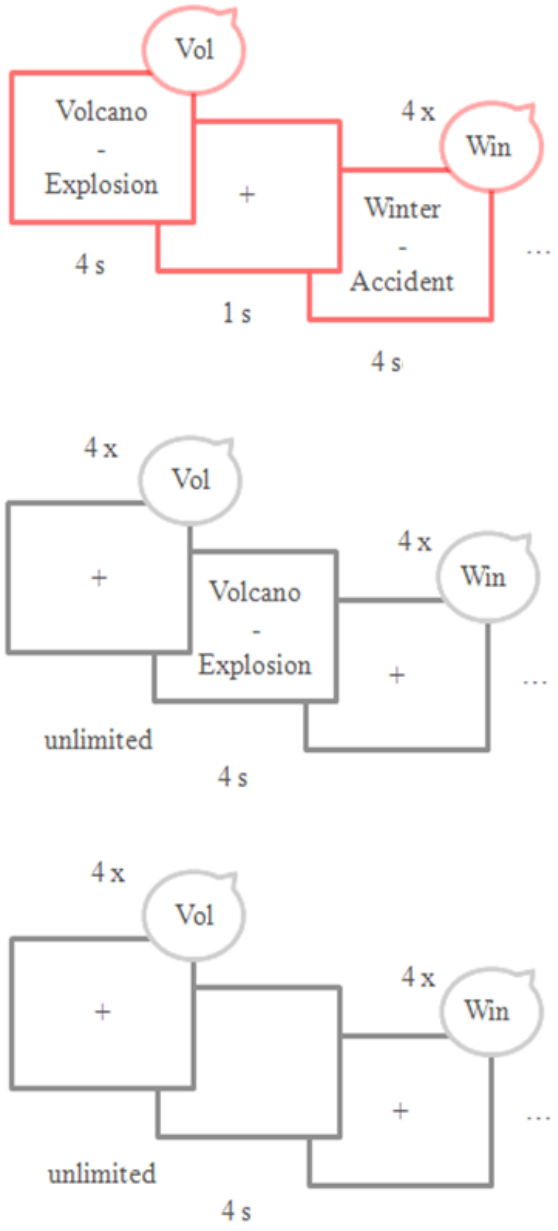


Figure 14: Schematic diagram of the memory task (PAL)

(A) Encoding
During learning, each word pair presentation lasted 4 s and interstimulus intervals were set for 1 s. Each syllable was presented 4 times during the 4-second word pair presentation, with each syllable lasting around 600 ms and with 500 ms long intervals between the repetitions.

(B) Recall with feedback
During immediate recall with feedback trials, syllables were presented 4 times, followed by unlimited thinking time for the subject performing. After giving a response, the subject pressed a key on the computer in order to be presented with the correct answer on the monitor (the corresponding word pair) and with the corresponding syllable played four times again over the in-ear headphones for further learning effect. Syllable and word pair presentations lasted the same amount of time as during learning trials.

(C) Recall without feedback
During the last immediate recall trial in the evening and the delayed recall trial in the morning after sleep, word pairs and syllables were presented in the same manner as in (B) but subjects were not presented the correct answer after giving their response (i.e., no feedback).
S: seconds.

The difference in the number of recalled word pairs during the last immediate recall in the evening and the delayed recall session the next morning was calculated to determine overnight declarative memory consolidation.
The German original word pair lists with corresponding syllables for both experimental nights are supplied in the appendix in the original language (see Appendix, A3).

2.2.2 Psychometric assessment and additional cognitive tests

Psychometric assessment

Subjects were instructed to rate themselves to assess psychometric data prior to encoding and delayed recall testing during both experimental nights using specific questionnaires, all of which are supplied in the appendix in the original language (i.e., German) (see Appendix, A4-6).

Positive and Negative Affect Schedule (PANAS)

To assess current feelings of tiredness and mood, subjects performed on a German version of PANAS, a questionnaire originally designed by Watson, Clark and Tellegen (Watson et al., 1988) and translated into German by Krohne, Egloff, Kohlmann and Tausch (Krohne et al., 1996). PANAS comprises of a list of 20 adjectives: 10 measuring positive affect, e.g., ‘interested’, ‘enthusiastic’ and ‘determined’, and 10 measuring negative affect, e.g., ‘irritable’, ‘ashamed’, ‘nervous’. Subjects responded using a 5-point scale from ‘not at all’ (1) to ‘extremely’ (5). Points for positive affect adjectives and for negative affect adjectives were summed up separately, resulting in a possible range from 10 to 50 for each category.

Sleeping Quality Questionnaire (SF-A-R)

In the morning after adaptation and experimental nights, subjects performed on SF-A-R, a self-report questionnaire created by Görtelmeyer (Görtelmeyer, 1986), which subjectively measures sleep quality. The questionnaire includes a report on, e.g., periods of wakefulness during the night, memorable dream content and the feeling of being refreshed after sleep. Subjects’ personal sleep quality ratings were awarded points from ‘1

= not good' to '5 = very good' (see question 9c in the original SF-A-R in the Appendix, A6).

Stanford Sleeping Scale (SSS)

To subjectively quantify their own sleepiness before and after each experimental condition, subjects rated themselves using the SSS scale (Hoddes et al., 1972) from '1 = feeling active, vital, alert, or wide awake' to '8 = asleep'.

Additional cognitive tests

To control extraneous variables, e.g., subjects' alertness, memory span and general vocabulary capacity, and therefore improve conditions for data comparison, subjects performed on additional cognitive tests and psychometric assessment tasks, which are presented below.

PVT

The Psychomotor Vigilance Task (PVT) was originally proposed by Dinges and Powell to objectively assess fatigue-related changes in alertness associated with sleep loss (Dinges and Powell, 1985). Subjects in this study performed on a shorter, 5 minute version of the original task (Roach et al., 2006), in which subjects were sat in front of a black computer screen. A red millisecond timer counting upward appeared repeatedly at random intervals on the black screen. Subjects were tasked to press a key to stop the timer as soon as they realized that it appeared on the screen. Subjects' reaction times were recorded in milliseconds. Random interstimulus intervals were set between 2 and 10 seconds. When calculating each subject's mean reaction time, the first 2 recorded reaction times of each subject were discarded for the reason of possible interference from the experimenter being in the same room. Reaction times <100 ms (prepress) and >30,000 ms (timeout) were not included in the calculation for subjects' mean reaction times either.

Digit Span

To assess attention efficiency and working memory capacity, subjects performed on a computerized version of the Digit Span Task (Sterne, 1969) in which subjects were presented with a sequence of single-digit numbers appearing on a computer screen whilst hearing them over headphones. Subsequently, subjects were tasked to recall the sequence correctly by entering it in order into the computer (forward span). The level of difficulty increased with the sequence length increasing in each trial, from 3 to a maximum of 9 numbers. If a sequence was recalled incorrectly, another sequence of numbers of the same length was subsequently presented. The task was automatically terminated after two failed trials of the same sequence length or after correct completion of the last sequence of 9 numbers. Each number appeared for a period of 1 second, with interstimulus intervals of 1 second.

After completion, the task was repeated, but with subjects entering the numbers in reverse order (backward span). The sequence length during the reversed Digit Span Task began from 2 and increased up to a maximum of 8 numbers.

For both forward and backward Digit Span Tasks, every sequence of numbers correctly recalled during the first attempt was awarded 2 points, and 1 point was awarded during the second attempt. 0 points were awarded when subjects failed to recall a sequence during their second attempt.

Regensburg Word Fluency Test (RWT)

To allow assessment of word fluency, subjects performed on the RWT (Aschenbrenner et al., 2000), in which they were instructed to write down as many words as possible in a specific category within 2 minutes. Categories were set as ‘job’, ‘hobby’, ‘words beginning with K’ and ‘words beginning with B’, each randomly balanced across both experimental conditions respectively. Naming of proper nouns, e.g., Kenya or Kevin, was not permitted. Words with the same stem, e.g., bean, bean sprout, were counted as one answer. When evaluating the result, subjects were awarded one point for each correctly spelled and comprehensible word written down. The German originals used in the study can be found in the appendix (see Appendix, A7).

2.2.3 Polysomnographic recordings

To measure and record electrical cortical activity, subjects' sleep was recorded by performing standard polysomnography.

Nine electrodes were attached according to the international 10-20 system at positions F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4. Additionally, 2 reference electrodes were placed on the mastoids (M1, M2) and 2 ground electrodes to the forehead (G1, G2). Furthermore, 4 electrodes registered vertical and horizontal eye movements (EOG1, 2) and muscle activity on the chin (EMG1, 2).

Positions for electrode attachment were measured with measuring tape and were marked on the scalp. These positions were then disinfected with disinfection spray (Softasept N, Braun, Hesse, Germany), scrubbed with abrasive conductive paste (EVERI conductive and abrasive paste, Spes Medica, Genova, Italy), and subsequently electrodes were fixed on using conductive electrode paste (Abralyt HiCl Abrasive Electrolyte Gel, Easycap GmbH, Herrsching, Germany) and tape. Impedances were always kept below 5 kΩ. Unipolar Ag-AgCl electrodes were used during all adaptation and experimental conditions.

During both stimulation conditions, EEG signals were continuously recorded with a BrainAmp DC Amplifier (Brain Products GmbH, Gilching, Germany) and using linked software 'Brain Vision Recorder' (Brain Products GmbH, Gilching, Germany).

All signals were sampled at 500 Hz, filtered between 0.03 and 250 Hz, and stored on a PC for later offline analysis together with the stimulation triggers.

During each adaptation night, electrodes were attached and recorded at positions Fpz, C3, Cz, C4, EOG1, EOG2, EMG1, and EMG2, referenced to the average potential from 2 electrodes attached to the mastoids. Additionally, 2 ground electrodes were attached to the forehead.

2.2.4 Detection algorithm and in-phase auditory stimulation

Detection algorithm

For detection of slow oscillations during experimental nights, an additional electrode was attached to position AFz on the scalp (on the connecting line between nasion and inion centered between Fpz and Fz). The corresponding prefrontal EEG signal was conducted to a second EEG recording system with a 'Digitimer D360' amplifier (Digitimer Ltd.,

Hertfordshire, UK) for signal amplification and filtering, connected to a separate PC. A high-performance data acquisition interface 'Power 1401mk-II' (Cambridge Electronic Design Ltd., Cambridge, UK) was used for detection, data analysis and stimulus generation. The prefrontal EEG signal was sampled at 200 Hz and filtered between 0.25 and 4 Hz to extract the SO signal. EEG signals were conducted from the second Digitimer recording system to a specially implemented algorithm in a custom-made script running under 'Spike2 Software Version 7' (Cambridge Electronic Design Ltd., Cambridge, UK), which together with the Power1401 mk-II enabled control of the stimulation in real time (compare Ngo et al., 2013; Besedovsky et al., 2017). Connecting this set-up with a connecting cable to the BrainAmp system, the signal from the Afz electrode was referenced to the two electrodes attached to the mastoids.

For a better overview of the experimental set-up, Figure 15 depicts both separate EEG recording systems with respective functions of the individual components:

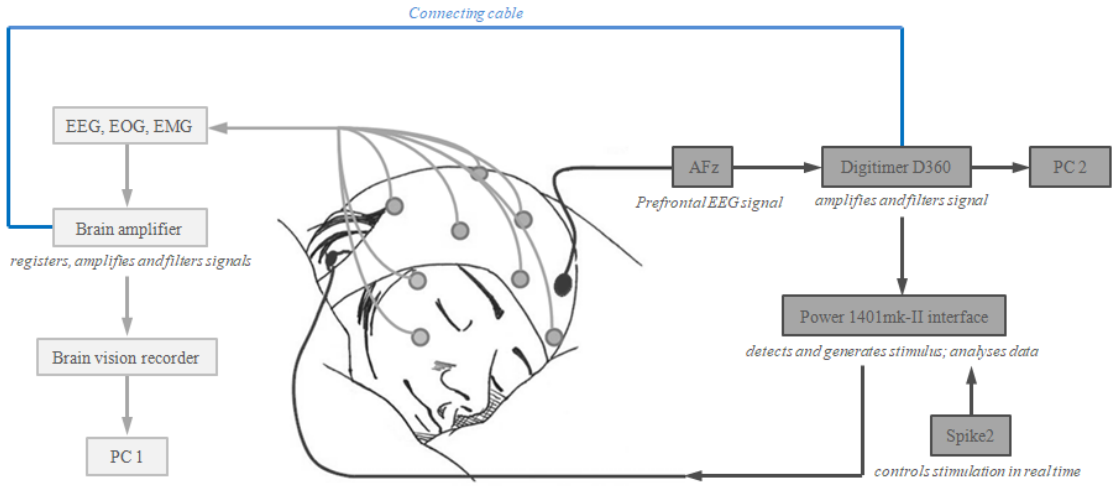


Figure 15: Experimental set-up

EEG: electroencephalography; EOG: electrooculography; EMG: electromyography; AFz: anterior-frontal reference electrode. (derived from Besedovsky, 2017)

In-phase auditory stimulation

Auditory stimulation was set to be triggered and presented over the subject's in-ear headphones once the EEG signal passed a threshold toward larger negative values set at -80µV. As described in Chapter 2.1.2 (Experimental set-up/ Design) of this study, closed-loop

auditory stimulation of SO signals in an up-phase stimulation condition was compared to those of a down-phase stimulation condition. Therefore, during up-phase stimulation conditions, the acoustic stimulus had to be adjusted to each subject's individual SO waveform average to ensure stimulation onset in phase with the following SO positive peak. Consequently, when the negative half wave of a SO passed the -80µV threshold and was detected, the stimulus was presented after each subject's individual delay time, i.e., the mean time between the detected SO negative peak and the following positive peak. During down-phase conditions, SOs were detected in the same way, but stimuli were sent in phase with subsequent negative peaks of SOs (see Figure 16).

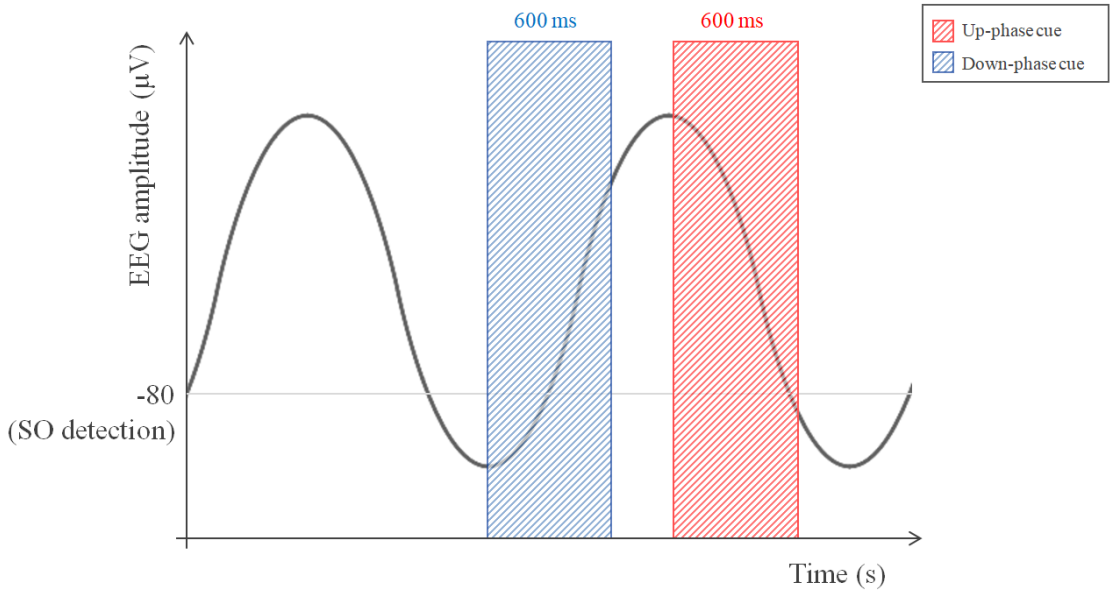


Figure 16: Closed-loop auditory stimulation protocol

Stimulation during experimental nights began with the detection of a SO when the EEG signal crossed a -80 µV threshold towards larger negative values (y-axis). Subsequent to SO detection, the auditory cues of 600 ms length each were presented to onset with the negative peak of the SO during down-phase stimulation conditions, or with the following positive peak of the SO during Up-phase stimulation conditions. SO: slow oscillation; µV: microvolts; s: seconds.

In order to determine this individual delay time, the SO detection algorithm was applied to the first SWS epoch of each subject during adaptation nights. During this process, the time points at which SOs were detected were marked in the EEG, but no auditory stimulation followed.

Auditory stimuli

On each experimental condition, one stimulus consisted of the first syllable of the first word in a word pair from the memorized and tested 40 word pair list.

Out of 40 available stimuli, 20 syllables were chosen to be presented over the headphones: half of the correctly remembered word pairs during the last immediate recall session and half of the incorrectly remembered word pairs on the same list were chosen randomly for stimulation. For example, assuming one subject correctly remembered 26 word pairs before sleep, then the first syllables of 13 words (half of the 26 remembered words) and 7 words (half of the 14 unremembered words) were chosen to be cued during sleep. If only one of the two words in a pair was remembered during the last testing session before sleep, the respective word pair was counted as incorrectly remembered. The 20 chosen syllables were played in random order once before the next random cycle began. The length of each single stimulus was set to 600 ms. A downtime period of 2.5 seconds followed successful stimulation. To ensure maximal stimulation effect, stimulation sound volume was set to begin 15 dB SPL above each subject's individual hearing threshold level determined during the adaptation night, and was calibrated upward until 3 dB below the volume under which subjects started showing signs of waking. By determining subjects' individual averaged evoked potential response to stimuli, responsiveness to stimulation was confirmed (see Figure 17).

Customary in-ear headphones (MDR-EX35, Sony, Germany) were used for binaural presentation of stimuli. In the morning after delayed recalled testing, 5 out of the 16 subjects reported having heard the auditory stimuli at least once in the night (3 times during up-phase and 4 times during down-phase stimulation conditions in total across all subjects). This occurrence may be caused by the continuance of stimulation during subjects' sudden brief arousals.

2.3 Data analysis

2.3.1 Analysis of polysomnographic recordings

Polysomnographic analysis was performed with Spike2 analysis program, Brain Vision Analyzer 2, and the program SchlafAus Version 1.5.0.1. The collected EEG and EOG data were filtered with a band pass between 0.3 and 30 Hz, and the EMG data filtered with a high pass of 5 Hz. Polysomnographic recordings of adaptation and experimental nights were then scored following the standard rules of Rechtschaffen and Kales (Rechtschaffen and Kales, 1968). Recordings from C3, Cz, C4, EOG and EMG channels were analyzed for subsequent 30-second epochs. Beginning with the 'lights off' and ending with the 'lights on' marker on the timeline, the polysomnographic recordings were scored into sleep stages 'awake', 'S1-S4', 'REM', 'Movement Time' and 'Movement Arousal', while epochs with artifacts were excluded from the analysis. EEG scoring was performed blind to the subjects and experimental conditions.

2.3.2 Statistical analysis

Data from 16 out of 20 subjects were included for statistical analysis. 3 participants had to be excluded due to significant sleeping problems, i.e., frequent or long-lasting wake phases, resulting in too less SWS during adaptation (2 subjects) or experimental nights (1 subject). Additionally, 1 subject's data had to be dismissed due to technical problems (sound file misfunction) during EEG recording procedures. Thus, the final number of usable data for analysis totaled n = 16.

Statistical data analysis was carried out using the Statistics Software SPSS 25 (IBM, Chicago, USA). Results are presented as mean values ± standard error (SEM). Analyses were conducted using either the two-tailed student's t test, or repeated measures analysis of variance (ANOVA), with the factor 'stimulation condition' (up-phase vs. down-phase) and the factor 'time' (before and after sleep). The Greenhouse-Geisser correction for degrees of freedom was performed when necessary. Post-hoc tests ensued when significant ANOVA effects were observed. Correlation analyses were performed using the Pearson product-moment correlation coefficient. The significance level was set to 0.05.

3 Results

3.1 Sleep stage analysis

The EEG data from the experimental nights were analyzed during the time period beginning with the "lights-off" marker and ending with the "lights-on" marker.

The average total sleep time during up-phase stimulation conditions amounted to 458.06 ± 7.23 min, and 454.38 ± 8.62 min during down-phase stimulation conditions ($p = 0.583$). The time subjects needed to fall asleep (onset of sleep time) was an average of 23.44 ± 6.32 min during up-phase stimulation conditions and 21.37 ± 4.65 min during down-phase stimulation conditions ($p = 0.549$). Table 1 displays the distribution of sleep stages during experimental nights.

Table 1: Distribution of sleep stages

	Up-phase	**Down-phase**	***p* values**
In minutes			
WASO	17.34 ± 4.46	17.50 ± 4.44	0.97
S1	22.16 ± 3.29	19.25 ± 2.51	0.35
S2	208.34 ± 7.85	197.44 ± 9.45	0.15
SWS	91.72 ± 8.02	101.06 ± 7.85	0.15
REM	70.38 ± 5.60	70.34 ± 4.24	1.00
Non-REM	300.06 ± 8.27	298.50 ± 7.76	0.83
MT	0 ± 0	0 ± 0	-
In %			
WASO	3.87 ± 1.05	3.90 ± 1.02	0.98
S1	4.88 ± 0.73	4.24 ± 0.55	0.37
S2	45.52 ± 1.59	43.41 ± 1.84	0.18
SWS	20.01 ± 1.66	22.35 ± 1.78	0.12
REM	15.25 ± 1.12	15.39 ± 0.82	0.91
Non-REM	65.52 ± 1.48	65.76 ± 1.34	0.86
MT	0 ± 0	0 ± 0	-

The results are presented as absolute numbers (minutes) and percentages (%) ± Standard Error of the Mean (SEM). WASO: wake after sleep onset, S1, S2: sleep stages 1 + 2, SWS: sleep stages 3 + 4, REM: rapid eye movement sleep, non-REM: S2 + SWS, MT: movement time. P values refer to two-sided pairwise comparisons between up-phase and down-phase stimulation conditions performing paired t tests.

To summarize, comparing up-phase and down-phase conditions, no significant changes in sleep architecture, total sleep time, onset of sleep time, the occurrence of arousals after sleep onset, and the duration and distribution of each sleep stage during experimental nights were found (see Table 1; all $p > 0.05$). There were also no significant correlations between S2, SWS or REM sleep stages and post-sleep memory performances during neither up-phase nor down-phase stimulation conditions (all $p > 0.05$) (for post-sleep memory performances see Chapter 3.4 Behavioral analysis).

3.2 Auditory stimulation (Cueing) analysis

The absolute number of auditory stimulations during a subject's second experimental night was adapted to the number of stimulations played during the first experimental night when possible. Table 2 displays the mean absolute number of auditory stimulations that were played to subjects during SWS and during non-REM sleep stages (S2 + SWS) for both conditions. During each experimental condition, 20 auditory cues were delivered in a randomized order in cyclic fashion, i.e., after the presentation of 20 cues, each cue had been delivered once before a new randomized cycle began. Consequently, dividing the absolute number of auditory stimulations performed by 20 equals the number of repetitions of all cues delivered. Accordingly, the mean number of SWS stimulations performed correspond to an average of 16.66 ± 2.11 repetitions of all cues during up-phase and 19.29 ± 2.28 during down-phase conditions. Non-REM stimulations correspond to an average of 21.13 ± 2.72 cueing repetitions during up-phase and 22.95 ± 2.58 during down-phase.

Table 2: Absolute number of auditory stimulations

	Up-phase	**Down-phase**	***p* values**
No. of stimuli			
SWS	333.06 ± 42.15	385.81 ± 45.65	0.010
Non-REM	422.50 ± 54.37	459.00 ± 51.67	0.015

Comparison of SWS and non-REM sleep phases during up-phase and down-phase conditions. The results are presented as mean values in numbers ± SEM. SWS: S3 + S4, non-REM: S2 + SWS. P values refer to two-sided pairwise comparisons between up-phase and down-phase stimulation conditions performing paired t tests.

As seen in Table 2, more auditory cueing was performed during down-phase stimulation conditions than during up-phase conditions on average. There is a statistically significant difference between the mean number of stimulations performed on subjects during SWS and during non-REM sleep phases when comparing both conditions (see Table 2; both $p < 0.05$). The absolute number of stimulations performed during SWS or during non-REM sleep did not significantly correlate with memory performances, neither during up-phase nor during down-phase conditions (all $p > 0.24$) (for memory performances see Chapter 3.4 Behavioral analysis).

Subjects' individual delay times, i.e., the mean time between detected SO negative peaks and subsequent positive peaks, were calculated to ensure stimulation in phase with the following SO positive peak during up-phase stimulation conditions (see Chapter 2.2.4 In-phase auditory stimulation). The mean delay time of all subjects amounted to 467.30 ± 13.02 ms. Stimulation onset during down-phase stimulation conditions was triggered automatically in phase with the following detected SO negative peak after the EEG signal crossed an amplitude threshold of -80µV towards larger negative values.

3.3 Analysis of auditory evoked potentials

To amplify and separate AEP signals from overlapping stimulus independent EEG signals, in this study, EEG signals (±SEM) were averaged at electrode positions Fz, Cz and Pz. The time interval of interest is the period of 3.25 s surrounding each presented stimulus (-1.5 s to 1.75 s around cue onset). The subjects' averaged AEP signals of this time period were then compared during up-phase and during down-phase stimulation conditions, as seen in Figure 17. Figure 17 depicts the typical immediate evoked potential responses for up-phase and down-phase stimulations, and the corresponding averaged SO phase angles during cue onset recorded at electrode positions Fz, Cz and Pz.

Each subject's individual averaged immediate evoked responses that were recorded at scalp position Fz are additionally supplied in the appendix (see Appendix, A8). Since four subjects (#1, #2, #6 and #15) showed no auditory evoked responses in their averaged AEPs, their data was removed from analysis. But even without these subjects' data, the result regarding behavioral and EEG analysis remained comparable to the original group of subjects. Limiting analysis to a group of subjects (n= 12) who showed evoked responses in their averaged AEPs did not change the result significantly.

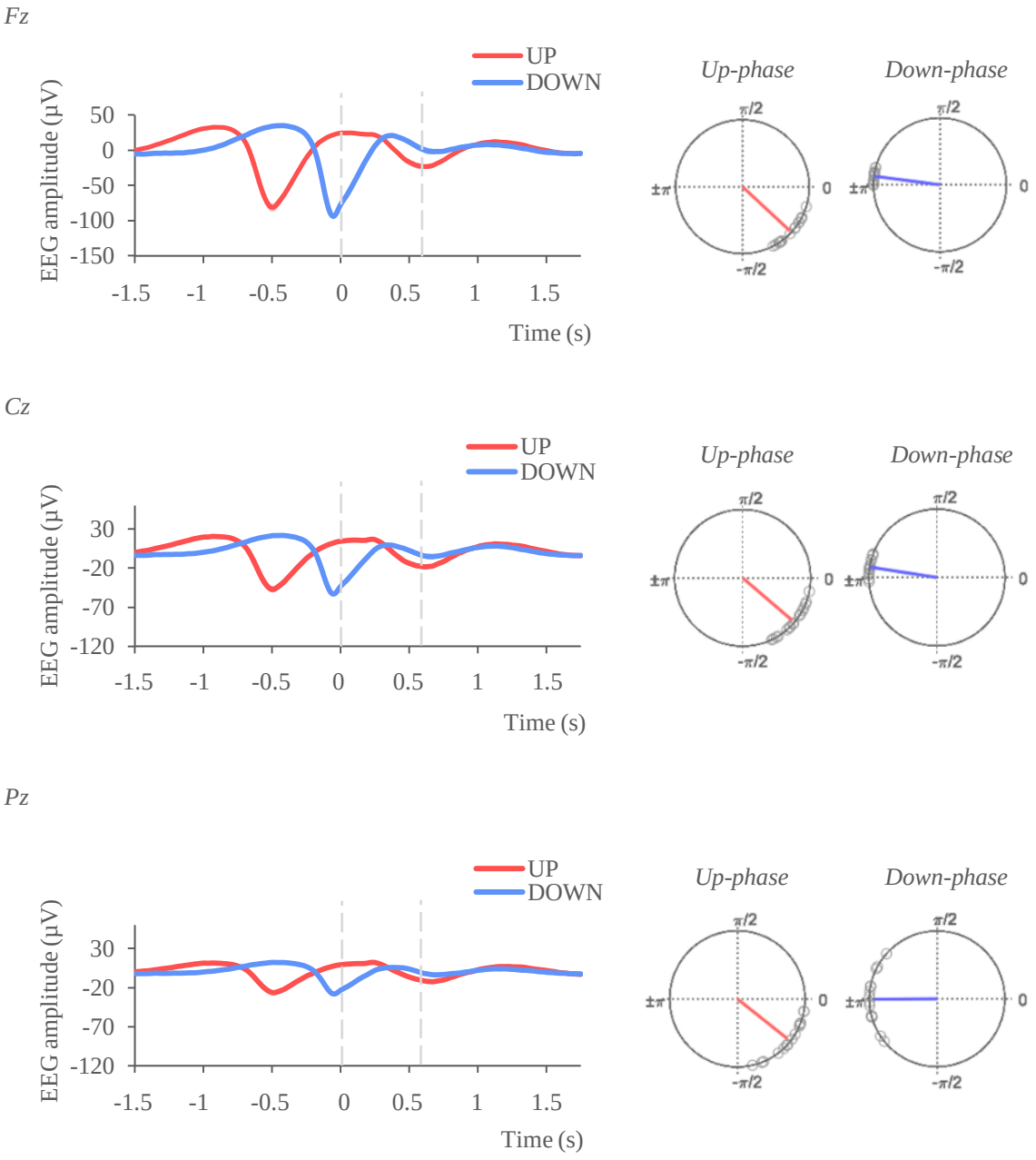


Figure 17: Averaged AEP signals and SO phase angle graphs during cue onset

Figure 17 depicts the mean averaged AEP signals across all subjects at electrode positions Fz, Cz and Pz during up-phase (red line) and down-phase conditions (blue line). SEM values are depicted as the light red rim of the red line (up-phase) and the light blue rim of the blue line (down-phase). The vertical dotted gray lines represent the moment of cueing onset and the end of cueing during both conditions. Fpz was used as the criterion electrode site for online detection of negative half-wave peaks of SOs. Therefore, depicted negative half-wave amplitudes appear smaller when recorded at posterior electrode sites (Pz) than at anterior sites (Fz) and do not always cross the -80mV threshold for online detection.
Averaged SO phase angles during cue onset, recorded at Fz, Cz and Pz, are depicted on the right for up-phase (red) and down-phase (blue) stimulation conditions. Phase angles at position "0" correspond to SO up states, "π" to SO down states. µV: microvolts; s: seconds.

3.4 Behavioral analysis

3.4.1 Memory task results

During pre-sleep learning sessions, subjects needed an average of 2.94 ± 0.25 learning rounds during up-phase and an average of 3.00 ± 0.24 learning rounds during down-phase stimulation conditions before reaching the 60% criterion for correctly recalled word pairs ($p = 0.75$, two-tailed paired t-test).

Analyses of the collected memory task data showed that the mean number of correctly recalled word pairs was lower after sleep than before sleep during both experimental conditions.

During up-phase stimulation conditions subjects correctly recalled an average of 32.13 ± 0.89 word pairs during the immediate recall session in the evening, and an average of 30.75 ± 1.01 word pairs during the delayed morning retrieval session. The post-sleep amount corresponds to an average of 95.77 ± 1.90 % of word pairs relative to learning performance before sleep during up-phase stimulation conditions.

During down-phase stimulation conditions the average amount of correctly recalled word pairs amounted to 33.31 ± 1.11 word pairs during the evening session and 31.75 ± 1.30 word pairs in the morning session. The post-sleep amount corresponds to an average of 95.18 ± 2.07 % of word pairs relative to learning performance before sleep during down-phase stimulation conditions.

The average memory performance results for both stimulation conditions are depicted in Figures 18 and 19 in absolute numbers and percentages respectively.

Contrary to expectations, both stimulation conditions showed no significant difference regarding the number of correctly recalled word pairs (main effect ‘up-phase/down-phase’: $F_{(1,15)} = 2.01$, $p = 0.18$). Time as a factor by itself, on the other hand, affected memory for word pairs significantly (main effect ‘before/after sleep’: $F_{(1,15)} = 8.00$, $p = 0.013$). Analyzing the effect of time for each experimental condition separately showed a significant difference for up-phase ($p = 0.04$, two-tailed t-test) and down-phase conditions ($p = 0.02$, two-tailed t-test). Analyzing interaction effects of time and stimulation conditions, the overnight loss of correctly recalled word pairs during up-phase stimulation conditions compared to the loss during down-phase stimulation conditions

revealed no statistically significant difference (interaction ‘up-phase/down-phase x before/after sleep’: $F_{(1,15)} = 0.09$, $p = 0.77$).

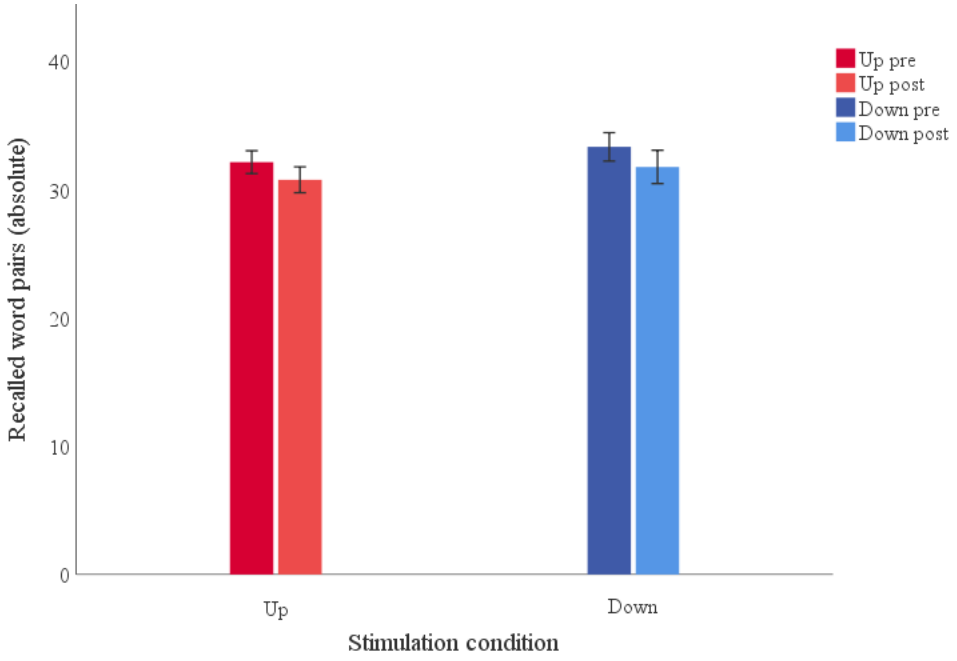


Figure 18: Correctly recalled word pairs (absolute)

The results are displayed as absolute numbers during up-phase and down-phase stimulation conditions, before and after sleep. Error bars represent SEM.

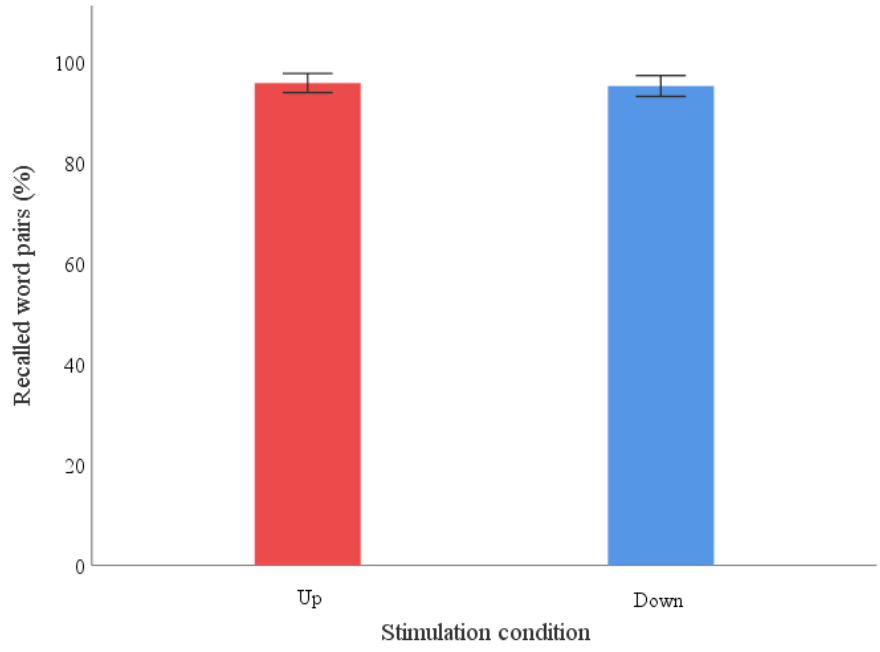


Figure 19: Correctly recalled word pairs (%)

Post-sleep results are displayed as percentages relative to learning performances before sleep during up-phase and down-phase stimulation conditions. Error bars represent SEM.

3.4.2 Cueing effect results

In order to evaluate the cueing effect itself, the mean values of the results of the delayed recall session in the morning were analyzed. All correctly recalled word pairs which were cued during the night were compared to the number of uncued word pairs correctly recalled.

During up-phase stimulation conditions subjects correctly recalled an average of 14.63 ± 0.63 cued word pairs and an average of 14.50 ± 0.56 uncued word pairs during the delayed morning retrieval session. The post-sleep amount corresponds to an average of 89.99 ± 2.29 % of recalled cued and an average of 90.96 ± 2.06 % of recalled uncued word pairs relative to learning performances before sleep during up-phase stimulation conditions.

During down-phase stimulation conditions subjects correctly recalled an average of 15.06 ± 0.81 cued word pairs and an average of 15.13 ± 0.77 uncued word pairs during the delayed morning retrieval session. The post-sleep amount corresponds to an average of 89.78 ± 2.46 % of recalled cued and an average of 90.19 ± 3.04 % of recalled uncued word pairs relative to learning performances before sleep during down-phase stimulation conditions. The average memory performance results comparing cued and uncued correctly recalled word pairs for both stimulation conditions are depicted in Figures 20 and 21 in absolute numbers and percentages respectively.

The cueing effect itself did not significantly modulate the number of correctly recalled word pairs (main effect ‘cued/uncued’: $F_{(1,15)} = 0.01$, $p = 0.93$). Comparing up-phase and down-phase stimulation conditions also showed no significant effect on memory performance for word pairs (main effect ‘up-phase/down-phase’: $F_{(1,15)} = 1.01$, $p = 0.33$, interaction ‘cued/uncued x up-phase/down-phase’: $F_{(1,15)} = 0.09$, $p = 0.77$). This implies that, contrary to expectations, no significant stimulation effect can be seen.

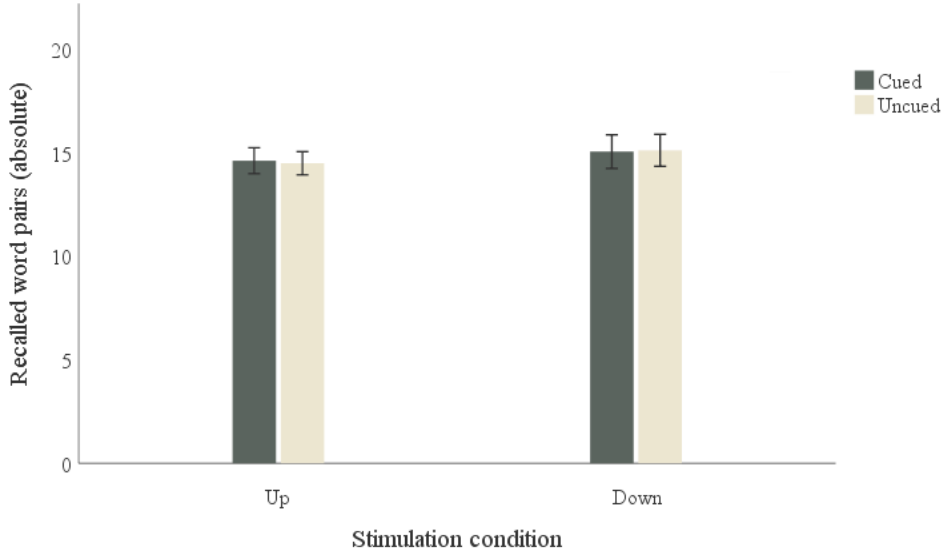


Figure 20: Cued vs. uncued correctly recalled word pairs (absolute)

The results are displayed as absolute numbers during up-phase and down-phase stimulation conditions, ***after*** *sleep. Error bars represent SEM.*

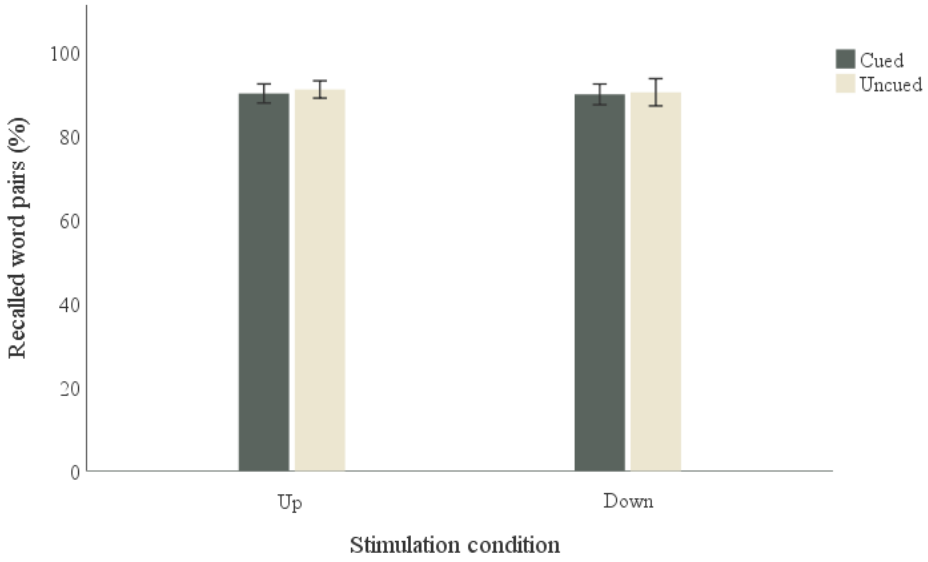


Figure 21: Cued vs. uncued correctly recalled word pairs (%)

Post-sleep results are displayed as percentages relative to learning performances before sleep during up-phase and down-phase stimulation conditions. Error bars represent SEM.

3.5 Additional cognitive tests and psychometric assessment analysis

3.5.1 Additional cognitive test results

Psychomotor Vigilance Test (PVT)

Table 3 depicts the average results of all PVT testing reaction times.

Table 3: Results of PVT testing

	Up-phase	Down-phase
Before Sleep	325.78 ± 7.91	323.22 ± 5.53
After Sleep	325.22 ± 6.19	326.47 ± 6.72

The results are presented as mean values in ms ± SEM during up-phase and down-phase stimulation conditions, before and after sleep.

Table 3 displays no statistically significant differences between subjects' individual reaction times (and thus behavioral alertness) during both conditions (main effect 'up-phase/down-phase': $F_{(1,11)} = 0.01$, $p = 0.91$). Comparing before and after sleep performance, no significant differences are discernible either (main effect 'before/after sleep': $F_{(1,11)} = 1.33$, $p = 0.27$). When analyzing before and after sleep PVT performances during up-phase compared to during down-phase conditions, no significant difference was revealed (interaction 'up-phase/down-phase x before/after sleep': $F_{(1,11)} = 0.03$, $p = 0.86$).

Digit Span

Table 4 depicts the average results of all Digit Span Tasks performed by subjects during up-phase and down-phase conditions.

Table 4: Results of Digit Span Task

	Up-phase	Down-phase
Forward		
Before Sleep	7.21 ± 0.28	7.58 ± 0.31
After Sleep	7.93 ± 0.25	7.82 ± 0.29
Backward		
Before Sleep	7.47 ± 0.27	7.33 ± 0.31
After Sleep	8.05 ± 0.24	7.50 ± 0.31

The results are presented as mean values in points ± SEM for the forward and backward Digit Span Task, during up-phase and down-phase conditions, before and after sleep.

No statistically significant differences on subjects' individual Digit Span Task scores (and thus attention efficiency and capacity) were shown when comparing the effects of evening and morning session results during up-phase and down-stimulation conditions:
Forward Digit Span: main effect 'up-phase/down-phase': $F_{(1,11)} = 0.03$, $p = 0.87$, main effect 'before/after sleep': $F_{(1,11)} = 3.09$, $p = 0.11$, interaction 'up-phase/down-phase x before/after sleep': $F_{(1,11)} = 2.11$, $p = 0.18$.
Backward Digit Span: main effect 'up-phase/down-phase': $F_{(1,13)} = 0.84$, $p = 0.38$, main effect 'before/after sleep': $F_{(1,13)} = 3.88$, $p = 0.07$, interaction 'up-phase/down-phase x before/after sleep': $F_{(1,13)} = 0.14$, $p = 0.72$.

Regensburg Word Fluency Test (RWT)

Table 5 presents the mean number of correctly spelled and conclusive words that subjects wrote down when performing on the RWT.

Table 5: Results of the RWT

	Up-phase	Down-phase
Before Sleep	18.94 ± 1.20	18.88 ± 1.28
After Sleep	18.63 ± 1.03	18.25 ± 0.81

The results are presented as mean values in points ± SEM during up-phase and down-phase conditions, before and after sleep.

Regarding performances on the RWT, no statistically significant differences during both stimulation conditions, as well as no significant differences between performances before and after sleep, were found (main effect 'up-phase/down-phase': $F_{(1,15)} = 0.12$, $p = 0.73$, main effect 'before/after sleep': $F_{(1,15)} = 0.23$, $p = 0.64$, interaction 'up-phase/down-phase x before/after sleep': $F_{(1,15)} = 0.09$, $p = 0.77$).

3.5.2 Psychometric assessment results

Positive and Negative Affect Schedule (PANAS)

Table 6 presents the results of the PANAS subjects performed on prior to and after sleep during both stimulation conditions.

Table 6: Results of the PANAS

	Up-phase	Down-phase
Positive Feelings		
Before Sleep	29.75 ± 1.40	29.13 ± 1.43
After Sleep	28.56 ± 1.36	28.38 ± 1.78
Negative Feelings		
Before Sleep	11.63 ± 0.45	12.38 ± 0.96
After Sleep	11.81 ± 0.52	12.44 ± 0.84

The results are presented as mean values of points ± SEM during up-phase and down-phase conditions, before and after sleep.

Table 6 shows no significant effect between up-phase and down-phase conditions (positive feelings: main effect 'up-phase/down-phase': $F_{(1,15)} = 0.21$, $p = 0.65$; negative feelings: $F_{(1,15)} = 1.12$, $p = 0.31$) and between before and after sleep performances for both positive and negative feelings (positive feelings: main effect 'before/after sleep': $F_{(1,15)} = 0.58$, $p = 0.46$; negative feelings: $F_{(1,15)} = 0.039$, $p = 0.85$). Also, there was no significant difference observable when comparing the before and after sleep performances during up-phase conditions with equivalent performances during down-phase conditions (positive feelings: interaction 'up-phase/down-phase x before/after sleep': $F_{(1,15)} = 0.10$, $p = 0.76$; negative feelings: $F_{(1,15)} = 0.019$, $p = 0.89$).

Sleeping Quality Questionnaire (SF-A-R)

Table 7 displays all subjects' mean awarded points for their personal sleep quality ratings in the SF-A-R after sleep during both stimulation conditions.

Table 7: Results of the SF-A-R

	Up-phase	**Down-phase**	***p* value**
After Sleep	3.36 ± 0.25	3.29 ± 0.26	0.75

The results are presented as mean values in points ± SEM during up-phase and down-phase stimulation conditions, after sleep.

Regarding personal sleep quality ratings in the SF-A-R after sleep, no statistically significant differences during both stimulation conditions were observed ($p = 0.75$ two-sided paired t-test).

Stanford Sleeping Scale (SSS)

Table 8 displays all subjects' mean results in the SSS, before and after sleep during both stimulation conditions.

Table 8: Results of the SSS

	Up-phase	**Down-phase**
Before Sleep	2.63 ± 0.27	2.69 ± 0.24
After Sleep	2.31 ± 0.18	2.44 ± 0.22

The results are presented as mean values in points ± SEM during up-phase and down-phase stimulation conditions, before and after sleep.

Table 8 showed no significant effects on subjects' results in the SSS when comparing up-phase and down-phase conditions (main effect 'up-phase/down-phase': $F_{(1,15)} = 0.30$, $p = 0.59$), or before and after sleep performances (main effect 'before/after sleep': $F_{(1,15)} = 1.06$, $p = 0.32$). Furthermore, comparing the effects of sleep on SSS results during

up-phase conditions and during down-phase conditions, no significant effect was observable (interaction ‘up-phase/down-phase x before/after sleep’: $F_{(1,15)} = 0.02$, $p = 0.90$).

3.6 Analysis of retention times

The time intervals between the immediate recall session in the evening and the delayed recall session in the morning were recorded for all subjects’ experimental nights and revealed an average retention time of 585.06 ± 6.94 min during up-phase and 589.67 ± 6.87 min during down-phase conditions. Comparing both conditions, no significant differences between retention times were observed ($p = 0.56$, two-sided paired t-test).

4 Discussion

The present study was aimed to build on previous findings in the field of declarative targeted memory reactivations. A closed-loop auditory stimulation was performed during SWS in order to improve declarative memory retention. The study specifically focused on examining the behavioral effect of auditory cues that were presented during different phases of single slow waves during SWS. In that regard, the effects of SO up-phase and SO down-phase dependent memory reactivations during SWS were compared. In order to enable the presentation of auditory cues precisely timed with respective SO phases, an established SO detection algorithm developed by Ngo and colleagues was used (Ngo et al., 2013).

4.1 Effects on sleep stages

Analyzing subjects' scored EEGs, no significant difference in sleep architecture during up-phase and down-phase stimulation conditions was observable. Particularly SWS, the sleep stage during which auditory cueing occurred, did not show any significant quantitative difference when comparing both conditions. This implies two possible non-exclusive conclusions. The auditory stimulation presented in this study either did not quantitatively effect SWS at all (or in that regard any other sleep stage), or sleep architecture was affected by the stimulation but indifferently to the timing of the stimulation in regard to slow oscillatory phases. However, since there were also no significant correlations between memory performances and the amount of sleep spent in any sleep stage observable during both conditions, it is suggested that there was no quantitative effect on sleep architecture after all. Moreover, the first conclusion is consistent with findings from previous closed-loop stimulation studies in which effects on sleep architecture during stimulation and sham conditions were compared (Shimizu et al., 2018; Ong et al., 2016), including Ngo and colleagues who suggested that "in-phase stimulation chiefly entrained SO activity, leaving the processes of initializing SOs unaffected" (Ngo et al., 2013; p. 546). Notably, only a recent closed-loop study by Göldi and colleagues, which compared SO up state and down state cueing effects similar to the present study, observed a significant correlation between memory performance during down state cueing and the amount of REM sleep during the night (Göldi et al., 2019). Consequently, they identified the

transition between SO positive peaks and negative peaks to be the most beneficial stimulation period for subjects who spent more time in REM sleep after non-REM cueing. They proposed that memory traces that were targeted and stabilized during down states of SOs might be dependent on their re-stabilization during REM sleep. Conversely, the present study revealed no indication whatsoever to confirm this conclusion since there was no correlation between the amount of time spent in REM sleep and memory performances during any stimulation condition observed.

4.2 Effects on auditory evoked potentials

Analyses of subjects' averaged AEP signals surrounding each presented stimulus (-1.5 s to 1.75 s around cue onset) showed the typical immediate evoked potential responses for up and down-phase stimulations, indicating correctly performed auditory cueing during both experimental conditions (Cash et al., 2009). Even though auditory stimulation was presented into opposite peaks of SOs during up-phase and down-phase conditions, the stimulations seemed to cause a similar EEG pattern comprising of an early positive depolarization and a late negative hyperpolarization (see Figure 17 and Appendix, A8). This similarity in evoked potentials suggests that the cues presented enforce similarly uniform neuronal firing patterns in the brain regardless of the preceding endogenous activity level of neurons. These results are consistent with previous observations of Göldi and colleagues. (Göldi et al., 2019)

The amplitude of the late hyperpolarization wave was descriptively higher during up-phase (i.e., cues presented in the transition from positive to negative peak) than during down-phase stimulation conditions (i.e., cues presented in the transition from negative to positive peak) on average (see Chapter 3.3 Analysis of auditory evoked potentials, Figure 17). This difference in amplitude might indicate a higher neocortical excitability for stimuli presented during SO up-phase and a lower excitability during down phase stimulations. This is in line with results of a previous closed-loop study which demonstrated that stimulation in phase with SO up states enhanced the slow oscillation rhythm and caused ERP responses of higher amplitudes than out of phase stimulation (Ngo et al., 2013). Descriptively, the evoked potentials during up-phase stimulation conditions also widened the depolarisation peak during which the cue was presented and caused a slightly shorter latency period of the following negative hyperpolarization peak in regard to the time of cue onset

on average (see Chapter 3.3 Analysis of auditory evoked potentials, Figure 17). This observation is consistent with findings of Rosanova and Timofeev, who concluded in their study in 2005 that “at the neocortical level, responses to peripheral stimuli varied in latency, amplitude, shape and ability to fire as a function of the phase of SO” (Rosanova and Timofeev, 2005; p. 580). They suggested that those responses were longer and more variable during down states of SOs, and shorter and less variable during up states of SOs, depending on the firing of thalamocortical neurons.

These findings might indicate that the variability of thalamic gating of sensory input depends on the phases of SOs during which the stimuli are transmitted to cortical areas and that slower and less sensory processing occurs during SO down states.

4.3 Behavioral effects

In accordance with the hypothesis of the present study, auditory cueing during SO up-phases was anticipated to improve declarative memory performance when compared to SO down-phase cueing. Contrary to expectations however, analysis of memory performance task results showed no significant differential effect between both conditions. Merely comparison of pre-sleep and post-sleep performances revealed that declarative memory retention was significantly lower after sleep (and hence after auditory stimulation) during both experimental conditions. Since there was no control group without stimulation, and since previous studies demonstrated improvement of post-sleep declarative memory recall for auditory closed-loop stimulation in phase with SO up states (Ong et al., 2016; Ngo et al., 2013, 2015) or SO down states (Leminen et al., 2017) compared to sham conditions, these results do not necessarily imply failed retention of declarative memory content overnight. They suggest, however, that the timing of auditory stimulation within a SO is irrelevant in regard to declarative memory performance.

This contrasts contradictory findings from previous auditory open-loop (Batterink et al., 2016) and closed-loop studies (Göldi et al., 2019; Ngo et al. 2013, 2015) which suggested the importance of precise timing for cueing within SOs.

4.3.1 The cueing effect

The cueing effect of the auditory stimulation itself was evaluated by comparing the number of cued and uncued correctly recalled word pairs during post-sleep performances for both stimulation conditions. Analyses of these post-sleep recall performances suggested that no cueing effect was observable in the present study.

One possible explanatory approach might also be the presumably most consequential possible source of error in the present study. EEG analysis revealed a statistically significant difference in the number of auditory cues presented during both experimental conditions, with significantly more cueing performed during down-phase stimulation conditions (see Chapter 3.2 Auditory stimulation analysis, Table 2). This error was caused by a conceptual flaw in the present study. The number of stimulations during the second experimental night was not always adapted to equal the number of stimulations during the first experimental night. When very deep SWS occurred towards the end of the stimulation period of the second experimental night, cueing continued until the end of that period to achieve maximal impact of the cues presented. Since significantly more stimuli were presented to subjects during down-phase stimulation conditions, this might explain the lack of expected stronger memory retention during up-phase conditions.

Another possible alternative explanation for the missing cueing effect might be the declarative memory task subjects performed on in the present study. A newly designed version of the PAL task, in which subjects were tasked with associating two words with one syllable, was used in the study. This version of the PAL task had never been used in a study before. Hence, it has never been demonstrated if memory performances in this specific task benefit from sleep in general and/or targeted memory reactivation at all. Consequently, the task itself might have caused the missing cueing effect.

4.4 Confounding factors

There are many possible confounding factors which might have contributed to the unexpected behavioral results of the present study.

Firstly, subjects' personal circumstances, i.e., their emotional states, motivation and cognitive skills are possible confounding factors in any study in which human subjects perform. Yet subjects' differences in cognitive skills and emotional states were analyzed

and assumedly ruled out as confounding factors (see Chapter 4.5 Effects of additional cognitive tests and psychometric assessment). Subjects' individual intrinsic motivation however, could not reliably be ruled out as a confounding factor since it is generally not objectively assessable as its proportion depends on inter alia personality traits, reward value and individual preferences (Miendlarzewska et al., 2016). Another possible confounding factor is pre-sleep memory strength. Since sleep for declarative memory consolidation has been shown to be more beneficial for weaker memory associations, a criterion of 60% correct responses was established for memory recall before sleep. The criterion was established to prevent over-learning, to allow for better comparability of recall results, and to reduce the standard error of the mean (SEM) for memory performances before sleep while at the same time enabling sufficiently strong memory associations between stimuli and reactivated memory content (Drosopoulos et al., 2007). Nonetheless, several subjects in this study achieved a result way above 60% during their immediate recall session before sleep, because they had already recalled just below 60% during the penultimate learning session. The ultimate learning session was additionally followed by a cued recall performance without feedback (see Chapter 2.2.1 Memory task), the result of which determined the average pre-sleep learning level. This might indicate that the learning task should have been conceptualized to be more difficult or that the recall criterion should have been adapted to a slightly lower percentage, such as 50%, such that one learning session less would have been sufficient to reach the criterion before the immediate recall session. The influence of the statistically significant differential number of auditory cues presented during both experimental conditions on memory performances can be disregarded since no significant correlations between the number of cues and memory performances were found (see Chapter 3.2 Auditory stimulation (Cueing) analysis).

4.5 Effects of additional cognitive tests and psychometric assessment

Since no significant differences between the results of the additional cognitive tests and psychometric assessment tasks for both conditions were observed, it is assumed that subjects' individual differences regarding cognitive abilities and psychological states can be excluded as confounding factors in this study. These include subjective tiredness, mood, sleep quality, sleepiness, as well as objectively measured alertness, attention efficiency,

working memory capacity, and word fluency before and after sleep during both conditions. It is additionally suggested that the auditory stimulation itself had no differential effect on these cognitive abilities and psychological states.

4.6 Reference to current research findings

Closed-loop auditory stimulations represent a rather novel approach to memory improvement during sleep that began with the development of an auditory closed-loop feedback system (ACLS) by Ngo and colleagues in 2013, which presented stimuli when crossing a set EEG wave amplitude threshold (Ngo et al., 2013). In their study, the researchers used an ACLS to present 50-ms bursts of pink noise (auditory stimuli) during SO up states. This allowed the precise induction of SOs and an increase in spindle activity phase-locked to SO up states, significantly improving declarative memory performance in a paired associate learning task compared to a sham group. More studies using similar ACLS algorithms followed and also successfully replicated these results. For example, Ong and colleagues demonstrated significantly less forgetting of word pairs after auditory cueing during 90-min afternoon naps subsequent to a paired-associate learning (PAL) task (Ong et al., 2016). During the task, subjects were sequentially presented with 40 semantically related word pairs in randomized order on a computer screen and were then tasked to recall the second word when presented with the first word of each pair during subsequent recall sessions. The cues presented during post-learning sleep consisted of 50-ms bursts of pink noise and were presented in blocks of five, with each cue phase-locked to a SO up state.

Similarly, Leminen et al. developed an automated auditory stimulation protocol to be applicable for ambulatory home use outside laboratory conditions (Leminen et al., 2017). Subjects performed on four memory tasks each (word pairs, serial finger tapping, picture recognition, and face-name association) and were presented with 50-ms noise bursts phase-locked to the negative peaks of SOs during subsequent SWS. Significantly increased memory consolidation was only observable in the word pair task (verbal associative memory) compared to a control group without stimulation.

These closed-loop auditory stimulation studies compared the effects of SO phase-coupled cues to the effects of sham conditions without cueing. They did not directly compare the effects of cues presented during different SO phases, as was done in the present study,

therefore not allowing the identification of one optimal time window for cueing during slow oscillatory activity. Additionally, the experimental set-ups of these studies differed in, inter alia, stimulation protocols, stimulus characteristics and learning tasks, which further complicates the identification of an optimal time frame for memory cueing in general.

4.6.1 Precise timing of the auditory stimulation

A previous study conducted by Ji and Wilson in 2007 emphasized the importance of precise timing for auditory cues for externally triggered memory reactivations. By analyzing neuronal firing patterns in the visual cortex and hippocampus during SWS in rats, they demonstrated a specific temporal order of the neuronal replay of previous memories occurring in both brain regions, with cortical replay preceding corresponding hippocampal memory reactivations by about 50 ms. Their findings emphasize how precise the temporal interplay between hippocampal and neocortical activity actually is. (Ji and Wilson, 2007) Therefore, addressing the issue of precise timing for auditory cues, the present study directly compared the effects of SO phase-dependent memory reactivations on memory consolidation. Auditory stimuli onsets were set to coincide with the negative peak of a SO during down-phase or the following positive peak during up-phase stimulation conditions. This means that, with each stimulus length set to 600 ms, auditory cues were presented precisely during the first part of the transition period from negative to positive peak of a SO (i.e., down-phase condition) and during the transition period from positive to negative peak (i.e., up-phase condition). The predicted significant memory benefit for auditory cueing in-phase with online detected SO up states was, however, not observed.
This predicted time window for auditory cues is not only based on the fact that SO up states represent a state of increased synchronized neuronal firing (Steriade, 2006), which presumably leads to preferable forwarding of information during this particular moment. It is also based on the assumption that thalamocortical spindles phase-locked to SO up states play a key role in the reactivation of memory representations (Bergmann et al., 2012).
A previous open-loop auditory stimulation study by Batterink and colleagues, which applied auditory stimuli during non-REM sleep independently of the endogenous brain rhythm, suggested the optimal time window for auditory cueing to occur during the second half of the transition period from SO up states to down states, “such that subsequent

memory-related processing would coincide with the slow-oscillation upstate" (Batterink at al., 2016; p. 1403). Their explanation took into account the time needed for stimulus presentation (500 ms), for the stimulus to reach the auditory cortex (about 80 ms) (Kraus and Nicol, 2009), and the time required for early auditory processing.

In order to more precisely cue one specific SO phase for memory reactivation, Shimizu and colleagues were one of the first research groups to combine targeted memory reactivation with closed-loop auditory stimulation (Shimizu et al., 2018). The auditory cues in their study were presented precisely during the transition period from down state to up state of SOs during non-REM sleep and were contextually linked to specific urban environments which subjects were confronted with when performing on a virtual reality navigation task. Shimizu and colleagues found significant improvement in subjects' navigation efficiency post-sleep compared to a control group which had not been cued during sleep.

However, the first study to directly compare the effects of auditory cues presented during up states and down states of SOs (and additionally the effects of non-cued words) was published by Göldi and colleagues in 2019 (Göldi et al., 2019). In a within-subject design closed-loop targeted memory reactivation study, they re-exposed previously learned foreign vocabulary to subjects during both stimulation conditions and additionally compared memory retention effects to memory performances on uncued vocabulary. They identified the most effective time window for auditory cueing to be the transition period from the SO negative to the positive peak, with stimulus onsets occurring shortly before positive peaks (i.e., 'up-state stimulation condition' in their study; similar to 'up-phase stimulation condition' in the present study (see Figure 17)). They hypothesized and demonstrated significantly improved declarative memory performances during their 'up-state' stimulation conditions. Their hypothesis was based on the idea that "neuronal down-states occur slightly before the negative peak of the surface slow wave, while neuronal up-states start with the negative-to-positive transition of the cortical slow-wave" (Göldi et al., 2019; p. 4).

When evaluating these varying results from previous auditory stimulation studies regarding the optimal time frame for auditory memory reactivations, it is worth noting that differences in experimental set-ups (subjects' age, gender, learning task, the type of memory

reactivated) and stimulus properties (length, intensity, repetition rate, content, complexity) could contribute to the observed optimal stimulation timing discrepancies.

4.7 Beneficial effects and potential future applications

Beneficial effects and potential future applications of targeted memory reactivation during sleep are vast and numerous, especially in regard to cognitive enhancement and, in a clinical setting, psychotherapy.

Since it is hypothesized that targeted memory reactivation during sleep depends on hippocampal reactivation processes, most studies in the past attempted to reactivate hippocampus-dependent memory contents. This technique has so far not only demonstrated to benefit declarative memory consolidation but also to accelerate the consolidation process (Diekelmann et al., 2012) and make newly consolidated memories more resistant to interference, i.e., less likely to forget (Diekelmann et al., 2011). Creery and colleagues additionally demonstrated that beneficial effects of cueing grow proportionally to subjects' initial performance levels as long as the pre-sleep performance is not already near-perfect. (Creery et al., 2015). One study showed that when cued memory reactivation during sleep is conducted, behavioral performance results increase by approximately 20% compared to regular sleep without cueing (Diekelmann et al., 2011). This 20% increase in performance could especially benefit children and adults with learning deficits (Sigman et al., 2014). Cued memory reactivation might also be able to facilitate learning of foreign languages (Schreiner and Rasch, 2015a), even though the existence of prior knowledge seems to be essential for a beneficial memory effect to occur (Groch et al., 2016b). Additionally, cued memory reactivation studies have shown possibilities to strengthen creative thinking and problem-solving skills (Ritter et al., 2012), and even to reduce gender and racial bias in individuals (Hu et al., 2015). Another direction for possible future applications of this technique lies within a more clinical setting. The average amount of SWS during the night decreases dramatically with increasing age, which seems to lead to a decline in declarative memory consolidation capabilities with growing age (Backhaus et al., 2007). Targeted memory reactivation could therefore potentially represent a new therapeutic approach to memory impairment of the elderly or even diseases such as dementia or Alzheimer's (Schouten et al., 2017). Targeted memory reactivation could prove to be a useful tool to alleviate symptoms of psychiatric disorders that are associated with

dysfunctional sleep and memory, e.g., post-traumatic stress disorder (PTSD), depression, and schizophrenia (Diekelmann and Forcato, 2015). One cued memory reactivation study demonstrated enhanced forgetting of unwanted memories and could thus be useful for patients with PTSD (Simon et al., 2018). This technique could also be incorporated into psychotherapy of social anxiety disorder (Groch et al., 2017) by changing the perception and interpretation of specific emotional situations into a more positive direction. (Diekelmann and Forcato, 2015) (Schouten et al., 2017)

Targeted memory reactivation has the potential to become a non-invasive, cost-effective drug-alternative method to enhance cognitive functions and improve efficiency of psychotherapy. Nonetheless, real-life applications of targeted memory reactivations are at present mainly theoretical, have been attempted only once so far (Göldi and Rasch, 2019), and have yet to achieve consistent behavioral results across studies. Further research is required before practical applications in the general population can succeed. Furthermore, many questions remain unanswered in regard to, inter alia, specific cueing conditions, underlying neural mechanisms, continuous long-term effects, limitations, and potential side effects of the cued memory reactivation technique.

Nonetheless, all of the above-mentioned reactivation studies demonstrating the potential benefits and future applications of targeted memory reactivation did not specifically present cues in-phase with a certain SO phase during post-learning non-REM sleep. They presented cues via an open-loop stimulation system, applying cues in a random fashion regardless of slow oscillation phases. Only one study considered the potential relevance of the application of cues at a specific phase of the slow wave and therefore added a random jitter of 0 to 0.4 s to a set interstimulus interval of 6 s for the presentation of auditory cues (Groch et al, 2016b). This ensured that auditory stimuli reached SOs at variable points, effectively eliminating the possible relevance of the temporal interplay between slow waves and auditory cues. Closed-loop auditory stimulation studies so far have reported improved memory retention compared to sham conditions, with cues presented phase-locked to SO up states (Ong et al., 2016; Ngo et al., 2013, 2015), cues phase-locked to the transition period from SO down states to up states (Shimizu et al., 2018), and cues phase-locked to the negative peaks of SOs (Leminen et al., 2017). Apart from the present study, one other closed-loop study directly compared the memory retention

effects of cueing during SO up states, down states, and uncued memories (Göldi et al., 2019). The next step in this line of memory research is to directly compare the effects of closed-loop and open-loop auditory stimulation, each combined with targeted memory reactivation. Further research should also examine whether closed-loop auditory stimulation combined with targeted memory reactivation results in greater memory retention effects than these techniques by themselves. Previous open-loop targeted memory reactivation studies could, e.g., be replicated using the exact same experimental set-up but with cues being presented in time with specific phases of SOs via a closed-loop detection algorithm. Future closed-loop targeted reactivation studies should also apply auditory stimuli which are standardized equally in regard to stimulus length, intensity, interstimulus interval and content in order to eliminate these differences from previous studies as potential confounding factors. This would allow possible replication of consistent results in order to determine the optimal time frame for cue presentation during non-REM sleep. Additionally, this kind of standardized set-up could be used to determine if specific timing of cues plays a role in regard to enhancement of slow oscillatory activity, phase-coupled spindle activity and consequently declarative memory consolidation compared to open-loop targeted memory reactivation.

4.8 Conclusion

In conclusion, the present study facilitated new insights into the relatively new field of targeted memory reactivation combined with auditory closed-loop stimulations for potential memory enhancement. Building on preceding research findings, the study focused on identifying the optimal time window for auditory reactivation within a single slow oscillation during SWS. In the end, the auditory cues presented in this study failed to successfully allow the identification of one definite SWS phase for significant beneficial effects on declarative memory. However, further research might draw on and benefit from the present study's findings, avoid possible sources of error and confounding factors that potentially occurred here and gain further insights into the effects of SO phase-dependent auditory cueing on declarative memory consolidation. Of particular note is the certainly conceivable introduction of new clinical therapeutic options using cued memory reactivation in the near future, especially in regards to psychotherapy and neurological disorders. Furthermore, a hopefully not too far into the future long-term objective would be a

portable automated targeted memory reactivation appliance that enhances deep sleep and memory consolidation overnight, accessible for the general population and suitable for everyday life if desired.

5 Summary

In the last decade, an increasing number of studies have demonstrated that using a relatively new technique called ‘targeted memory reactivation’ can modulate and enhance memory consolidation during sleep. This technique is performed by matching specific externally applied sensory stimuli (e.g., sound cues) with target information (e.g., associated word pairs) during wakefulness and then presenting the learned cues during subsequent non-REM sleep. The present study combines this technique with an auditory closed-loop stimulation (ACLS) algorithm, which detects slow oscillations (SOs) and triggers the presentation of the auditory cues precisely during a specific phase of a SO once the EEG signal passes a certain amplitude threshold. SOs are high-amplitude, low-frequency undulating EEG signals, which represent the hallmark oscillations during slow-wave sleep (SWS). Following the widely accepted assumption that SOs play a central role in the consolidation of memory by driving memory reactivation processes during SWS, the present study aimed to identify the optimal time window for the presentation of auditory cues within a single SO during SWS in order to optimize targeted memory reactivation and thus overnight consolidation. The main focus of the study lay thereby on the comparison of the differential benefits on declarative memory retention during two stimulation conditions: SO up-phase and down-phase. For this purpose, auditory stimuli were presented to subjects during non-REM sleep, timed to set in with SO negative peaks during down-phase stimulation conditions, and with SO positive peaks during up-phase stimulation conditions.

As SO up states are assumed to represent a state of increased neuronal firing, the hypothesis of this study proposes that auditory cueing in-phase with online detected SO up states leads to better declarative memory consolidation than cueing during SO down states.

During the evening of each experimental condition, subjects performed on a paired-associate learning task (PAL task), during which they were presented with each word pair on a screen while the first syllable of the first word was played simultaneously over in-ear headphones. They were first tasked to learn to match syllables with corresponding word pairs and then to recall a word pair when presented with the matching syllable only. Following this learning session of declarative memory contents after reaching a criterion of 60% correctly recalled word pairs, memory performance was tested once immediately

and once during a delayed recall session the next morning. The syllables used in the task where of 600-ms length each and were later presented as cues during post-learning non-REM sleep.

Contrary to expectations, analyses of behavioral results during up-phase conditions showed no significant declarative memory benefit compared to down-phase conditions. Also, no significant cueing effect could be observed during both conditions. Analyses of subjects' averaged auditory evoked potential signals showed the typical immediate evoked potential responses for up-phase and down-phase stimulations, indicating correctly performed auditory cueing during both experimental conditions. Additionally, subjects performed on additional cognitive tests and psychometric assessment tasks, the results of which showed no significant differences for both conditions.

The hypothesis of the present study, stating that up-phases represent a superior time window for auditory stimulations compared to down-phases of SOs, was not validated. However, the study facilitated new insights into the relatively new field of closed-loop targeted memory reactivation for potential memory enhancement. As was demonstrated in previous research, targeted memory reactivation combined with precise closed-loop auditory stimulation seems not only to represent a non-invasive and effective method to enhance memory consolidation during sleep, but also to hold various potential benefits, particularly in regard to clinical applications in the field of psychotherapy and neurological disorders. As a result, the present study has undeniably demonstrated that further research is still necessary and might benefit from the present study's findings as many questions remain unanswered, particularly in regard to specific cueing conditions, underlying neural mechanisms, long-term effects, limitations, and potential side effects of the cued memory reactivation technique.

6 Zusammenfassung

Mehrere in den letzten zehn Jahren entstandene Studien zeigen, dass sich durch „Zielgerichtete Gedächtnisreaktivierung“ die Gedächtniskonsolidierung im Schlaf verändern und verbessern lässt. Bei dieser Technik werden im Wachzustand sensorische äußere Reize (z.B. Tonsignale) mit Lerninhalten (z.B. assoziierte Wortpaare) verbunden und die verknüpften Reize im nachfolgenden non-REM-Schlaf den Probanden dargeboten. Die vorliegende Studie verbindet diese Technik mit einem auditorischen Stimulationsalgorithmus in einem geschlossenen Regelkreis. Langsame Oszillationen - Schwingungen des Tiefschlafes - bewirken hochamplitude, langsamfrequente, wellenförmige EEG-Signale. Sobald während des Schlafes diese EEG Signale auftreten und einen bestimmten Amplitudenschwellenwert überschreiten, werden Stimuli gezielt ausgelöst und folglich während einer spezifischen Phase einer langsamen Oszillation dargeboten.

Es ist weitgehend anerkannt, dass langsame Oszillationen Gedächtnisaktivierungsprozesse während des Tiefschlafes fördern. Aufbauend auf dieser Erkenntnis hatte die vorliegende Studie das Ziel, zur Optimierung der zielgerichteten nächtlichen Gedächtniskonsolidierung das optimale Zeitfenster für die Darbietung von Stimuli innerhalb einer einzelnen langsamen Oszillation während des Tiefschlafes zu identifizieren. Der Hauptfokus lag dabei auf dem Vergleich der Gedächtniswirkung während zweier Stimulationsbedingungen: *Up-phase* und *Down-phase* langsamer Oszillationen. Zu diesem Zweck wurden Probanden während des non-REM Schlafes auditorische Stimuli präsentiert, die zeitlich auf die Minima langsamer Oszillationen während der *Down-phase* und auf die Maxima langsamer Oszillationen während der *Up-phase* Stimulationsbedingungen abgestimmt waren. Allgemein wird angenommen, dass *Up states* langsamer Oszillationen einen Zustand erhöhter neuronaler Feuerungsraten repräsentieren. Folglich wird in der vorliegenden Studie die Hypothese angenommen, dass auditorische Stimulation, phasengekoppelt mit online detektierten *Up states* langsamer Oszillationen (*Up-phase* Stimulationsbedingungen), im Vergleich zu Stimulationen während *Down states* langsamer Oszillationen (*Down-phase* Stimulationsbedingungen) zu einer besseren Gedächtniskonsolidierung führen.

Probanden bearbeiteten abends vor den Experimentalnächten Paar-Assoziations-Lernaufgaben, in denen ihnen einzelne Wortpaare nacheinander auf einem Bildschirm präsentiert

und gleichzeitig die erste Silbe des ersten Wortes jedes Paares über In-Ohr-Kopfhörer vorgespielt wurden. Zuerst wurden die Probanden gebeten, die Silben mit den zugehörigen Wortpaaren zu lernen und dann die Wortpaare abzurufen, sobald ausschließlich die entsprechenden Silben vorgegeben wurden. Nachdem in dieser Lernrunde 60% korrekt abgerufener Wortpaare erreicht wurden, wurde die Gedächtnisleistung einmal sofort und einmal in einer verspäteten Abrufrunde am nächsten Morgen getestet. Die in der Aufgabe benutzten Silben waren jeweils 600 ms lang und wurden als Stimulus während des nachfolgenden non-REM Schlafes vorgespielt.

Wider Erwarten zeigten die Ergebnisse der Verhaltensanalysen der *Up-phase* Bedingungen keinen signifikanten Gedächtnisgewinn im Vergleich zu den *Down-phase* Bedingungen. Zudem konnte in beiden Bedingungen kein signifikanter Stimulationseffekt nachgewiesen werden. Analysen der gemittelten akustisch evozierten Potenziale von Probanden zeigten die charakteristischen unmittelbaren evozierten Potenzialantworten für *Up-phase* und *Down-phase* Stimulationen und legen somit korrekt ausgeführte auditorische Stimulationen während der beiden experimentellen Bedingungen nahe. Zudem absolvierten Probanden kognitive Tests und psychometrische Bewertungsaufgaben, deren Ergebnisse keine signifikanten Unterschiede für beide experimentellen Bedingungen aufwiesen.

Die Annahme dieser Studie, dass *Up-phase* gegenüber *Down-phase* langsamer Oszillationen ein überlegenes Zeitfenster für auditorische Gedächtnisreaktivierungen darstellen, hat sich nicht bestätigt. Dennoch brachte die Studie neue Erkenntnisse auf dem relativ neuen Feld der zielgerichteten Gedächtnisreaktivierungen im geschlossenen Regelkreis. Wie aus früheren Studienergebnissen hervorgeht, scheint die zielgerichtete Gedächtnisreaktivierung nicht nur eine nicht-invasive und effektive Möglichkeit zur Verbesserung der Gedächtniskonsolidierung im Schlaf darzustellen, sondern bietet auch potenzielle Vorteile, in Bezug auf klinische Anwendungen im Bereich der Psychotherapie und neurologischer Krankheiten. Daher verdeutlicht die vorliegende Studie, dass auf diesem Gebiet weiterführende Forschung notwendig ist. Diese könnte aufbauend auf den Ergebnissen der vorliegenden Studie zahlreiche noch offene Fragen klären, besonders in Bezug auf spezifische Stimulationsbedingungen, zugrundeliegende neuronale Mechanismen, Langzeitfolgen, Grenzen und potenzielle Nebenwirkungen der Technik der zielgerichteten Gedächtnisreaktivierung.

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Declaration

The study was conceptualized by Dr. Jing-Yi Wang, former research associate at the Institute for Medical Psychology and Behavioural Neurobiology, and Prof. Dr. Jan Born, head of the Institute for Medical Psychology and Behavioural Neurobiology at the Eberhard Karls University Tübingen. The study design was developed by Dr. Jing-Yi Wang in collaboration with Dr. Susanne Diekelmann and Dr. Hong-Viet V. Ngo, research associates at the Institute for Medical Psychology and Behavioural Neurobiology at the Eberhard Karls University Tübingen.

All experiments were performed by myself after initial training by Dr. Jing-Yi Wang. Analyses of polysomnographic recordings were performed by me after initial introduction by Dr. Hong-Viet V. Ngo, and reviewed and corrected where necessary by Dr. Jing-Yi Wang.

Statistical analysis was conducted by me with some assistance by Dr. Susanne Diekelmann, Dr. Jing-Yi Wang, and Dr. Hong-Viet V. Ngo.

I hereby declare that I have produced the present work on my own and that I have used no further sources and aids than indicated and marked as such.

Tübingen, ……..… ……..……

Date Signature

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Appendix

A1 Protocol adaptation night

Ablaufprotokoll – Probenacht

Proband / Session: ____ / ____Datum:_____

		Zeit(spanne)	Anmerkungen
	Vorbereitung (des Labors)*		Verstärker & PCs anschalten!
□	Ankunft des Probanden	Zeit: _____	
□	**Fragebogen :** • Probandeninformation • Einverständniserklärung		
□	Allg. Fragen	Gesund/fit	□ Ja □ Nein
		Aufstehzeit:	□ Ja □ Nein
		Mittagschlaf:	□ Ja □ Nein
		koffeinartige Getränke nach 14 h	□ Ja □ Nein
		24 Std. kein Alkohol	□ Ja □ Nein
		(Prüfungs-)stress:	
□	Elektrodenapplikation	Zeit :_____ - _____	Kappengröße: _____ Kopfumfang: _____
□	BrainAmp Elektroden einstecken		
□	EEG Software starten, Widerstände überprüfen		Display-Filter Interne Widerstandsmessung
□	EEG und Stimulations-Software starten		Stimulation: D360 Client, Spike2
□	Probanden gelb-grünes Kabel zur Stromableitung halten lassen		
□	Digitimer Elektroden einstecken		„Deblock“-Taste beim Einstecken
□	Aufnahme starten	(PC-Zeit: _____)	
□	Ableitung überprüfen		EOG, EMG
□	Hörschwelle bestimmen		**Hörschwelle: _____ dB**
□	Licht aus > Versuchsbeginn	(Zeit: _____)	LichtAus Marker!
□	Bestimmung der Verzögerung		**Verzögerung: _____**
□	Proband wecken Aufnahme beenden,	(Zeit: _____)	Nicht in REM oder SWS-Phasen „LichtAus“-Marker!
□	Erneute Widerstandsmessung		**Fehlerhafte Elektroden:** _____
□	Elektroden entfernen		
□	**Fragebogen:** SF-A-R		
□	Aufräumen		

A2 Protocol experimental night

Ablaufprotokoll – Experimentalnacht

Proband / Session: ____ / ____Datum:_____

		Zeit(spanne)	Anmerkungen
	Vorbereitung (des Labors)*		Verstärker & PCs anschalten!
□	Ankunft des Probanden	Zeit: _____	
□	**Fragebogen :** • Probandeninformation • Einverständniserklärung		
□	Allg. Fragen	Gesund/fit	□ Ja □ Nein
		Aufstehzeit:	□ Ja □ Nein
		Mittagschlaf:	□ Ja □ Nein
		koffeinartige Getränke nach 14 h	□ Ja □ Nein
		24 Std. kein Alkohol	□ Ja □ Nein
		(Prüfungs-)stress:	
□	Elektrodenapplikation	Zeit :_____ - _____	
	SSS, PANAS	10 min	
	PVT Digit Span (Forward & Backward) RWT	10 min	Digit Span_forward: _____ Digit Span_backward: _____ RWT (Kategorie): ___, Count: ___
	Lernen (1x) + direkter Abruf	Zeit: _____ - _____	Liste: Reaktivationsbedingung: □ Nein □ + 0.5 □ + 0.5
□	BrainAmp Elektroden einstecken		
□	EEG Software starten, Widerstände überprüfen		Display-Filter Interne Widerstandsmessung
□	EEG und Stimulations-Software starten		Stimulation: D360 Client, Spike2
□	Probanden gelb-grünes Kabel zur Stromableitung halten lassen		
□	Digitimer Elektroden einstecken		„Deblock“-Taste beim Einstecken
	Ableitung überprüfen		Ground □ Ref □ Fehlerhafte Ableitungen: _____ _____
□	Aufnahme starten	(PC-Zeit: _____)	
□	Licht aus > Versuchsbeginn	(Zeit: _____)	LichtAus Marker!
□	Beginn der Stimulation	(5-10 min ab der ersten S3 Epoche)	Bedingung/ Volumen: _____ / _____
	Lautstärke um 15 dB erhöhen	(ggf. in 3er Schritten verringern)	**Antwort vom EEG** **□ Ja □ Nein** **Finale Lautstärke: _____ dB**
	Ende der Stimulation		3-3 1/2 Std. ab dem ersten Stimulus
□	Proband wecken Aufnahme beenden,	(Zeit: _____)	Nicht in REM oder SWS-Phasen Stimulationsanzahl: _____ „LichtAus“-Marker!

	Frage zur Stimulation:	Stimulation wahrgenommen?	□ Ja □ Nein
□	Erneute Widerstandsmessung		**Fehlerhafte Elektroden:** _____
	Duschen Zeit	(ca. 30 min)	
□	SF-A-R, PANAS, SSS		
□	PVT, DS (Forward & Backward), RWT		
	Lernabfrage	Zeit: _____ - _____	Mind. 30 min nach dem Aufwecken
□	Aufräumen		

Notizen/ Auffälligkeiten

_____
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A3 Word pair lists with corresponding syllables

List 1

	Wort 1	Wort 2	1st Syllable
1	VULKAN	EXPLOSION	Vul
2	TÄUSCHUNG	ECHTHEIT	Täu
3	INDUSTRIE	BRANCHE	In
4	ANSICHT	MEINUNG	An
5	LEIDENSCHAFT	KUSS	Lei
6	HELDENMUT	TAPFERKEIT	Hel
7	FORDERUNG	GEHALT	For
8	PAPIER	BRIEF	Pa
9	GESUNDHEIT	IMPFUNG	Ge
10	ARMUT	ELEND	Ar
11	RICHTER	GERECHTIGKEIT	Rich
12	BEWEIS	TATSACHE	Be
13	ALKOHOL	OPIUM	Al
14	VERGLEICH	GLEICHNIS	Ver
15	AUTO	PRESTIGE	Au
16	PROFIL	PHOTOGRAPHIE	Pro
17	WOLLE	KLEIDUNG	Wol
18	STILLE	EINSAMKEIT	Stil
19	CHAOS	STRUKTUR	Cha
20	ZEITUNG	DRUCK	Zei
21	BARGELD	WERT	Bar
22	ABSPRACHE	VERTRAG	Ab
23	NUTZEN	KOSTEN	Nut
24	KUGEL	QUADRAT	Ku
25	VOGEL	KATZE	Vo
26	ERDE	STEIN	Er
27	DEMOKRATIE	SYSTEM	De
28	LÖSUNG	PROBLEM	Lö
29	SÄNGER	KÜNSTLER	Säng
30	MANGEL	VERZICHT	Mang
31	NÄSSE	GEWITTER	Näs
32	RÜSTUNG	ANGRIFF	Rüs
33	FIGUR	BRETT	Fi
34	FÄHIGKEIT	VERANLAGUNG	Fä
35	PUPPE	KIND	Pup
36	THEATER	REIHE	The
37	KÜCHE	EIMER	Kü
38	MUSEUM	ÄGYPTEN	Mu
39	LADEN	REKLAME	La
40	DIAMANT	GOLD	Di

A3 Word pair lists with corresponding syllables

List 2

	Wort 1	Wort 2	1st Syllable
1	WINTER	UNFALL	Win
2	EISENBAHN	SCHIENE	Ei
3	DYNAMO	LICHT	Dy
4	TONNE	REGEN	Ton
5	TANNE	RINDE	Tan
6	VERORDNUNG	BESCHEID	Ver
7	REVOLVER	KALIBER	Re
8	RINGE	BAUM	Ri
9	NÄHEN	KREUZSTICH	Nä
10	BIBLIOTHEK	SIGNATUR	Bi
11	GRILLEN	SOMMER	Gril
12	ORKAN	WIRBEL	Or
13	KÜSTE	DÜNE	Küs
14	INSEKT	LIBELLE	In
15	LABOR	PIPETTE	La
16	RADIO	STIMME	Ra
17	GRUPPE	VERSAMMLUNG	Grup
18	SCHÜLER	DOZENT	Schü
19	SALAT	GARTEN	Sa
20	ANGEBOT	MARKT	An
21	LAUNE	HUMOR	Lau
22	AUFGABE	ERLEDIGUNG	Auf
23	THEORIE	AUSNAHME	The
24	NAGEL	METALL	Na
25	ERGÄNZUNG	ZUSATZ	Er
26	KLIPPE	ABGRUND	Klip
27	BETRAG	WECHSEL	Be
28	ZIMMER	ECKE	Zim
29	GÖTTIN	GEBET	Göt
30	MUSIKER	AKKORDEON	Mu
31	DIENER	HALTUNG	Die
32	FAHNE	EROBERUNG	Fah
33	POLIZIST	WACHE	Po
34	GENUSS	ZIGARRE	Ge
35	APFEL	PFIRSICH	Ap
36	SEGEN	SCHÖPFER	Se
37	PUDDING	SÜSSIGKEITEN	Pud
38	MASCHINE	APPARAT	Ma
39	EMPFEHLUNG	RAT	Emp
40	JUNGE	MÄDCHEN	Jung

A4 Stanford Sleepiness Scale

Stanford-Schläfrigkeits-Skala

Datum: _____Uhrzeit: _____

Im folgenden soll der Grad der Schläfrigkeit (wie wach fühlen Sie sich?) erhoben werden:
Kreuzen Sie bitte das entsprechende Kästchen an.

Schläfrigkeitsgrad	Punktwert
Ich fühle mich aktiv, lebhaft, aufmerksam oder sehr wach	1
Ich kann konzentriert arbeiten, habe aber kein Leistungshoch	2
Ich fühle mich wach, entspannt und aufnahmefähig aber nicht voll konzentriert	3
Ich fühle mich irgendwie träge	4
Ich fühle mich träge, verlangsamt, und könnte mich hinlegen	5
Ich fühle mich schläfrig, benebelt, kämpfe gegen die Müdigkeit und würde mich lieber hinlegen	6
Ich bin kurz vor dem Einschlafen und habe bereits Traumdeutungen	7
Ich schlafe	8

A5 Positive and Negative Affect Schedule

Pnum:_____

O vor Lernen O vor Abruf

PANAS

Datum:_____ Uhrzeit: _____

Dieser Fragebogen enthält eine Reihe von Wörtern, die unterschiedliche Gefühle und Empfindungen beschreiben. Lesen Sie jedes Wort und tragen dann in die Skala neben jedem Wort die ***Intensität*** ein. Sie haben die Möglichkeit zwischen fünf Abstufungen zu wählen.

Geben Sie bitte an, wie Sie sich ***gerade jetzt*** fühlen.

	Gar nicht	ein bisschen	einigermaßen	erheblich	äußerst
1. aktiv	1	2	3	4	5
2. bekümmert	1	2	3	4	5
3. interessiert	1	2	3	4	5
4. freudig erregt	1	2	3	4	5
5. verärgert	1	2	3	4	5
6. stark	1	2	3	4	5
7. schuldig	1	2	3	4	5
8. erschrocken	1	2	3	4	5
9. feindselig	1	2	3	4	5
10. angeregt	1	2	3	4	5
11. stolz	1	2	3	4	5
12. gereizt	1	2	3	4	5
13. begeistert	1	2	3	4	5
14. beschämt	1	2	3	4	5
15. wach	1	2	3	4	5
16. nervös	1	2	3	4	5
17. entschlossen	1	2	3	4	5
18. aufmerksam	1	2	3	4	5
19. durcheinander	1	2	3	4	5
20. ängstlich	1	2	3	4	5

Pnum:_____

O vor Lernen O vor Abruf

A6 SF-A-R

Pnum:_____

Fragebogen zur Schlafqualität (SF-A-R)

Datum:_____ Uhrzeit: _____

Anleitung:
Die folgenden Fragen beziehen sich darauf, wie Sie in der letzten Nacht geschlafen haben. Kreuzen Sie bitte die Antworten an, die für Sie am ehesten zutreffen. Gehen Sie bei der Beantwortung der Fragen zügig voran und lassen Sie keine Frage aus. Bitte sofort nach dem Aufwachen morgens ausfüllen!

1. **Konnten Sie, nachdem Sie sich schlafen gelegt hatten, gleich einschlafen?**

Ja.	
Nein, erst nach 10 min.	
Nein, erst nach 20 min.	
Nein, erst nach 40 min.	
Nein, erst nach 1 Stunde.	
Nein, erst nach mehr als 1 Stunde.	
Ich konnte überhaupt nicht schlafen.	

1.a) Falls Nein, welches waren die Gründe? (Mehrfachnennungen möglich)

Persönliche / berufliche Probleme	
Geräusche im Zimmer oder von draußen	
Beschäftigung mit Tagesereignissen	
Ungewohnte Schlafumgebung	
Sonstige:	

2. **In der Einschlafphase hat man hin und wieder plötzlich deutliche Bildeindrücke. War dies gestern Abend bei Ihnen so?**

Nein	Bin nicht sicher	Ja, sehr deutlich

3. **Hatten Sie während der Einschlafphase Muskelzuckungen in den Armen oder Beinen?**

Nein	Leicht	Stark

4. **Sind Sie gestern nach dem Einschlafen nachts wieder aufgewacht?**

Nein	1x	2x	3x	>3x

4.a) Falls Ja, welches waren die Gründe? (Mehrfachnennungen möglich)

Persönliche / berufliche Probleme	
Geräusche im Zimmer oder von draußen	
Ich musste zur Toilette	
Ich hatte schlecht geträumt	
Sonstige:	

4.b) Falls Ja, wie lange waren Sie ungefähr wach? (Schätzen Sie bitte.)

1. Aufwachen	Dauer (min):	
2. Aufwachen	Dauer (min):	
3. Aufwachen	Dauer (min):	
4. Aufwachen	Dauer (min):	

5. **Können Sie sich erinnern, ob Sie heute Nacht geträumt haben?**

Nein, ich kann mich nicht erinnern geträumt zu haben	
Ja, ich habe geträumt, kann mich aber nicht mehr an den Trauminhalt erinnern.	
Ja, ich habe geträumt und kann mich an den Trauminhalt erinnern.	

5a.) Falls ja, welche Gefühle hatten Sie während des Träumens (Mehrfachnennungen möglich)

Angenehm	Neutral	Unangenehm

5b) Falls ja, was war (grob) der Inhalt der Träume

_____

_____

_____

_____

6. **Haben Sie in der letzten Nacht geschwitzt?**

Nein	Leicht	Stark

7. **Haben Sie heute Morgen Kopfschmerzen?**

Nein	Leicht	Stark

8. **War der gestrige Tag für Sie anstrengend?**

Nein	Ein wenig	Sehr

Anleitung:
Auf dieser Seite finden Sie einige Wörter, mit denen Sie beschreiben können, wie Sie sich gestern Abend fühlten, wie Sie heute Nacht geschlafen haben und wie Sie sich heute Morgen fühlen. Kreuzen Sie hinter jedem Wort an, in welchem Ausmaß es für Sie zutrifft. Bitte antworten Sie zügig und lassen Sie keine Zeile aus!

9. **Wie haben Sie letzte Nacht geschlafen?**

	Sehr	Ziemlich	Mittel	Wenig	Nicht
a) gleichmäßig					
b) tief					
c) gut					
d) entspannt					
e) ungestört					
f) ruhig					
g) ausgiebig					

10. **Wie fühlten Sie sich gestern vor dem Schlafengehen?**

	Sehr	Ziemlich	Mittel	Wenig	Nicht
a) sorglos					
b) erschöpft					
c) schlafbedürftig					

d) überfordert					
e) ausgeglichen					
f) ruhig					
g) müde					
h) entspannt					

11. **Wie fühlen Sie sich heute Morgen?**

	Sehr	Ziemlich	Mittel	Wenig	Nicht
a) Ausgeglichen					
b) Dösig					
c) Tatkräftig					
d) munter					
e) frisch					
f) ausgeschlafen					
g) entspannt					

A7 RWT

RWT_1 Untertest: † Berufe †Hobbys

Probanden-Code:

Datum:

Uhrzeit:

Bei dieser Aufgabe sollen Sie innerhalb von 2 Minuten möglichst viele verschiedene Wörter aus einer bestimmten Kategorie aufschreiben, die Ihnen der Versuchsleiter nennen wird. Dabei dürfen Sie keine Wörter mehrfach nennen und die Wörter dürfen nicht mit dem gleichen Wortstamm anfangen (z.B. Fischer, Fischverkäufer, Fischhändler wäre falsch).

Bitte versuchen Sie möglichst schnell viele verschiedene Wörter aufzuschreiben.

RWT_2 Untertest: † K-Wörter †B-Wörter

Probanden-Code:

Datum:

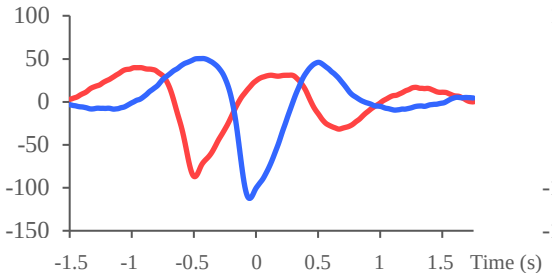
Uhrzeit:

Bei dieser Aufgabe sollen Sie innerhalb von 2 Minuten möglichst viele verschiedene Wörter mit einem bestimmten Anfangsbuchstaben aufschreiben, den Ihnen der Versuchsleiter nennen wird. Dabei dürfen Sie keine Wörter mehrfach nennen, keine Eigennamen benutzen (z.B. Paris oder Peter wäre falsch) und die Wörter dürfen nicht mit dem gleichen Wortstamm anfangen (z.B. Sport, Sportplatz, Sportschuhe wäre falsch).

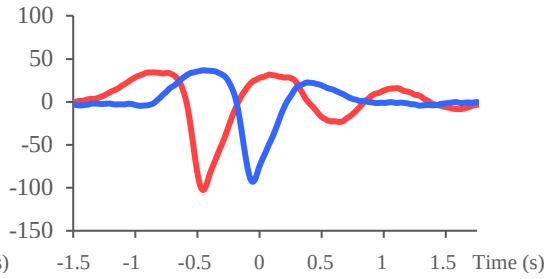
Bitte versuchen Sie möglichst schnell viele verschiedene Wörter aufzuschreiben.

A8 Individual averaged evoked responses (Fz)

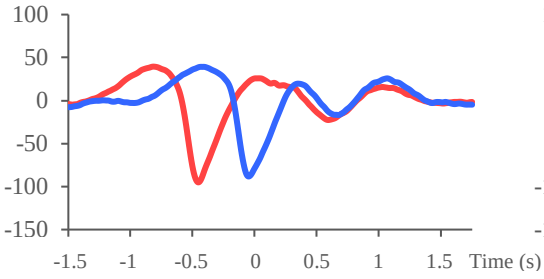
Subject #1: No down-phase response



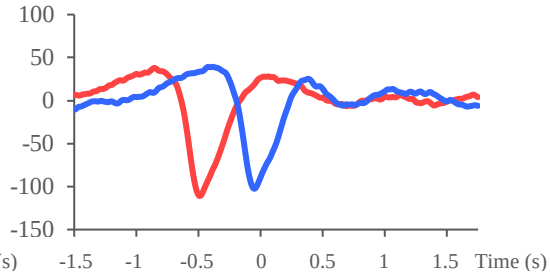
Subject #2: No down-phase response



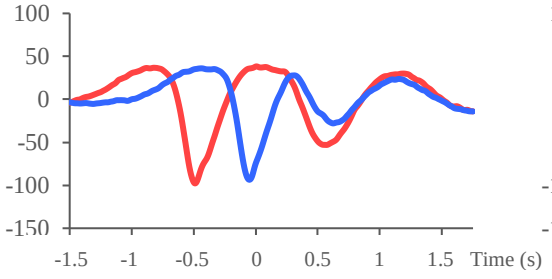
Subject #3



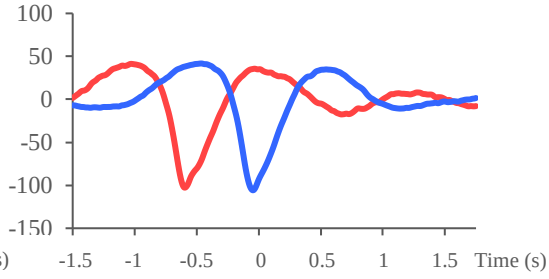
Subject #4: Weak responses



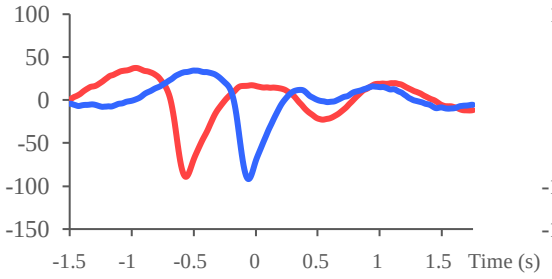
Subject #5



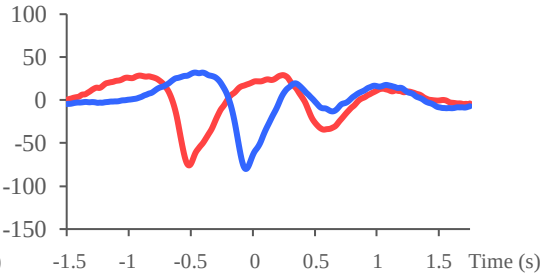
Subject #6: No down-phase response



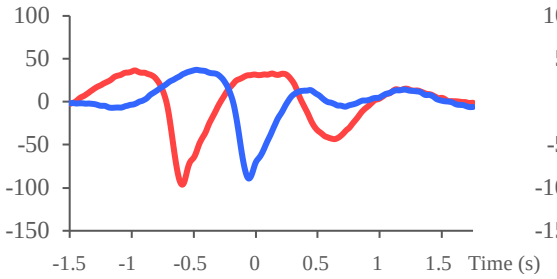
Subject #7: Weak down-phase response



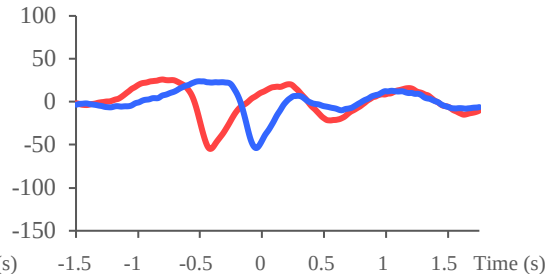
Subject #8



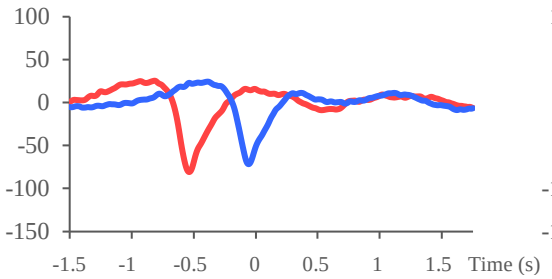
Subject #9: Weak down-phase responses



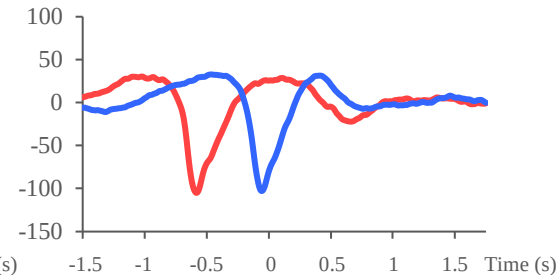
Subject #10: recorded at Cz



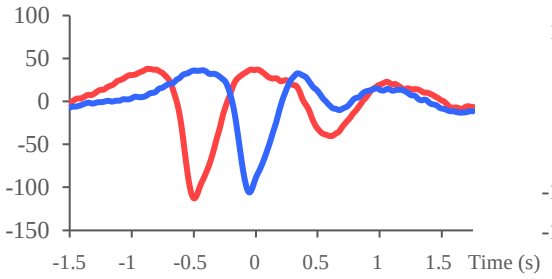
Subject #11: Weak responses



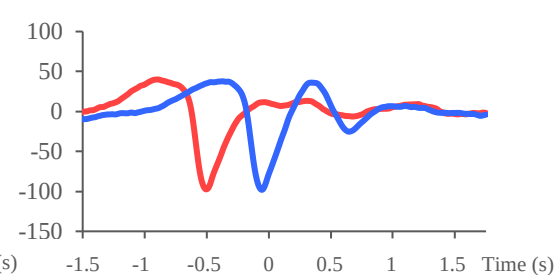
Subject #12



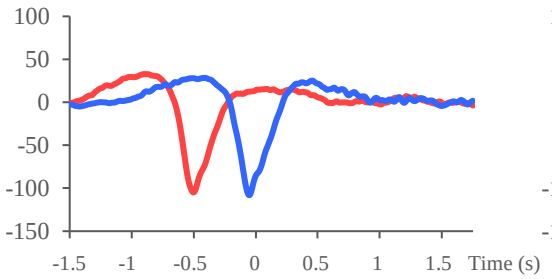
Subject #13



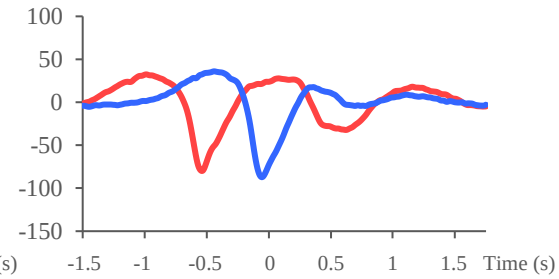
Subject #14: Weak up-phase response



Subject #15: No responses



Subject #16: Weak down-phase response



Individual averaged evoked responses

The mean averaged AEP signals for each single subject at electrode position Fz during up-phase (red line) and down-phase stimulation conditions (blue line) are depicted. Only the depicted AEP signals of subject #10 were recorded at electrode position Cz due to Fz electrode detachment causing a lost Fz signal during the up-phase condition. The time marker '0 s' represents the moment of cueing onset (the moment the acoustic stimulus starts being presented) during both conditions. s: seconds.