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Single closed-loop acoustic stimulation during NREM sleep in mice

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Abstract

Sleep rhythms can elucidate the neurophysiological processes underlying sleep-dependent memory consolidation, and their neuromodulation. Enhanced slow oscillation (SO) activity, as well as increased phase-coupled spindle and hippocampal sharp wave-ripple (SWR) activity, are associated with improved memory consolidation during sleep across mammalian species. In humans, closed-loop acoustic stimulation (CLAS) has been shown to facilitate the grouping of sleep spindles to SOs using EEG scalp electrodes. In the present study, single closed-loop acoustic stimulation (sCLAS) was implemented in mice, in order to investigate the modulation of SOs, sleep spindles as well as SWRs. Memory retention performance of mice measured in an object place recognition (OPR) task did not differ significantly from the control session under sCLAS, although differential responses were found for Up- versus Down-State Stimulation. Application of sCLAS in the 3h sleep interval did not affect the measured sleep parameters significantly. In all conditions, the acoustic stimuli produced an acute increase in SWR activity followed by a significant suppression of at least 1 second. The only electrophysiological outcome indicating a possible relationship to mnemonic function was the SO-SWR coupling. Further experiments are necessary to investigate the translational relevance of (s)CLAS in mice.

Keywords: closed-loop acoustic stimulation (CLAS), mouse model, object place recognition (OPR), slow oscillation (SO), sleep, NREM sleep, sleep-spindles, sharp wave-ripples, memory consolidation

Zusammenfassung

Schlafrhythmen gewähren einen Einblick in die neurophysiologischen Prozesse, die der schlafabhängigen Gedächtniskonsolidierung zugrunde liegen, sowie in deren Neuromodulation. Eine erhöhte Aktivität der langsamen Oszillationen (SO) sowie eine erhöhte Aktivität der phasengekoppelten Spindeln und des hippocampalen Sharp Wave-Ripples (SWR) sind bei Säugetierarten mit einer verbesserten Gedächtniskonsolidierung während des Schlafs verbunden. Beim Menschen hat sich gezeigt, dass die Closed-Loop-Akustische-Stimulation (CLAS) die Gruppierung von Schlafspindeln zu SOs mithilfe von EEG-Kopfhautelektroden erleichtert. In der vorliegenden Studie wurde die single Closed-Loop-Akustische-Stimulation (sCLAS) in Mäusen eingesetzt, um die Modulation von SOs, Spindeln sowie SPWRs zu untersuchen. Die Retentionsleistung von Mäusen, die in einer Object- Ort- Anerkennung (OPR) Aufgabe gemessen wurden, unterschied sich nicht signifikant von der Kontrollsitzung unter sCLAS, obwohl unterschiedliche Reaktionen für die Stimulation der Up- und Down-State gefunden wurden. Die 3-stündige sCLAS-Periode hatte keinen signifikanten Einfluss auf die gemessenen NREM Schlafparameter. Unter allen Bedingungen führten die akustischen Reize zu einem akuten Anstieg der Spindeln- und SWR-Aktivität, gefolgt von einer signifikanten Unterdrückung von mindestens 1 Sekunde. Das einzige elektrophysiologische Ergebnis, das auf einen möglichen Zusammenhang mit der Gedächtnisfunktion hinwies, war die SO-SWR-Kopplung. Weitere Experimente sind notwendig, um die translationale Relevanz von (s)CLAS bei Mäusen zu untersuchen.

Schlüsselwörter: Closed-Loop Akustische Stimulation (CLAS), Mausmodell, Objekt- Ort-Anerkennung (OPR), langsame Oszillationen, Schlaf, NREM-Schlaf, Schlafspindeln, Sharp wave-ripples, Gedächtniskonsolidierung

Abbreviations

CLAS	Closed-Loop Acoustic Stimulation
i.p.	Intraperitoneal
LFP	Local Field Potential
NOR	Novel Object Recognition
NREM	Non-Rapid-Eye Movement
PreREM	Pre-Rapid-Eye Movement
PVC	Polyvinyl Chloride
REM	Rapid Eye Movement
RMS	Root Mean Square
s.c.	Subcutaneous
sCLAS	Single Closed-Loop Acoustic Stimulation
SO	Slow Oscillation
SWR	Sharp Wave-Ripple
SWA	Slow-Wave Activity

Key Term Definitions (ordered by relevance)

Closed-loop acoustic stimulation (CLAS): This term refers to the delivery of acoustic stimulation during NREM sleep in synchrony with the brain's own endogenous slow oscillation (SO) rhythm. In this thesis CLAS is to be understood as SO-CLAS, although CLAS is a general term in neuroscience research and is not only limited to SOs.

Single closed-loop acoustic stimulation (sCLAS): This term describes a specific method of delivering CLAS, wherein a single pulse per stimulus is applied instead of consecutive pulses. This term is utilized in the scientific paper associated with this thesis.

Acoustic Stimulation: This term refers to results generated in response to (unmuted) acoustic stimuli only. Consequently, these results reflect a subsection of the sCLAS application used in this study.

Pseudo Stimulation: This term refers to results generated in response to muted acoustic stimuli only. As a result, it reflects a subset of the sCLAS application utilized in this study.

Stimulation session: This term denotes a session during which acoustic stimuli and pseudo stimuli were alternately applied throughout NREM sleep.

Control session: This term refers to a session in which only muted stimuli were applied consistently throughout NREM sleep. In the scientific paper associated with this thesis, this is referred to as a "sham" session.

Sleep rhythms/sleep oscillations: The three sleep rhythms within the scope of this thesis are slow oscillations (SOs), sleep spindles, and sharp wave-ripples (SWRs).

1 Introduction

1.1 Sleep

1.1.1 Sleep definition and function

Sleep is defined on electrophysiological and behavioral levels, although its full functions remain to be elucidated (Wang et al., 2011). Behavioral characteristics of sleep include an increased arousal threshold for external stimuli, reversibility, relative immobility, and homeostatic regulation (Rasch & Born, 2013). Sleep and sleep-like behavior has been observed not only in vertebrates but also in invertebrates such as flies, bees and cockroaches (Cirelli & Tononi, 2008). The fact that this state is evolutionarily highly conserved, although the accompanying loss of consciousness puts the individual at risk against predators, points toward its fundamental function. Supporting immune defense, regulating calory usage, restoring brain energy stores, serving glymphatic system and supporting brain connectivity can be given as some examples of functions attributed to sleep (Krueger et al., 2016). Regarding cognitive functions sleep deprivation studies have shown the relevance of sleep most predominantly for attention and psychomotor vigilance, and behavioral response variability. Sleep deprivation has also led to increased formation of false memories (Killgore, 2010; Lo et al., 2016), and sleep is known to facilitate many facets of memory consolidation (Chambers, 2017). The electrophysiological brain activity serving memory is the focus of this thesis.

1.1.2 Sleep electrophysiology

The two main phases of mammalian sleep, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep are distinguishable by their electroencephalogram (EEG) and muscle tone electromyogram (EMG) patterns (Zielinski et al., 2016). NREM sleep is characterized by slow and high- amplitude EEG encompassing delta rhythms (0.5–4 Hz) accompanied by low muscle tone. Rapid and low amplitude EEG dominated by theta activity (4-8 Hz) is observed along with muscle atonia and rapid eye fluctuations in REM sleep

(R. Zielinski et al., 2016). Unlike humans, rats and mice are nocturnal and have multiple sleep periods throughout a 24-hour period. Additionally, their individual sleep episodes and cycles of NREM and REM sleep are considerably shorter (Rayan et al., 2024).

1.1.3 Sleep brain rhythms

Mammalian cortical networks exhibit a wide range of oscillations. The phylogenetic preservation of these oscillations suggests their functional relevance (Buzsáki & Draguhn, 2004). Information integration occurs when convergent inputs are delivered synchronously. Neurons have the ability to preferentially respond to inputs of a specific rhythm and phase of an oscillating network oscillation. Thus, oscillator activity in the brain can facilitate the integration of information (Buzsáki & Draguhn, 2004). The oscillations during sleep of relevance for memory consolidation, emerge predominantly from the thalamocortical and hippocampal systems. Slow oscillations, sleep spindles and sharp wave-ripples are such rhythms occurring during NREM sleep whose activity will be investigated in this thesis. They will be shortly described in the following sections **1.1.3.1- 1.1.3.3.**

1.1.3.1 Slow oscillations

The most prominent electrophysiological events during sleep are undoubtedly the slow oscillations (SO, ~ 1 Hz). Intracellular animal recordings reveal a bimodal membrane potential distribution of cortical neurons: a hyperpolarized membrane potential level (SO down-state) associated with neuronal silence for several hundred milliseconds, and a depolarized up-state lasting similarly long (Steriade, Nunez, et al., 1993). The source of SOs was initially reported to be solely the cerebral cortex (Steriade, Contreras, et al., 1993) involving an interplay of intrinsic currents and network interactions (Bazhenov et al., 2002; Timofeev et al., 2000). Yet, recently the contribution of the thalamus to SO generation has been reviewed in (Neske, 2016). SOs are predominantly local, travel mainly from anterior to posterior direction and are reflected in subcortical structures like hippocampus (Massimini et al., 2004; Nir et al., 2011; Ruch et al., 2022). Non-invasive brain stimulation studies in humans have revealed the causal contribution

of SOs in memory consolidation process where increases in SO activity led to enhanced memory promotion in sleep (Marshall et al., 2006; Ngo et al., 2013; Perrault et al., 2019). Functionally, SOs are proposed to group sleep-spindle and sharp wave-ripple events, thereby enabling crosstalk between cortical and subcortical regions (Bartos et al., 2007; Girardeau & Zugaro, 2011). This function will be revisited in the section **1.2.1 Memory and the Active System Consolidation Hypothesis** .

1.1.3.2 Sleep-spindles

Another oscillatory hallmark of NREM sleep are sleep-spindles (~10-15 Hz; please note that this range is not strictly confining the spindle activity) which are generated through the coordinated activity within the thalamo-cortical loop, consisting of the thalamus, the thalamic reticular nucleus and neocortex (Fernandez & Lüthi, 2020; Schönauer & Pöhlchen, 2018). During sleep cycles of inhibition and excitation between neurons of the reticular nucleus and thalamo-cortical neurons are initiated, that are observed as waxing and waning sleep-spindles in cortical recordings. Sleep-spindles tend to co-occur with the SO up-state of NREM sleep (Fernandez & Lüthi, 2020; Schönauer & Pöhlchen, 2018). Spindle density (Eschenko et al., 2006) as well as the SO-spindle coupling were increased after learning (Mölle et al., 2009) in humans. Klinzing and colleagues describe the time window where spindles co-occured with SO up-states as the most effective time to enhance memory consolidation (Klinzing et al., 2019). Importance of spindle and ripple coupling for memory consolidation was emphasized in a study in which disrupting their co-occurrence but not the discrete events separately, reduced the beneficial effect on memory of sleep (Bartos et al., 2017).

1.1.3.3 Hippocampal sharp wave-ripples

A further relevant oscillatory sleep pattern are the hippocampal sharp wave-ripples (SWR) which occur predominantly during NREM sleep but also during quiet wakefulness (Klinzing et al., 2019). The ripple component (100 – 250 Hz) of this activity is the result of an interplay between

activated pyramidal cells and interneurons in the hippocampal CA1 layer whereas the conjointly occurring sharp-wave component arises from CA3 input into stratum radiatum (Buzsáki, 2015). Hippocampal CA1 pyramidal cells which are coactivated during exploration are reactivated as assembles during subsequent sleep within SWR patterns, a phenomenon termed reactivation (Wilson & McNaughton, 1994). A study showed that the optogenetic silencing of CA1 pyramidal cells during SWR occurrence in NREM sleep was enough to disrupt consolidation of novel spatial maps acquired prior to sleep (van de Ven et al., 2016). Rothschild and colleagues recorded neural activity in both the auditory cortex (AC) and hippocampus during a sound-guided task and sleep in rats. They found that activation patterns in the AC precede and predict hippocampal activity during sharp wave-ripples (SWRs), while SWR-associated patterns in the hippocampus forecast subsequent activity in the AC (Rothschild et al., 2017). Taken together, representations reactivated in neocortex during NREM sleep are biased by hippocampal memory traces as reflected by hippocampal SWRs (Lewis & Bendor, 2019; Rothschild et al., 2017).

1.2 Sleep and Memory Consolidation

1.2.1 Memory and the Active System Consolidation Hypothesis

Memory is the capacity to encode, store and retrieve information (Grimaldi et al., 2020; Squire, 2009). Encoding defines the storage of newly established memory traces in both hippocampus (short-term store) and neocortex (long-term store) (Antony & Paller, 2017; Squire et al., 2015). At this stage, memories are very labile and can be easily decayed, i.e., be forgotten. The following stage, consolidation, serves to strengthen these memory traces and to integrate them to long term storage in neocortex (Antony & Paller, 2017; Squire et al., 2015) which requires that information is sorted and reinforced in the absence of external information barrage (Girardeau & Lopes-dos-Santos, 2021). The integration of memory traces into existing neural networks is indispensable (Zlotnik & Vansintjan, 2019). Over time recall may become hippocampus independent, at least for the declarative memory (Wiltgen et al., 2010). Other

results argue toward continued engagement of the hippocampus in recall (Slotnick, 2022). In humans, intervention procedures aimed to understand and/or improve sleep-associated memory function are primarily focused on declarative memory and NREM sleep (Feld & Diekelmann, 2015; Rasch & Born, 2013). Declarative memory involves semantic and episodic memories, which relies mainly on medial temporal lobe structures including the hippocampus whereas non-declarative or procedural memories depend mostly on striatum, cerebellum, and cortical association areas (Cohen & Squire, 1980). Due to the many interactions and overlaps between key subcomponents of learning and memory, a perfect taxonomy is impossible to achieve (Brem et al., 2013). Yet, the essential component of declarative memories are their dependance on the hippocampus. For instance, the extent of impairments in verbal learning, logical memory, and visual reproduction have been linked to reductions in hippocampal volume (Petersen et al., 2000) and challenges in declarative memory tasks have been connected to hippocampal degeneration across diverse animal species (Squire & Zola-Morgan, 1991). In line with this distinction, in animal studies, the terms hippocampus-dependent vs. non-hippocampus-dependent memory processes are used.

The active system consolidation hypothesis refers to how the transfer of information between two different memory stores, a fast temporary and slower long-term storage, takes place. According to this theory, newly established hippocampal memory traces are reactivated in NREM sleep during hippocampal SWRs. SOs are thought to synchronize both thalamo-cortical spindles and SWRs and thereby enable the redistribution of information through a coordinated dialogue between the hippocampus and neocortex (Rasch & Born, 2013). In this triple nesting event, spindles are nested into SO up-state where SWRs are nested into the troughs of spindles which is thought to optimize both transmission of replayed information from hippocampus to neocortex and the reorganization of them through synaptic consolidation (Klinzing et al., 2019). The nesting of ripples in spindles may allow for a bidirectional information transfer, where cortical inputs bias replay activity in ripples nested in spindles, and hippocampal outputs modulate cortical activity during spindles. Despite the challenge of competing with local

inhibition, hippocampal inputs may still bias activity at the population level. This complex interplay underscores the importance of temporal precision in the SO-spindle-ripple coupling for effective memory consolidation (Todorova & Zugaro, 2020).

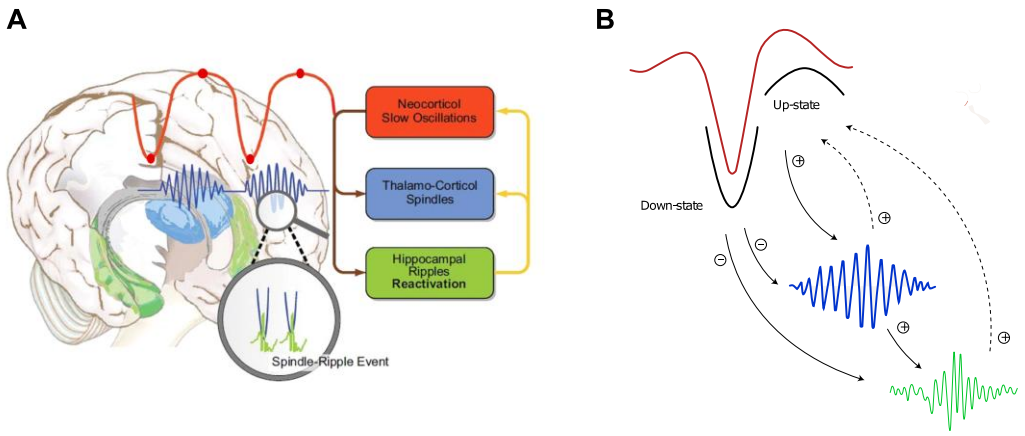


Figure 1.1. The Active Systems Consolidation Hypothesis

A. The illustration shows the interaction between the main brain structures of cortico-thalamo-hippocampal loop: neocortex, thalamus and hippocampus, and the sleep rhythms associated: slow oscillations (SOs), spindles and sharp wave-ripples (SWRs). Triple coupling of these sleep brain rhythms facilitates memory consolidation during NREM sleep. Sleep spindles nest into the SO up-states, simultaneously SWRs are nested in the troughs of sleep spindles and thereby the transfer of information from the hippocampus to the neocortical long-term store is mediated in synchronization. The red dots are marking the SO up- and down-states. **B.** Potential regulation loops between sleep oscillations are emphasized. Top down (solid arrows): SO down-state drives the generation of sleep spindles and in turn SWRs, in contrast SO down-state suppresses the other two sleep oscillations. Bottom up (dotted arrows) both spindles and SWRs can induce SOs in the cortex. (Figure adapted from Klinzing et al., 2019, Nat Neurosci, Figure 2 and Rasch & Born, 2013, Physiol Rev,Figure 3).

1.2.2 Object Place Recognition Task (OPR)

To obtain comparable results, care toward implementing closely comparable task procedures in both humans and rodents should be taken (Petersen et al., 2000; Squire & Zola-Morgan, 1991). Sleep, in particular NREM sleep has been shown to play a selective role in enhancing hippocampus-dependent memory consolidation in rodents as in humans (Cai et al., 2009). Investigating hippocampus-dependent memory consolidation in rodents also promises to be

rewarding, since hippocampal ripple events and their coupling to other sleep rhythms can be electrophysiologically well monitored (Todorova & Zugaro, 2020). Only a few human laboratories world-wide with associated neurological departments and invasive recordings have this possibility.

The role of the hippocampus in the spatial object place recognition (OPR) task has been provided by studies involving hippocampal damage (Barker & Warburton, 2011; Mumby et al., 2002). The hippocampus-dependent OPR task leverages rodents' innate preference for novel locations, thereby eliminating the need for extensive training. Additionally, this task is relatively low-stress and can be repeated with different objects for each animal (Save et al., 1992). Hence, in this study, it was opted to employ the OPR task and implemented it using a within-subject design.

It is noteworthy that in rats, it has been demonstrated that only NREM sleep-enriched periods improve memory retention above chance level in OPR tasks (Binder et al., 2012).Additionally, an intriguing study revealed that sleep deprivation for 3 hours can be compensated for when applied immediately after the OPR task, but not when a 1 - hour sleep opportunity is introduced in between (T. M. Prince et al., 2014). In this study, a 3 hours sleep interval commenced right after the sample phase of the OPR task, coinciding with the beginning of the light phase when sleep pressure is typically at its peak for mice.

For the OPR task it is important to mention, that it aims to assess whether the animals' preference for the displaced object in the test run is or is not above chance performance. In a subsequent step differences between Preference Indexes (PIs) can be tested (Dere et al., 2007).

1.2.3 Interventions to enhance sleep-dependent memory consolidation

1.2.3.1 General information

Considering the described and generally accepted relationship between sleep and memory consolidation, modulating sleep has been a focus of attention and the methods of intervention are ever-growing. Enhancing memory consolidation during sleep can have important implications, especially in certain subpopulations (e.g., elderly people, people with neurocognitive and/or neuropsychiatric disorders). SOs and sleep-spindles were the main target in most techniques. The most common procedures investigated include (weak) electrical stimulation, magnetic stimulation, acoustic stimulation, and targeted memory reactivation (Malkani & Zee, 2020). Below only studies regarding acoustic stimulation, as used in this study, are described.

1.2.3.2 Acoustic stimulation in humans

Acoustic stimulation to modulate sleep-dependent memory is a rapidly developing field (Harrington & Cairney, 2021). In this technique, a sub-arousal sensory (acoustic) stimulus is introduced to synchronize neuronal cortical activity during NREM sleep (Ngo et al., 2013). Firstly introduced in the seminal work of Ngo and colleagues, the acoustic stimulation was delivered in synchrony with the endogenous SOs in healthy young adults. When a supra-threshold EEG SO down-state peak was detected algorithmically, two 50 ms pulses of pink noise were given targeting the two subsequent SO up-states (two pulses per stimulation, **Figure 1.2.A**). To ensure precise, phase-locked stimulation (closed-loop manner), the timing of the stimuli was individually adjusted to the temporal SO characteristics (i.e., the average time per subject between the SO- down-state and up-state peaks, corresponding to the SO down- and up-states respectively). In the sham condition, which comprised a separate session, the time point of potential stimuli were marked as time stamps, but stimuli were not delivered. This application of CLAS enhanced the SO rhythm by inducing ‘trains’ of successive SO-cycles (**Figure 1.2.B**) but did not increase overall SO density, increased the amplitude of SO-cycles and their slope

magnitude, and amplified the phase-coupled fast spindle activity during SO up-states. Furthermore, overnight memory retention was improved in comparison to the sham condition. It must be noted that CLAS application phase-locked to down-state disrupted SO activity and did not improve memory performance (Ngo et al., 2013).

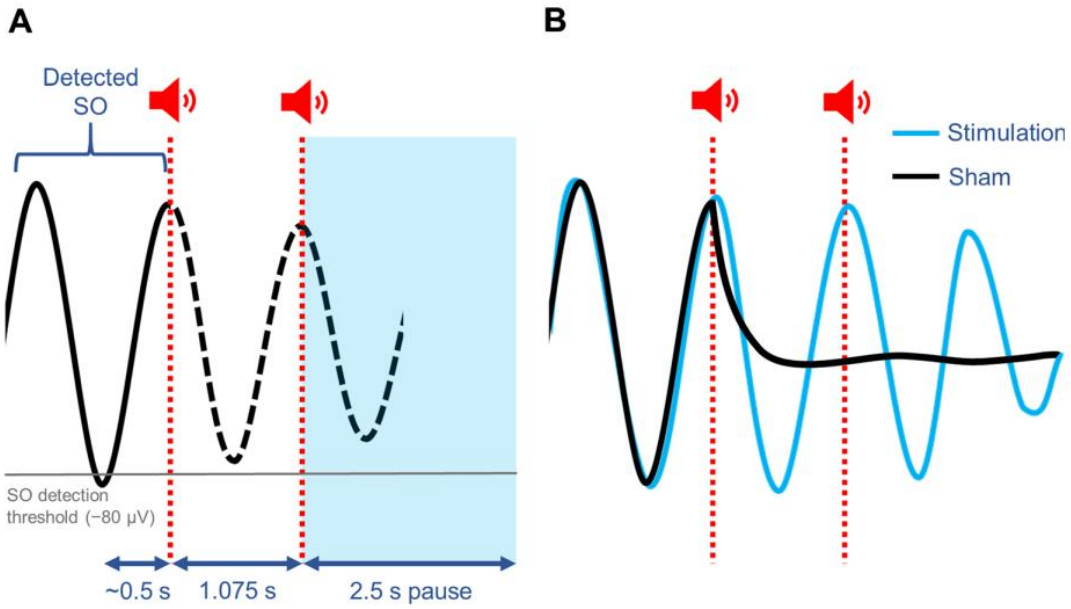


Figure 1.2. The CLAS protocol used by Ngo and colleagues and its implications on the SO-rhythm.
A. The detection of a supra-threshold SO down-state peak (−80 µV; grey line) triggers the delivery of two in-phase 50ms pulses of pink noise (red lines). The first stimulus occurs during the predicted SO up-state, according to the average delay between the SO down-state and up-state peak (~0.5 s) and the second after a fixed interval (1.075 s) corresponding to the typical duration of a full SO-cycle in humans. In the sham condition, potential stimuli were marked as time stamps, but they were not delivered. The detection protocol paused for 2.5 s after the second stimulus. **B.** Entraining of three SOs and hence the improvement in the SO rhythm, is shown in contrast to the sham condition. (From Harrington & Cairney, 2021, Curr Sleep Med Rep, Figure 1).

1.2.3.3 Acoustic stimulation in rodents

As described above, interventions to investigate and modulate the sleep-dependent memory consolidation in rodents involved only electrical and optogenetic stimulation until recently. A rat study implemented closed-loop acoustic stimulation (CLAS) over chronic periods and tested its effects on the sleep spectral characteristics, and on the procedural memory (Moreira et al., 2021). Persistent alterations were observed in both delta (reflecting slow oscillations) and sigma

(representing sleep spindles) power. While CLAS applied at SO up-states did not elicit a significant change in memory performance, CLAS applied at SO down-states affected the overall engagement and success rate in the memory test detrimentally and evoked spectral changes which were positively correlated with learning performance. Similarly as in humans, CLAS delivery during the up-state entrained slow oscillations, in contrast to down-state delivery in which these oscillations were disturbed.

1.2.3.4 Different stimulation parameters of acoustic stimulation

Below, the differential acoustic stimulation parameters employed essentially in human CLAS studies are categorized, highlighting differences observed. The only SO-CLAS study found in the literature in rodents prior to this study applied a single acoustic stimulation in a closed-loop manner, targeting either the up- or down-state of the SO cycle and involving a motor learning task (Single-Pellet-Reaching,SPR) to measure memory performance in rats (Moreira et al., 2021). The present study employed similarly a single acoustic stimulation in a closed-loop manner, targeting four different time points on the SO cycle per session, and involving the hippocampus-dependent Object-Place Recognition) OPR task in mice.

Number of pulse per stimulation

Given the observed effects above, follow-up studies questioned whether applying more subsequent stimuli without pausing would enhance the benefits of acoustic stimulation further (Ngo et al., 2015; Ong et al., 2016; Papalambros et al., 2017). However, additional improvement of neither SO activity nor memory performance was observed. This could be imposed by protective mechanisms in the brain that prevent hyper synchronicity (also seen in epileptic seizures) during SO activity (Cairney et al., 2015; Papalambros et al., 2017). On the other hand, some studies also applied single pulse per stimulation (Cox et al., 2014; Henin et al., 2019; Krugliakova et al., 2020) and one reported beneficial effect both on NREM sleep rhythms (increased slow wave and spindle activity) and memory retention (Leminen et al., 2017).

Closed-loop vs Open-loop methods

Acoustic stimulation was primarily applied in a closed-loop manner, with one study using the open-loop method (Weigenand et al., 2016). In this method, the first pulse was independent of the ongoing SO-rhythm, while the second and third pulses targeted the predicted SO up-state evoked by the first stimulus. This approach entrained SOs and enhanced phase-coupled spindle activity during the first SO-cycle. However, overall fast spindle power decreased during the entire stimulation period and had no significant impact on memory retention compared to the sham condition.

Timing of stimulation

Recently, a study investigated the optimal SO-state to deliver acoustic stimuli in order to maximally enhance the oscillatory activity during NREM sleep (Navarrete et al., 2020). Any stimuli applied anywhere on the positive portion of the SO (EEG) increased the amplitude of subsequent SOs and increased the likelihood of detecting sleep-spindles which were phase-coupled to it. However, these effects were maximal when the delivery was closest to the SO up-state, which points out the criticality of precise timing.

Memory tasks

Given the association of SOs with memory reactivation in the hippocampus, most studies have focused on changes in hippocampus-dependent declarative memories, including paired associate learning with related word-pairs, unrelated word-pairs, face-name associations, picture recognition tasks, and spatial navigation tasks. Additionally, investigations have explored the impact of acoustic stimulation on other memory domains, such as procedural, emotional, and working memory reviewed by Harrington & Cairney (2021).

Findings in the declarative domain indicate that consolidation benefits from stimulation are observed best when learned information aligns with pre-existing knowledge (as seen in paired

associate learning with related word-pairs, (Leminen et al., 2017; Ngo et al., 2015) or when a reward is presented to create value for associating information(Prehn-Kristensen et al., 2020).

1.3 Overarching objective

The main objective of this study is to take the first steps in developing a stimulation protocol to further investigate the electrophysiological effects of single closed-loop acoustic stimulation (sCLAS) as introduced in humans (Ngo et al., 2013). For this purpose, acoustic-evoked changes on endogenous sleep rhythms and mnemonic function during NREM sleep are to be assessed. The successful development of a stimulation protocol with comparable results to that in humans would enable the electrophysiological responses of deeper lying structures, e.g., the hippocampus, to be analyzed, which in human subjects (except for patients with electrodes implanted for clinical reasons, e.g., for monitoring epilepsy) is not feasible.

In this framework, the optimal stimulation time point (corresponding to different states of the SO) within a SO-cycle are to be analyzed. For this purpose, sCLAS is delivered at four distinct time points following the detected SO down-state peak; 2 ms, 120 ms, 180 ms or 300 ms thereafter. The modulatory effect of each application is tested followingly. From now on these deliveries will also be mentioned as 2 ms, 120 ms, 180 ms and 300 ms -stimulation- conditions. The 2 ms condition is aimed to target the SO Down-State whereas the 180 ms condition is supposed to correspond to SO Up-State Stimulation. On the other hand, 120 ms and 300 ms are introduced to target transitional states, namely the Down-to-Up-State Transition, and Up-to-Down-State Transition. In addition, a control condition is introduced where no acoustic stimuli were delivered (please see **Figure 2.1**).

1.4 Hypotheses

In the section **1.2 Sleep and Memory Consolidation**, the literature on sleep highlights the significance of delivering CLAS during SO up-state in influencing various sleep-event parameters and memory retention. For instance, studies have indicated that up-state CLAS is associated with an increase in mean SO amplitude and enhancement of SO-coupled fast spindle activity (Navarrete et al., 2020; Ngo et al., 2013). Additionally, it has been noted to lead to an increase in mean SO slope and the acute entrainment of SOs (Ngo et al., 2013), as well as an augmentation of slow-wave and sleep spindle power (Leminen et al., 2017). In several cases, up-state stimulation has been associated with increased memory retention (Leminen et al., 2017; Ngo et al., 2013).

Conversely, down-state delivery of CLAS, previously associated with the disruption of SO activity (Ngo et al., 2013) has been linked with a decrease in slow wave and spindle power (Moreira et al., 2021) and either no changes (Ngo et al., 2013) or negative changes (Moreira et al., 2021) in memory retention.

Furthermore, the importance of SO-spindle as well as spindle-ripple coupling for memory consolidation has been emphasized in several studies (Bartos et al., 2017; Klinzing et al., 2019). Building upon these insights, this study advances the following hypotheses. Please note that the stimulation condition which would be found to be associated with strong memory consolidation in this study, potentially could reveal not yet distinguished sleep-oscillatory patterns.

1.4.1 Main Hypotheses

Hypothesis I. sCLAS delivery at each specific SO-state will affect electrophysiological responses differently. Specifically:

a) **Although the sCLAS delivery at different SO-states will lead to the modulation of electrophysiological responses, sleep structure will remain unaffected.**

CLAS in humans modulated SO activity without affecting the processes initiating SO generation, as evidenced by comparable time spent in each sleep stage between the stimulation and control conditions (Ngo et al., 2013). Therefore time spent in sleep stages; NREM, PreREM, REM and Wake will be investigated. There will not be any significant changes across all conditions.

b) **Up-State Stimulation will enhance sleep rhythms and their coupling.**

Both acute responses to stimulation and spontaneous activity within the sessions (i.e., not in response to acute stimulation) will be investigated. Acutely, stimulus-event correlations will be increased for the sleep-events, i.e., **i)** cortical SOs **ii)** cortical spindles and **iii)** hippocampal ripples under the Up-State (180 ms) Stimulation compared to Pseudo Stimulation. The responses in spontaneous activity, namely, **iv)** SO density, **v)** SO slope, **vi)** SO amplitude, **vii)** spindle density, **viii)** SO-spindle-coupling and **ix)** SO-ripple coupling will be increased under Up-State Stimulation compared to the control session.

c) **Down-State Stimulation will reduce sleep rhythms and their coupling.**

Both acute responses to stimulation and spontaneous activity within the sessions (i.e., not in response to acute stimulation) will be investigated as given in **Hypothesis I.b**. Acutely, stimulus-event correlations will be decreased for the sleep-events, **i)** cortical SOs **ii)** cortical spindles and **iii)** hippocampal ripples under the Down-State (2ms) Stimulation in comparison to Pseudo Stimulation; continuously,

iv) SO-spindle-coupling and **v)** SO- ripple-coupling will be decreased under Down-State Stimulation in comparison to the control session.

d) The stimulation conditions will differentially affect the sleep rhythms and/or their coupling.

The statements outlined in **Hypothesis I.b** are constructed under the assumption that delivering sCLAS to mice, in reference to the SO-states of the local field potential, will induce similar effects on sleep rhythms as observed in human EEG. It is conceivable that in mice, the augmentation of sleep rhythms might align with a different temporal point within the SO cycle than the SO Up-State. Consequently, if **Hypothesis I.b** cannot be validated, it will be tested whether parameters in its scope were differentially modulated by the four stimulation conditions in comparison to the control condition.

Hypothesis II. sCLAS delivery at each specific SO-state will affect memory performance as compared to the control session differently. Specifically:

a) Up-State Stimulation will increase the Preference Index.

This hypothesis will be examined by comparing the preference indexes (please see **2.7.1 Object Place Recognition Task**) of the control condition and Up-State (180 ms) stimulation. It is hypothesized that the PI of the 180 ms condition will be above chance level and higher than the PI under the control condition.

b) Down-State Stimulation will not affect the Preference Index.

This hypothesis will be examined by comparing the preference indexes of the control condition and Down-State (2 ms) stimulation. It is hypothesized that the PI of the 2 ms condition will not be lower or higher than the PI under the control condition.

c) The stimulation conditions will differentially modulate the Preference Index

For the same reason which was rationalized in **Hypothesis I.d**, if **Hypothesis II.a** are not validated, it will be tested whether the preference index was differentially modulated by the four stimulation conditions in comparison to the control condition.

1.4.2 Exploratory Hypothesis

Hypothesis III. Investigating the sleep rhythms in early- and late-NREM sleep separately will reveal the modulatory effect of sCLAS in more detail. Since early NREM sleep right after learning is critical for memory retention for OPR, if electrophysiological changes stated in **Hypothesis I.d** cannot be detected for a stimulation condition compared to the control over NREM sleep in 3 h sleep interval, NREM sleep will be dissected into two pieces as early- and late-NREM sleep (Please see **2.7.2 Sleep Architecture** for more information) and the continuous changes on **i)** SO density, **ii)** SO slope, **iii)** SO amplitude, **iv)** spindle density as well as on **v)** SO-spindle-coupling and **vii)** SO- ripple coupling in comparison to the control session will be investigated for all stimulation conditions separately.

2 Materials and Methods

2.1 Animals

Male C5BL/6N mice (Janvier, France) were taken as subjects, which were 8–10 weeks old on arrival. Due to technical setbacks, only 10 mice of 12 mice entered the study. Surgery was performed when the mice were 10–12 weeks of age. Type II-Long IVC cages (Greenline, Tecniplast, GM500) were used as housing on a 12 h light/dark cycle (lights on: 7:00 h or 8:00 h) with ad libitum access to food and water. Mice were housed initially with littermates and individually after electrode implantation. Animals were handled at least 5 min per day individually for 5 days before the surgery. All animals were treated identically, and all procedures were concordant with the European and national guidelines (EU Directive 2010/63/EU) and were approved by the local state authority (Ministerium für Energiewende, Landwirtschaft, Umwelt und ländliche Räume, Schleswig-Holstein).

2.2 Electrode Implantation

Animals were anesthetized with Isoflurane (induction: 3.5%, maintenance 0.8-2% at 1-1.3 l/min O2) and were placed into a stereotactic frame (David Kopf Instrument) during the surgery. To ease the breathing 0.04 mg/kg atropine (Atropinum Sulfuricum, Eifelfango) was administered s.c. right after the head fixation. After shaving and disinfection of the implantation area, lidocaine (1% solution, B. Braun Melsungen) was injected s.c here, before any scalp incision. Every 30 min of surgery, 0.1 ml of warm saline was s.c. to substitute fluid loss. An array of five tungsten wires (40 µm, California Fine Wire) was implanted into cingulate cortex, CC (AP: 1.2, L: 0.3, DV: -0.75) and another array of five tungsten wires, cut slightly diagonally, was implanted into the dorsal hippocampus, HC (AP: -1.94, L: 1.5, DV; deepest wire: -1.35) for recording LFP activity. Two stainless-steel screws (Bilaney, Germany) were used as reference and ground electrodes, implanted above the cerebellum (AP: −4.80, L: 0.00) and the somatosensory cortex (AP: −2, L: 2), respectively. A polyimide-insulated stainless-steel wire (0.125mm diameter, Plastics One)

inserted into the neck muscles was used for recording the EMG. Wire ends of all electrodes were soldered to a plug-connector and secured with dental acrylic (Super-Bond, Sun Medical or Relyx Unicem 2 Automix, 3M; Grip Cement, 3M; and Palapress, Heraus Kulzer). Finally, the headstage was encircled by copper wire mesh. Before cessation of the isoflurane induction, Carprofen (5 mg/kg, Rimadyl, Pfizer) was given i.p. for pain relief. To substitute for fluid loss, 0.5 ml of warm saline were given s.c. at the end of the surgery. Animals were kept under red light in their home cage until they became mobile and were then transferred back to the animal holding room where they spent at least 7 days for recovery.

2.3 Electrophysiological Data Acquisition

Recording sessions took place in a dark gray recording PVC box (20 × 20 × 31 cm; Fachhochschule Lübeck PROJEKT, Germany) with ad libitum access to food and water. The electrode connector was attached to a head stage micro amplifier (µPA16, Multi Channel Systems MCS GmbH, Germany). Electrophysiological signals were amplified (amplifier gain: 400) and digitally sampled with 4 kHz through a portable ME16 System (ME16-FAI-µPA, Multi Channel Systems MCS GmbH, Germany) and stored on an assigned computer (MC_Rack software, Multi Channel Systems MCS GmbH, Germany).

Real time analog signals from one cortical and one hippocampal electrode were transferred through two audio outputs of the ME16 System to the data acquisition interface CED Power1401-3 (Cambridge Electronic Design Limited, Cambridge, England) which was connected to a second computer. The CED Power1401-3, its application program Spike 2 and a custom-made program based on the built-in script language were used for online calculation of the hippocampal delta/theta ratio and the cortical SO detection.

2.4 Threshold determination for the sCLAS during NREM sleep

The second habituation recording (see **2.6.2 Experimental Recording Habituation**) was used for offline determination of the hippocampal delta/theta ratio threshold and the initial threshold for the SO-events. The first was used in the experimental recording sessions for online NREM sleep (stage) detection, and the latter to detect the SO-events online. The threshold of the delta/theta ratio was set by visual inspection from two histograms showing the distribution of all delta/theta ratio values of all sleep stages, one histogram for NREM sleep and another histogram for all other sleep stages (Wake, REM, PreREM, see **Supplementary Data D1**). Analyses to determine the SO detection threshold were limited to NREM sleep, and the same SO detection algorithm was applied for all offline SO analyses This algorithm is described in detail further under the section **2.7.4 Offline Event Detection**.

2.5 Closed-Loop Acoustic Stimulation

Closed-loop acoustic stimulation (sCLAS) algorithm introduced stimuli only when a SO-event detected during NREM sleep, i.e. upon cortical signal surpassing the adapted SO detection threshold and when the calculated delta/theta ratio was above the threshold for NREM sleep at the same time. Threshold values for SO-event and delta/theta ratio were determined for each animal individually. Half of the stimuli were devoid of an acoustic component, hence they were only timestamps and called pseudo-stimuli. In order to avoid overstimulation, an acoustic-stimulus was followed by a pseudo-stimulus and vice versa (**Figure 2.1**). After each stimulus, sCLAS was automatically paused for 2,5 second, which leaves a buffer between two consecutive stimuli (not shown). For Acoustic Stimulation an analog signal, consisting of 10 ms acoustic white noise, was sent from the CED Power1401-3 data acquisition interface to two dome tweeters (TW 6 NG 8 ohm, Visaton, Germany) inside the recording box through a custom-made stereo audio amplifier (2 x 5 W) at 58 dBA volume.

Online detection of SOs was conducted by a custom-made script running under Spike 2 software together with a sequencer in the CED Power1401-3 data acquisition interface, as it is described in Ngo et al. (2013). In brief, for each animal an initial down-state peak threshold value for SO detection was calculated according to their second habituation recording (see **2.6.2 Experimental Recording Habituation**). This initial threshold value was updated every 0.5 second i.e. was decreased to the minimal (i.e., the largest negative) instantaneous amplitude of the cortical signal within the preceding 2 s interval, when this amplitude exceeded the initial threshold. This algorithm ensured a continuously reliable detection of gradually increasing and decreasing SO amplitude during NREM sleep. Each time the cortical LFP signal crossed the adapted threshold toward smaller negative values, the Acoustic Stimulation was triggered, i.e. acoustic or pseudo-stimulus was introduced.

The timing of all SO detections was marked digitally in the Spike 2 data file, and these markers were also transferred to the ME16 System to be saved with the MC_Rack Software along with the animal's cortical and hippocampal data on a separate channel. The delay between the SO detection and the delivery of the stimuli varied between each condition, e.g.; 2 ms, 120 ms, 180 ms and 300 ms -stimulation- conditions. 2 ms targets the SO Down-State whereas 180 ms targets SO Up-State. 120 ms and 300 ms are introduced to target the transition between Up- and Down- States. It is important to note that control condition consisted of only pseudo-stimuli **(Figure 2.1)**.

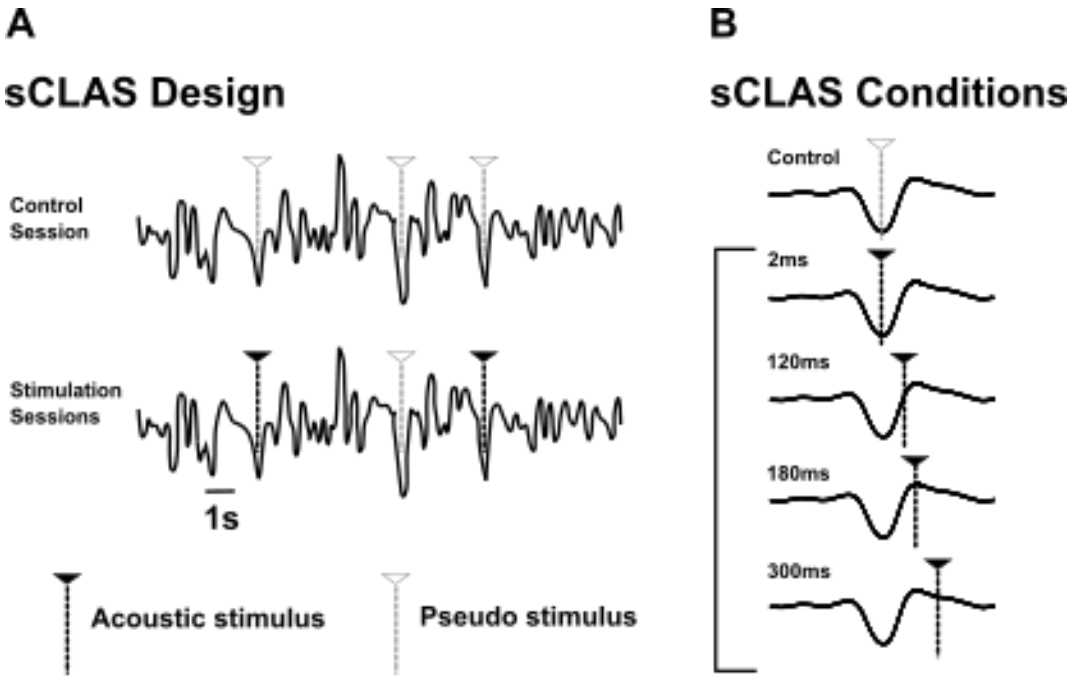


Figure 2.1. sCLAS paradigm in this study.
A.The application of sCLAS in control session and stimulation sessions are marked on low-pass filtered cortical LFP in real time. In the control session only pseudo stimuli are introduced, whereas in stimulation sessions, acoustic and pseudo stimuli are applied in alternating manner. **B.** The timing of each condition is given in reference to SO-event-averages in higher time resolution.

2.6 Behavioral Task

2.6.1 Apparatus and Objects

A dark gray open-field box (37 cm wide× 37 cm deep × 35cm high, Ewald Kongsbak GmbH + Co. KG) made of nontransparent PVC was used as the experimental apparatus. For all experiments, the light intensity was set to 100 lux above the open field. A webcam (Logitech, C270 HD), was mounted above the open-field for visual recording purposes. Two visual cues, one intramaze figure on a maze wall and, one extramaze by the camera stand, were represented. Glass bottles of various shapes, texture and size (height 11 – 20 cm, bottom diameter 5 – 6 cm) were used as experimental objects in pairs. Each identical bottle-pair was filled with decorative sand of a different color. Objects had sufficient weight to ensure that the

mice could not displace them. Prior behavioral testing with the same type of mice had shown that these bottle-pairs were equally preferable for the mice.

2.6.2 Experimental Recording Habituation

After the post-surgery recovery period of seven days, mice were ready to be involved in the experimental procedure. They were taken to the recording box in the experimental room during the light phase and spent the consecutive dark phase here untethered with ad libitum access to food and water. After lights on i.e. in the morning, they were connected to the head stage and electrophysiological activity was monitored for the next 3 hours, i.e. sleep interval. Habituation recordings were defined as the sleep recordings where the electrophysiological activity was captured, but sCLAS was not yet introduced. There are two consecutive habituation recordings prior to sCLAS application. The last habituation recording was used to determine the individual SO threshold and delta/theta ratio threshold for NREM sleep.

2.6.3 OPR Habituation

After the completion of the two habituation recordings, mice become admissible to the habituation for the object place recognition task. For this procedure, firstly they were left at least one hour in the experimental room to spatially adapt. Then they were taken from their home cage into a clean transfer cage to be placed in the open field (37 x 37 x 35 cm, Ewald Kongsbak GmbH + Co. KG) and allowed to explore the open field for 10 minutes. Followingly, they were taken back first to the transfer cage and to the home cage and brought to the holding room. This procedure was repeated on 3 consecutive days.

2.6.4 OPR Task

All object place recognition (OPR) tasks took place at the beginning of the light phase (08:00). In brief, in the OPR sample phase (10 min) mice were exposed to two identical objects, which were placed in two corners of the open field. Special care was taken that the surface of the objects that animals contacted would not differ. For the test phase (5 min) one of the objects

was then displaced to one of the other two free corners, whereas the other object remained at its position. Both objects were cleaned with 70% ethanol before each sample and test phase, as well as after each OPR session. Electrophysiological recording took place within the 3 hours sleep interval between the two OPR phases. Each animal participated in 5 separate OPR and sleep sessions. These sessions were set 5–8 days apart in order to prevent the interference from previous memories. Positioning of the displaced object as well as the order of sCLAS condition was pseudo-randomized **(Figure 2.2)**.

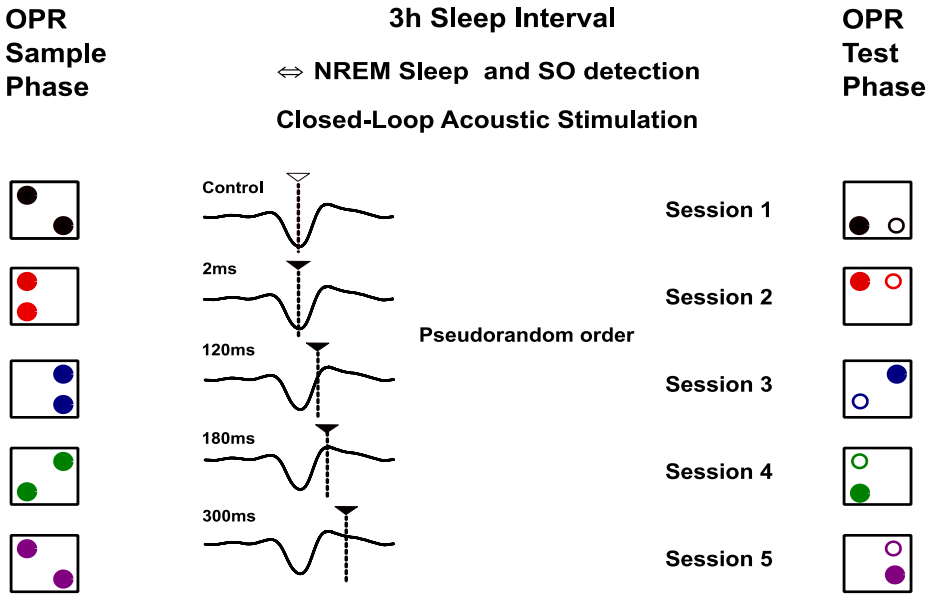


Figure 2.2. OPR experimental design within 3h sleep interval and exemplary OPR sessions.
Left, OPR sample phase is represented with two identical objects placed at two corners of the square open field. In the middle, after this exploration phase, animals are taken into the recording box to spend the retention time with sleep. During sleep, either the Acoustic Stimulation is omitted (control) or one of the four stimulation conditions is applied. Right, when the retention period is over animals are taken back into the open field, but this time one of the objects was replaced. The previous placement is marked with an empty circle. This procedure was repeated on separate days with certain intervals until each animal completed the five sessions. Color change indicates a new object. The order of the sCLAS condition is pseudorandomized.

2.7 Data Analysis

2.7.1 OPR Task

Video-recorded spatial exploration was scored manually by an experimenter blind to the sCLAS condition and previous placement of the objects, using a tracking software (AnyMaze, Stoelting, version 4.72). Only the active exploration i.e. direct approach, sniffing and contact to the objects with nose and forepaws were taken into account where grooming and freezing behavior in close vicinity of the objects were dismissed. Spatial retention performance was reflected in the preference index (PI), which is the ratio of exploration time of the displaced object to the total exploration time of both objects during the OPR test phase. Followingly, PI for each condition (stimulation and control) was compared to chance level to determine if the memory retention took place in this condition. Furthermore, PI for control condition was compared to PI for each stimulation condition in order to investigate whether a stimulation condition could improve or reduce the memory retention. Please note that conditions cannot be compared when the performance lies at the chance level for both.

2.7.2 Sleep Architecture

An experimenter assigned the sleep stages to all 5s epochs of the sleep recordings according to the standard criteria using the cortical and hippocampal LFP as well as EMG from the neck muscle using a specialized software (SleepSign for Animals, Version 2, Kissei Comtec). Briefly, the sleep stages were characterized as follows: wakefulness (Wake) by high EMG activity and desynchronized cortical LFP; NREM sleep by low EMG activity and high-amplitude low frequency cortical LFP activity consisting mostly of delta activity (0.75-4 Hz); PreREM sleep by low EMG activity and high-amplitude cortical sigma (10-15 Hz) activity before REM sleep or Wake; and REM-sleep by low EMG activity and high theta activity (6-10 Hz) in the HC LFP. Furthermore, NREM sleep was investigated in two separate sections which named as “early NREM” (average duration : 48,15 ± 1,31) and “late NREM” (average duration: 47,50 ± 1,58) in

order to explore the potential effect of sCLAS on sleep-event parameters and event-coupling in more detail. Special care was taken to not split the continuous NREM epochs in this process.

2.7.3 Electrophysiology

Electrophysiological analyses for sCLAS were conducted on the digital LFP data from one cortical and one hippocampal channel of each animal across different conditions. The channels for each region were selected out of 5 channels based on the data quality in the second habituation recording. For the analyses, Spike2 software (Cambridge Electronic Design) and custom scripts written with the built-in script language were used.

2.7.4 Offline Event Detection

SO, spindle and SWR events were identified, as explained in Binder et al. (2019). In brief, to identify SOs in the cortical LFP signal, a low-pass finite impulse response (FIR) filter of 30 Hz was applied, and the resultant signal was down-sampled to 100 Hz. Subsequently, a low-pass FIR filter of 3.5 Hz was used to produce the SO signal. In the slow oscillation signal all two succeeding negative-to-positive zero crossings separated by 0.45–1.43 s (corresponding to 0.7–2.22 Hz) were marked and the down-state and the up-state peak potentials between these marked negative-to-positive zero crossings were registered. SO events were defined as those intervals that displayed (1) a positive peak amplitude of 1.25 times the average positive peak amplitude of these animals control session or higher (2) a positive-to-negative peak amplitude difference of at least 1.25 times the average positive-to-negative peak amplitude difference of the respective control session.

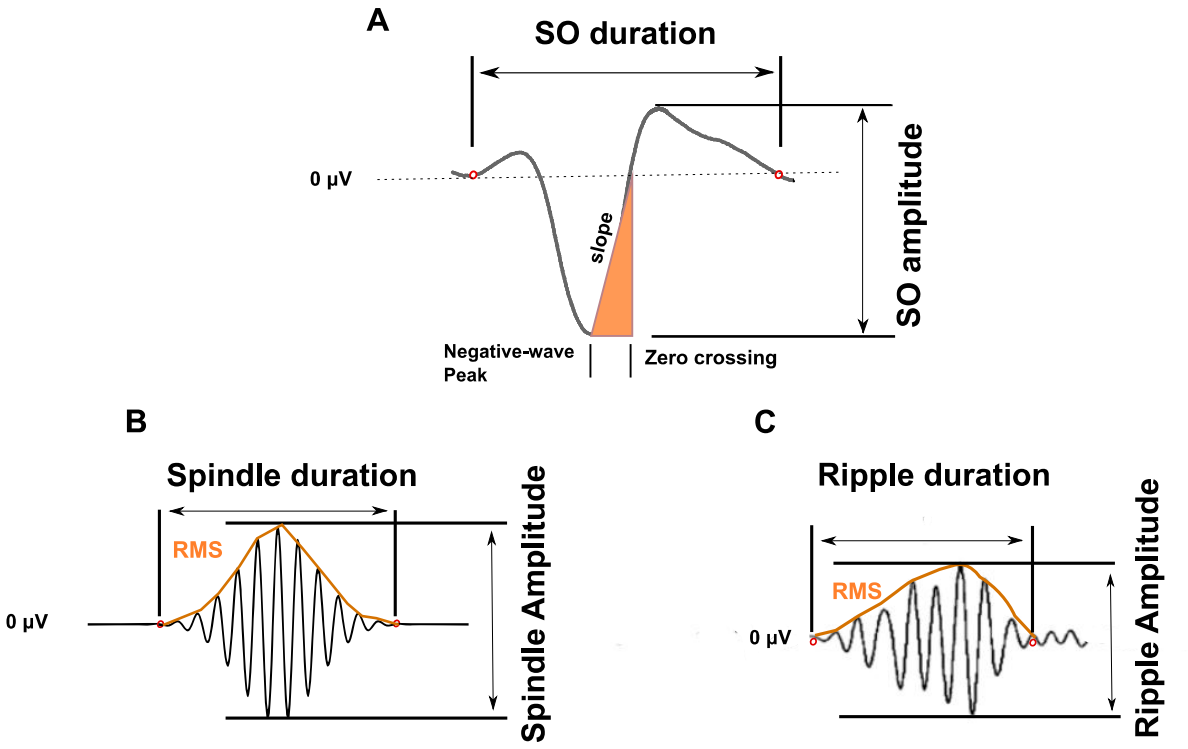


Figure 2.3. Detected events and parameter definitions.
A. Visualization of individual SO parameters: SO duration, SO amplitude and SO slope. **B.** Visualization of individual sleep-spindle parameters: spindle duration, spindle amplitude and spindle RMS. **C.** Visualization of individual sharp wave-ripple parameters: ripple duration, ripple amplitude and spindle RMS. Please note that the sleep rhythms are not given on the same scale.

Spindle identification also required first low-pass (<30 Hz) filtering and down-sampling to 100 Hz of the cortical LFP. Subsequently, a FIR bandpass filter of 9 – 15 Hz was applied and a root mean square (RMS) representation of the filtered signal was generated by using a 0.2 s long sliding window. The resulting RMS signal was smoothed additionally with a sliding window average of 0.2 s. Time frames were considered as spindle intervals if the RMS signal exceeded a threshold of 1.25 SD of the bandpass filtered signal for 0.5 – 3 s and if the largest value within the frame was > 2 SD of the bandpass filtered signal. For each animal, individual thresholds were calculated from the bandpass filtered signal of the control session. Two succeeding spindles were counted as one spindle when the interval between the end of the first spindle and

the beginning of the second spindle was shorter than 0.5 s and the resulting (merged) spindle was not >3 s. Detected events were not accepted as spindles, when the difference between the largest and smallest potential of the low-pass filtered signal (< 30 Hz) within the frame was 5 times larger than 2 SD of the bandpass filtered signal and the time between these two extrema was equal or shorter than one-half of an oscillation cycle of 15 Hz (0.033 s). For event-coupling analyses the peaks and troughs of every spindle were marked as the maxima and minima of the bandpass filtered signal (between the beginning and end of the spindle), and the deepest trough was designated as the “spindle peak” that represented the respective spindle in time, i.e., the time point taken for referencing event correlation histograms (see description of event-coupling histograms later in this section).

Identification of SWRs initially required the application of a low-pass FIR filter of 300 Hz and down-sampling to 1 kHz of the dorsal hippocampal LFP. Subsequently, a bandpass FIR filter of 150 – 200 Hz was applied and the RMS signal was calculated with a sliding 0.02 s time window. The RMS signal was then smoothed with a sliding window average of 0.02 s. Time frames were considered as SWR intervals if the RMS signal exceeded a threshold of 1.25 SD of the bandpass filtered signal for 0.025 – 0.1 s and if the largest value within the frame was > 5 SD of the bandpass filtered signal. For each animal, individual thresholds were calculated from the bandpass filtered signal of the control session. Detected events were not accepted as SWR, when the difference between the largest and smallest potential of the low-pass filtered signal (< 300 Hz) within the frame was 5 times larger than 5 SD of the bandpass filtered signal and the time between these two extrema was equal or shorter than one-half of an oscillation cycle of 200 Hz (2.5 ms). For event-coupling analyses, the peaks and troughs of every SWRs were marked as the maxima and minima of the bandpass filtered signal, and the deepest trough was designated as the “SWR peak” that represented the respective SWR in time.

Durations of SO, spindle and SPWR events correspond to the time from beginning to end of the detected oscillatory event, respectively. Density values refer to the mean density of the

corresponding event across all NREM sleep epochs. SO, spindle and SWR amplitudes are defined as the mean peak-to-peak values of events detected from the corresponding filtered frequency band signal. Spindle and SWR RMS represent the average values across all detected oscillatory events. The SO slope was derived from the SO upstate peak to SO downstate peak to the following zero crossing of the SO filtered signal. Grand mean averages of the detected SOs, spindles, and SWR across all animals were calculated for the different conditions. Also, averages of the original signal, spindle RMS and SWR RMS were calculated for Acoustic Stimulation of all delays and corresponding Pseudo Stimulation.

2.7.5 Stimulus-event correlation histograms

Stimulus-event correlation histograms were calculated for SO (total number of up-state and down-state peaks), spindle and SWR activity (total number of peaks and troughs) in intervals of -1.0 — 2.5 s around the online detected and stimulated (either with acoustic or pseudo stimuli) SOs. These histograms, with a bin size of 50 ms, were separately calculated for all acoustic- and all pseudo- stimuli during NREM sleep over the entire 3 hours recording session. The individual histograms were z-scored by the corresponding mean and SD of the SO, spindle and SWR activity, respectively, for each animal during the -1.0 — 2.5 s interval to eliminate the considerable variability across animals and conditions. These histograms represent the acute modulatory effect of sCLAS on event activity. Grand mean averages of the stimulus-event correlation histograms across all animals were calculated for the different conditions.

2.7.6 Event-coupling histograms

Event-coupling histograms were calculated for spindle and SWR activity (total number of peaks and troughs) with reference to the time of the SO down-state peak as identified in the cortical LFP signal. Further event-coupling histograms were calculated for SWR activity (number of peaks and troughs) in the dorsal hippocampal LFP with reference to the spindles (i.e., spindle peaks, see description of spindle identification in the prior section) and for spindle activity (number of peaks and troughs) in the cortical LFP with reference to the SWRs (i.e., SWR peak,

see description of SWR identification in the prior section). For all event-coupling histograms, 3 second windows were used with an offset of 1.5 second and a bin size of 50 ms. Again, these histograms were calculated for all NREM sleep over the entire 3 hours sleep interval and additionally for early- and late NREM sleep. The individual histograms were z-scored by the corresponding mean and SD of the spindle and SWR activity, respectively, for each animal during the ±1.5 s interval to eliminate the considerable variability across animals and conditions. In contrast to the stimulus-event correlation histograms, the event-coupling histograms were independent of the timing of sCLAS and compared the control session to the other stimulation sessions. The histograms represent a measure for the probability of activity of one event at a given time to proceed or follow another event, i.e., for the coupling of SOs, spindles and hippocampal SWRs.

2.7.7 Statistical Analysis

The study adapted the within-subject design for 5 conditions of sCLAS application (4 stimulation and 1 control condition). For statistical analyses of the preference index, the mean value of each condition was first compared to the chance level (0.5) by a one sample t-test. Preference Index of each stimulation condition was then compared to the control group through paired t-tests. Additionally, mixed- effects model for repeated measures analyses of variance (ANOVA) and post hoc paired t-tests were applied.

Event parameters of SOs, spindles, and SPWRs; duration of sleep stages; and number of stimulation triggers given at a certain sleep stage for the 3h sleep interval or for early and late NREM sleep periods were subjected to mixed effects models repeated measures analyses of variance (ANOVA) with the factors Condition (control, 2 ms, 120 ms, 180 ms, 300 ms), and when applicable Time (Early, Late). Correction for multiple comparisons was conducted using the Holm-Sidak method.

Statistical comparisons of event-related activity, stimulus-event correlation histograms of Acoustic- and Pseudo-Stimulation and event-coupling histograms were conducted through paired t-tests. False discovery rate was controlled by Benjamini-Hochberg procedure.

All data are expressed as mean ± SEM and analyzed using the software package Prism 8 (GraphPad Software, Inc.). A p value < 0.05 was considered significant.

3 Results

3.1 NREM sleep was not interrupted by sCLAS

One concern with sCLAS delivery would be that it could disturb NREM sleep. To assure that it did not, the proportion of the assigned sleep stages was investigated; NREM, PreREM, REM and Wake within the 3h sleep interval across all conditions (**Table 1.A**). An effect of Condition ($F [4,29] = 2.911$, $p = 0.038$) was found on the duration of PREM sleep only, where it was longer under 180 ms condition than at 300 ms condition (mean difference 2.46 ± 0.80 min, $p = 0.044$, post-hoc Holm-Sidak multiple comparison test).

Ideally, the sCLAS delivery should be only in NREM sleep and the number of delivered stimuli should not differ across conditions. Therefore, the distribution of acoustic stimuli in each assigned sleep stage for all conditions was investigated (**Table 1.B**). No differences were found across differential sCLAS conditions ($F [4,28] = 1.242$, $p = 0.316$), consecutively, the data was collapsed. Across all sessions, a mean number of 727.21 ± 38.86 acoustic stimuli were delivered. Further analysis has shown that a substantial proportion of acoustic stimuli fell under the Wake-stage (16.86 ± 1.584 %). The result could be associated with poor online distinction between NREM sleep and quiet wakefulness.

Table 1. Distribution of sleep stages and acoustic stimuli

A

Total time	NREM (%)		PreREM (%)		REM (%)		Wake (%)	
control	50.94 ± 2.23		6.24 ± 0.71		2.22 ± 0.67		40.6 ± 2.94	
2ms	51.47 ± 3.89		6.09 ± 0.69		1.9 ± 0.48		40.53 ± 4.32	
120ms	55.3 ± 3.3		7.51 ± 1.04		2.11 ± 0.54		34.87 ± 2.95	
180ms	53.8 ± 1.4		7.51 ± 0.64*		2.49 ± 0.44		36.22 ± 1.76	
300ms	50.91 ± 2.9		5.81 ± 0.93*		1.85 ± 0.34		37.8 ± 1.77	
	F	***p***	***F***	***p***	***F***	***p***	***F***	***p***
condition (4,29)	0.432	0.784	2.911	0.038*	1.054	0.396	0.710	0.591

The duration of each sleep stage in percentage for a total of approximately 3h sleep interval. The sleep interval was on average 186.06 ± 1.03 min. *p < 0.05 for post-hoc Holm-Sidak multiple comparison test. F and p values of mixed effects models are given. Number of animals in each group: 2 ms, 180 ms, n = 10; 120 ms, n = 7; 300 ms; n = 6; control, n = 9. Session triggers marking acoustic - and pseudo stimuli could not be retrieved for one animal in the control group. Mean ± SEM (From Aksamaz et al., 2024, EJN, Table 1).

B

Total count of acoustic stimuli (#)			NREM (%)		PreREM (%)		REM (%)		Wake (%)	
all conditions	727.21 ± 38.86		80.53 ± 1.7		0.54 ± 0.14		2.05 ± 0.30		16.86 ± 1.584	
	F	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
condition (4,28)	2.617	0.056	1.108	0.372	1.242	0.315	0.678	0.612	0.346	0.844

Distribution of acoustic stimuli across respective sleep stages given in **A**, averaged across conditions. *p < 0.05 for post-hoc Holm-Sidak multiple comparison test. F and p values of mixed effects models are given. Number of animals in each group: 2 ms, 180 ms, n = 10; 120 ms, n = 7; 300 ms; n = 6; control, n = 9. Session triggers marking acoustic - and pseudo stimuli could not be retrieved for one animal in the control group. Mean ± SEM

3.2 Acoustic Stimulation evoked changes in event- related activity

In this section the responses to the sCLAS delivery were analyzed through the changes on cortical local field potentials, and thalamo-cortical spindle and hippocampal ripple root mean square (RMS) values. Here the results are given time-locked to either acoustic or pseudo stimuli, separately. Since Acoustic- and Pseudo-Stimulation was delivered alternately during NREM-sleep, this visualization allows one to capture the acute effects.

Cortical LFP confirms that differential stimulation conditions were successful to target four specific SO-state, Down-State, Down-to-Up-Transition-State, Up-State and Up-to-Down-Transition-State respectively (**Figure 3.A**,**B**, top rows). Here, the SO down-state peak is given upwards. Additional changes on mean cortical LFP seen in **Figure 3.A** for Acoustic Stimulation in contrast to **Figure 3.B** for Pseudo Stimulation are the acoustic evoked potentials upon sCLAS delivery. Unsimilar to human subjects, Up-State stimulation did not entrain SOs. Upon application, Acoustic Stimulation introduces an acute increase in both spindle and ripple RMS which is followed by a longer suppression across all stimulation conditions (**Figure 3.A, B; second and third row**).

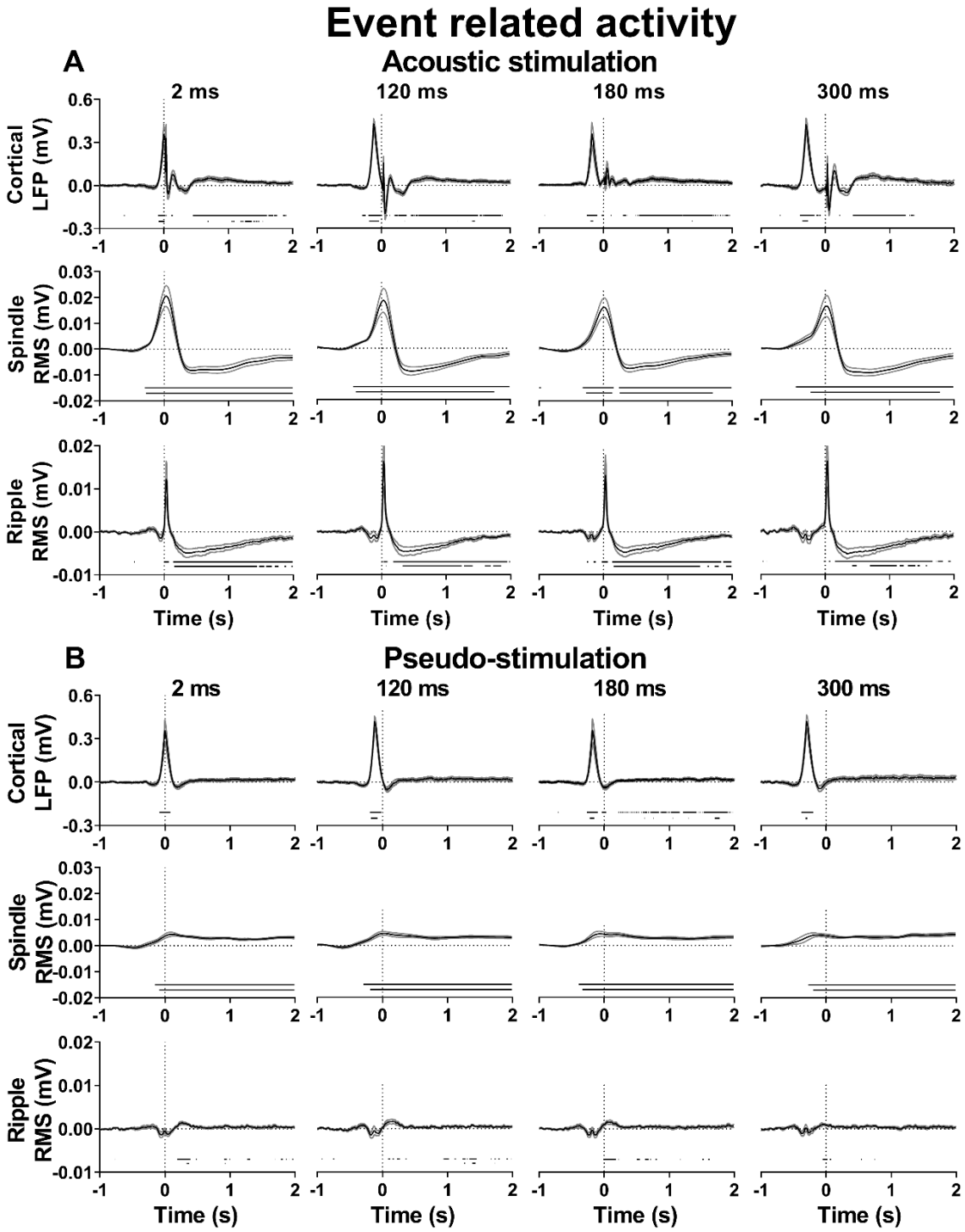


Figure 3. sCLAS delivery time-locked to the differential SO-states modulated the cortical local field potential (LFP), cortical spindle and hippocampal ripple activity.
A. Acoustic Stimulation. **B**. Pseudo Stimulation. **A**,**B**. From top to bottom, baseline normalized mean (± SEM) cortical LFP, spindle RMS, and CA1 ripple RMS. Horizontal lines correspond to significant differences from the baseline level (-1 to -0.5 s; paired t-tests) for false discovery rates set to 0.05 (top line) and 0.01 (bottom line). T = 0 s corresponds to the time of stimulation. 2 ms, 180 ms, n = 10; 120 ms, n = 7; 300 ms, n = 6. (From Aksamaz et al., 2024, EJN, Figure 2).

3.3 sCLAS application in mice did not alter the average sleep-event parameters in NREM sleep

Certain sleep-event parameters are closely associated with memory retention in sleep literature. Since the sCLAS did not interfere with NREM sleep, they could be analyzed further. A significant effect was not detected across different stimulation conditions for any of the sleep-event parameters (each $p \geq 0.05$, mixed-effects model, **Table S1)**. Paired comparisons of each stimulation condition with control group did not reveal any changes either (for Holm-Sidak's multiple comparisons test $p \geq 0.05$, data not shown). Therefore, the sleep-event parameter data was collapsed across all sleep sessions (**Table 2**) to have a better overview of the sleep-events.

Table 2. Mean sleep-event parameters of SOs, spindles and SWRs

Event	Density (min^{-1})	Amplitude(mV)	Duration (s)	RMS (mV)	Slope (mV/s)
SO	12.32 ± 0.69	0.62 ± 0.03	0.703 ± 0.004	-	0.83 ± 0.04
Spindle	2.36 ± 0.15	0.40 ± 0.01	0.843 ± 0.013	0.093 ± 0.003	-
SWR	15.60± 1.13	0.11 ± 0.01	0.070 ± 0.001	0.039 ± 0.002	-

Event parameters of density, amplitude, duration, and slope (only for SOs) or RMS (for spindles and SWRs) were analyzed in NREM sleep of the 3h sleep interval. For SO and ripple parameters, n = 43, for spindle parameters n = 41. Spindle density values of one animal for two delay conditions were spuriously high, thus values of this animal were omitted. Mean ± SEM (From Aksamaz et al., 2024, EJN, Table 2).

3.4 Acoustic Stimulation caused sustained suppression of spindle and ripple event activity

The top-down driving influence of SOs to group spindles and ripples was shown to promote memory consolidation, therefore the coupling of these sleep-event activities was investigated. Here, the bin-wise comparison of the event activities time-locked to either Acoustic or Pseudo Stimulation enables us to capture the modulatory impact of Acoustic Stimulation statistically.

For SOs, the event activity is calculated as the total number of the down-state and up-state peaks. Since the time-zero marks the detection of SO down-state peak, the event activity reaches its maximum at this time point for all conditions (**Figure 4.A**, **Supplementary Figure 1.A.**). Deviations from the endogenous SO activity are observed right after (<100ms) the acoustic stimuli delivery at the Down-State (2 ms condition).

For spindle and ripple activity, the total number of troughs and peaks of these events are taken. Acoustic Stimulation does not necessarily enhance the spindle activity, but for all conditions it causes a suppression with varying latencies (**Figure 4.B, Supplementary Figure 1.B.**). As it was expected from **Figure 3.A**, hippocampal activity increased drastically with Acoustic Stimulation as compared to Pseudo Stimulation for all conditions. Stimulus-event correlation histograms also revealed a suppression of ripple event-activity after Acoustic Stimulation, up to ~ 1 s (**Figure 4.C., Supplementary Figure 1.C.**).

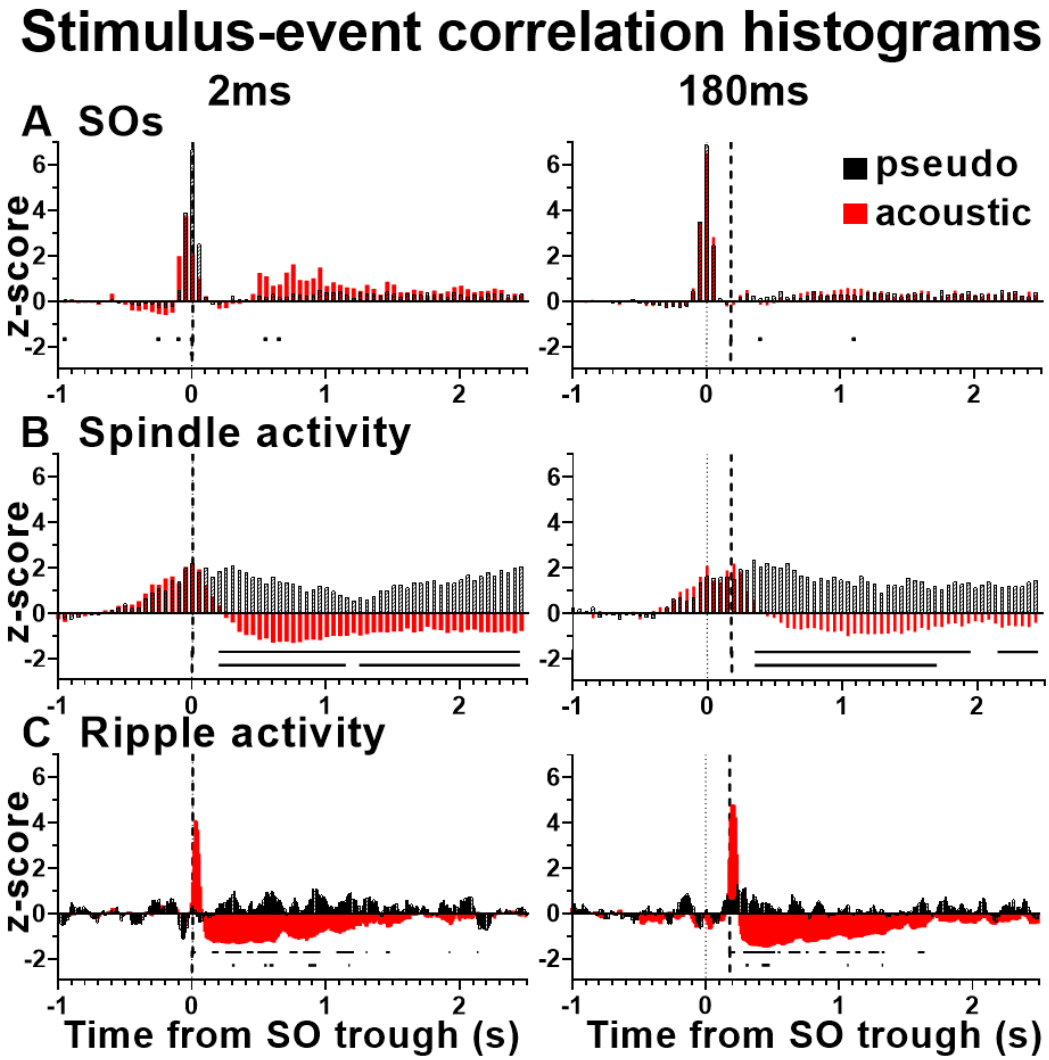


Figure 4. SO, thalamo-cortical spindle and hippocampal ripple event-activity time-locked to sCLAS delivery.

A-C. mean stimulus-event correlations for SO, spindle and CA1 SPWR events (z-transformed and baseline normalized, -1 to -0.5 s) for Acoustic and Pseudo Stimulation averaged across the 3h sleep interval for the 2 ms and 180 ms stimulation conditions, respectively. t = 0 s corresponds to the detected SO down-state peak. Horizontal lines correspond to significant differences between Acoustic and Pseudo Stimulation (paired t-tests) for false discovery rates set to 0.05 (top line) and 0.01 (bottom line). Results are presented for the two delay conditions with a behavioral preference index significantly above (180 ms) and not significantly different from the chance level (2ms), and for which data from all ten animals are available. (From Aksamaz et al., 2024, EJN, Figure 4).

3.5 The coupling-dynamics of the sleep-events under sCLAS application

Although the individual sleep-event parameters were not affected, the change in their timing in reference to each other can modulate the memory retention. Thus, the relative timing of event-pairs was compared to have an overview of the temporal coupling dynamics in NREM sleep. In order to investigate the grouping effect of SOs on other sleep rhythms, the troughs and peaks of concurrent spindle or SWR events were reflected on a time frame where each SO event (as detected in cortical LFP) was taken as a reference. This process was repeated through whole NREM sleep to generate a grand average (**Figure 5**). The histograms depict the likelihood of an event when another respective event concurrently occurs. The depictions are in line with the sleep literature where the mean spindle activity decreases in SO down-state, as it is minimized at the SO down-state peak and increases again as the SO up-state approaches (**Figure 5.A**,**B, left**). A similar temporal change can be tracked for SWRs (**Figure 5.A**,**B, right**). Furthermore, SWRs are more likely to coincide with spindle troughs (nesting) and spindles are more likely to co-occur with SWRs, respectively.

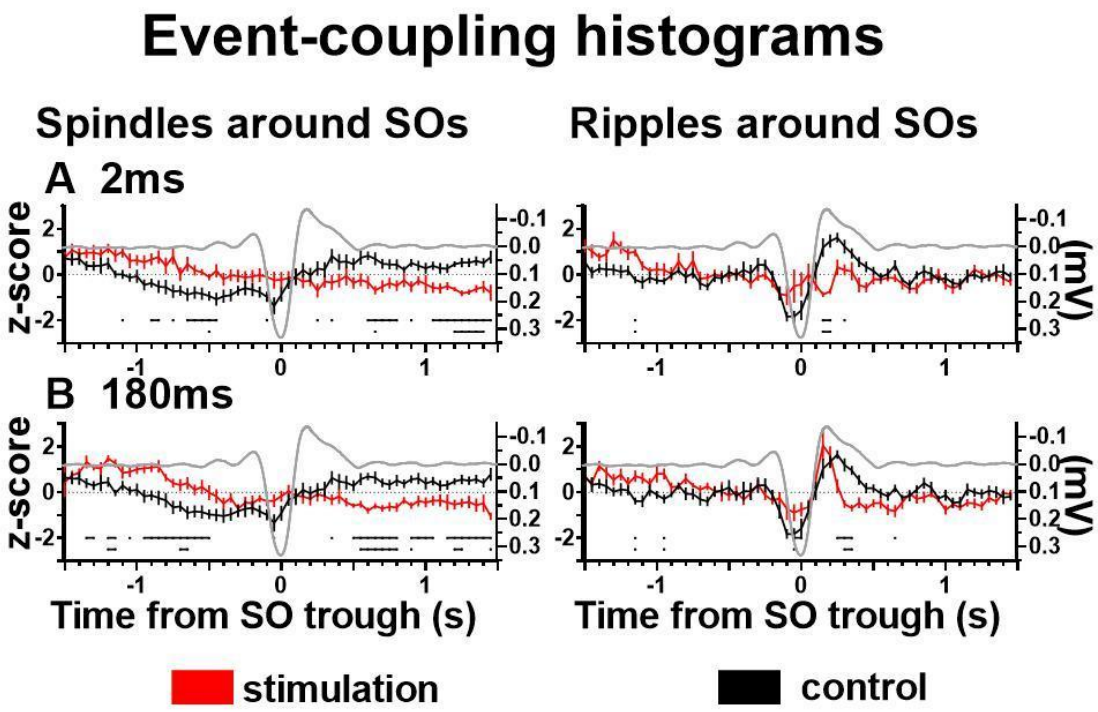


Figure 5. Event-coupling histograms between SO, spindle and SWR events respectively. **A**. SO-spindle coupling (left) and SO-SPWR coupling (right) time-locked to the Down-state peak of the SO (t = 0, SO trough) for the 2 ms stimulation and control conditions. **B**. Same as A, but for the 180 ms stimulation condition. Horizontal lines correspond to significant differences between control and stimulation condition (paired t-tests) for FDRs set to 0.05 (top line) and 0.01 (bottom line). Mean ± SEM for event-event coupling, mean for control SO (grey line). Note, to facilitate comparisons with overlaid event-event coupling, the SO trough is depicted downward. (From Aksamaz et al., 2024, EJN, Figure 5).

3.6 Preference Index in OPR task after sCLAS in NREM sleep

Figure 6. shows the preference index, used as a measure for memory retention in the OPR task, across all sCLAS conditions. Mice performed above chance level in the control condition as well as in the 120 ms and 180 ms stimulation conditions (one sample t-test; theoretical mean: 0.5, control: t (9) = 2.525, p = 0.0325; 120 ms: t (6) = 5.039, p = 0.0024; 180 ms: t (9) = 4.613, p= 0.0013) but not in 2 ms or 300 ms stimulation conditions (2 ms: t (9) = 1.784, p = 0.1081; 300 ms: t (5) = 1.375, p = 0.2275). Behavioral performance did not differ between the control condition and any stimulation condition under sCLAS treatment (paired t-test; control vs. 2 ms: t (5) = 0.7276, p = 0. 4995; control vs. 120 ms: t (5) = 0.3851, p = 0.7160; control vs. 180 ms: t (5) =1.137, p = 0.3069; control vs. 300 ms: t (5) =1.343, p = 0.2370). Effect of sCLAS on PI

values across all conditions only revealed a trend (mixed effects model; F [4,38] = 2.105, p = 0.099, not corrected). When an exploratory analysis was conducted exclusively four conditions (2 ms, 120 ms, 180 ms and 300 ms) the p value was smaller than 0.05 mixed effects model; F [2.633, 17.55] = 3.366, p = 0.0471, Geisser-Greenhouse`s correction). Further post-hoc t-tests for the PI between the four stimulaton conditions revealed a difference between the behavioral performance the 180 ms condition and the 300ms condition (t (5) = 3.362. p = 0.020, not corrected).

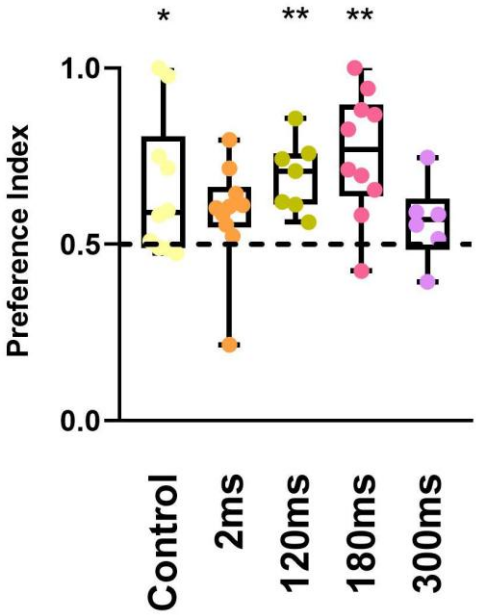


Figure 6. Preference Indexes in comparison to chance level
Preference Indexes across different sCLAS conditions (from left to right: control condition and 2 ms, 120 ms, 180 ms and 300 ms stimulation condition) in reference to chance level (0.5). *p < 0.05, **p < 0.01, for one sample t-test.

3.7 Time variable effects on sleep-event parameters

The investigation of sleep-event parameters within early and late NREM sleep revealed characteristic temporal changes, independent of condition, for all parameters except spindle density, spindle amplitude and spindle RMS (mixed effects model analysis, please see **Supplementary Table 2, 3 and 4** for F and p values). Notably, no significant effect of time was observed for the three aforementioned parameters.

In early NREM sleep, significant differences were observed, with higher SO density and SO amplitude, as well as a steeper SO slope (SO density: mean difference = 1.719, p < 0.001;

SO amplitude: mean difference = 0.008, p < 0.0001; SO slope: 0.019, p < 0.0001shown in **Figure 7**.). Furthermore, SWR duration, SWR density, SWR amplitude, and SWR RMS were all higher in early NREM sleep (duration: mean difference = 0.0009, p < 0.0001; density: mean difference = 1.155, p < 0.0001; amplitude: mean difference = 0.009, p < 0.0001; RMS: mean difference = 0.0016, p < 0.0001). Conversely, SO duration and spindle duration were found to be longer in late NREM sleep (SO duration: mean difference = 0.027, p < 0.0001; spindle duration: mean difference = 0.0287, p < 0.0001).

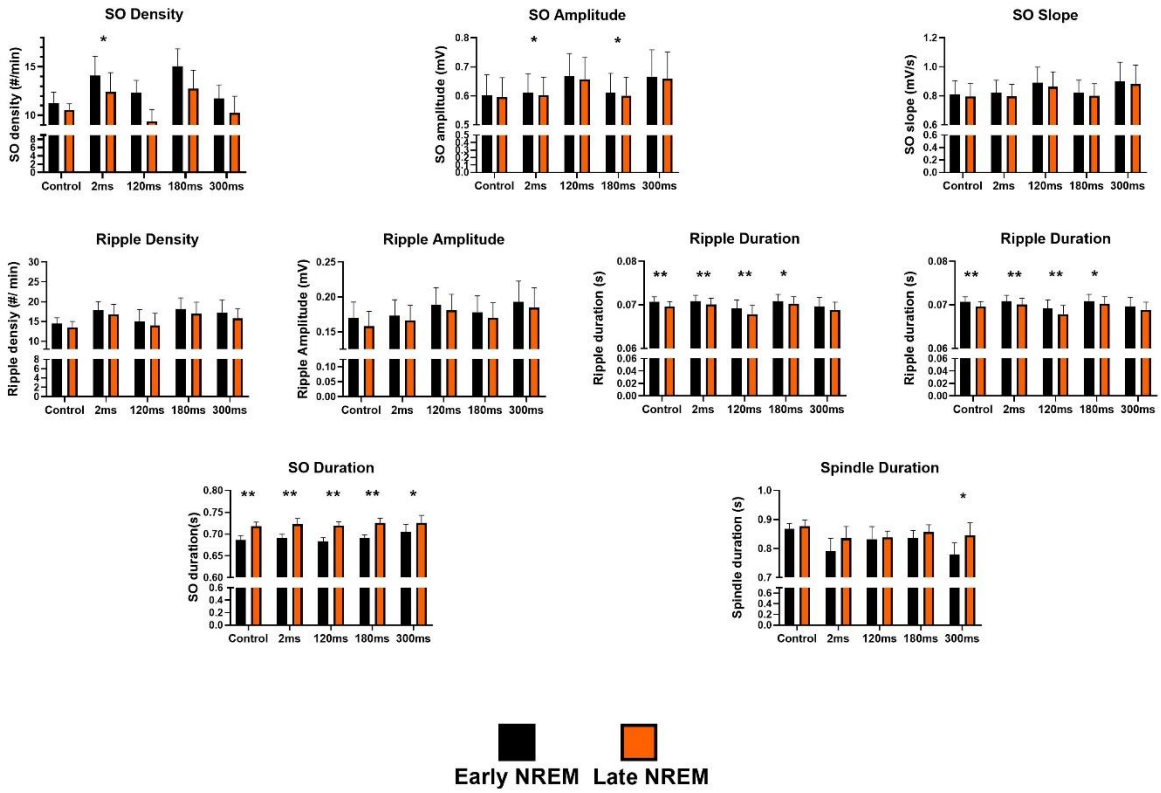
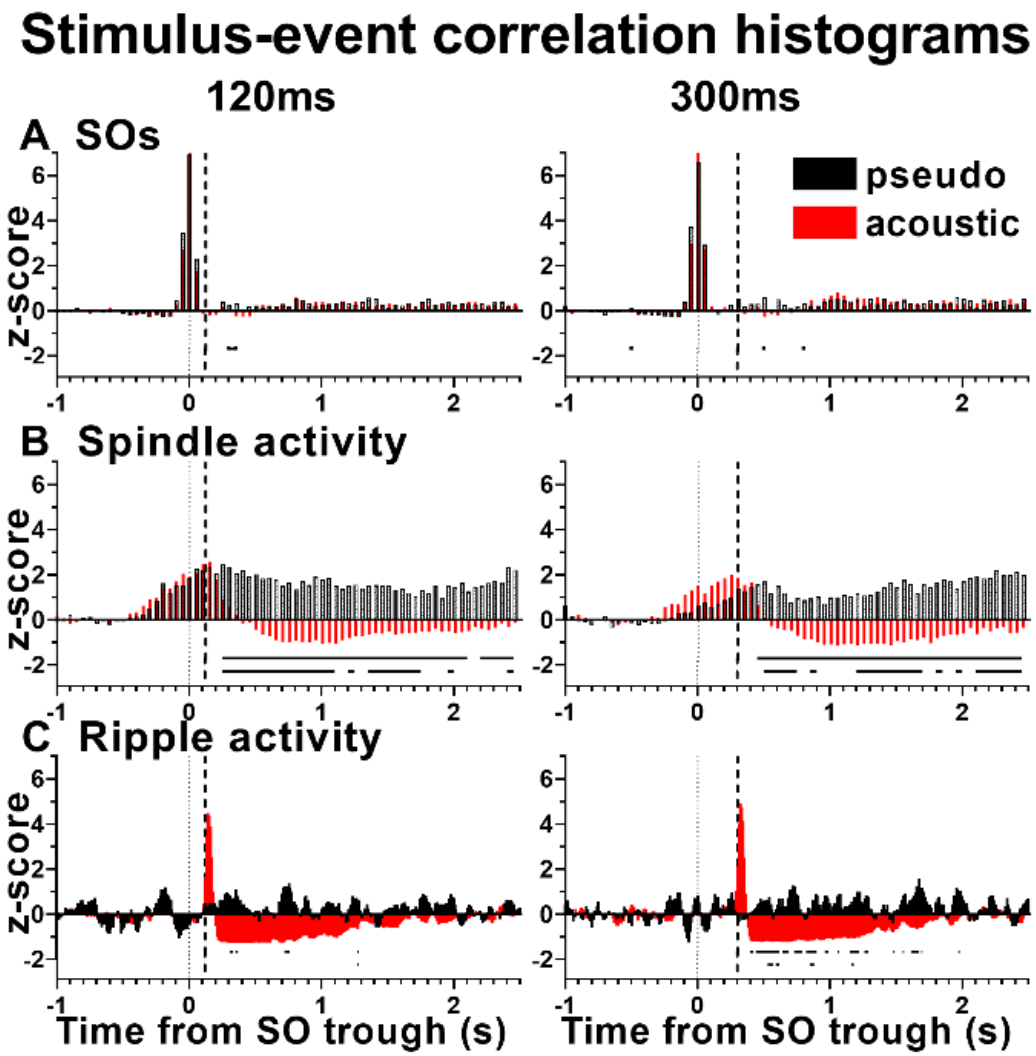


Figure 7. Changes in event parameters in early and late NREM sleep.
Top lines 1-2: The event parameters showing a decrease across time. Bottom Line: The event parameters showing an increase across time. *p < 0.05, ** p< 0.01 for Holm-Sidak's multiple comparisons test. Control, 2ms, 180 ms, n=10; 120 ms, n=7; 300 ms, n= 6 (for spindle parameters only: 2 ms, 180 ms, n = 9). Mean ± SEM

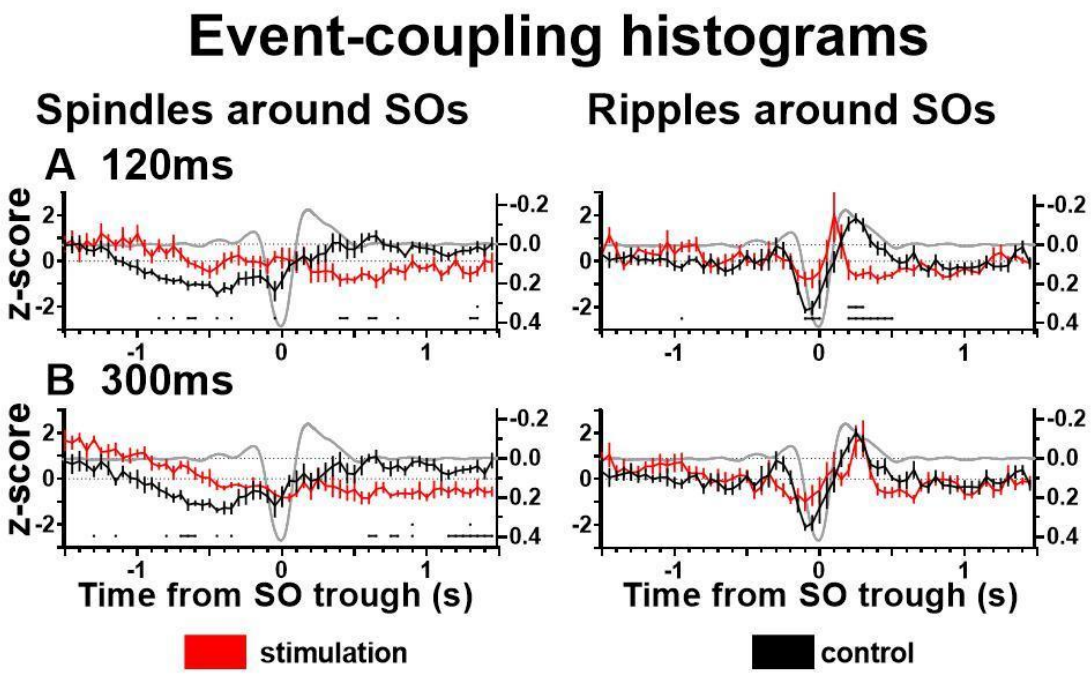
4 Supplementary Results

Supplementary Figure 1.



S1. SO, thalamo-cortical spindle and hippocampal ripple event-activity relative to sCLAS. **A-C.** Mean stimulus-event correlations for SO, spindle and CA1 SPWR events (z-transformed and baseline normalized) for Acoustic and Pseudo Stimulation averaged across the 3 h sleep interval for the 120 ms and 300 ms stimulation conditions, respectively. t = 0 s corresponds to the detected SO down-state peak. Horizontal lines correspond to significant differences between Acoustic and Pseudo Stimulation (paired t-tests) for false discovery rates set to 0.05 (top line) and 0.01 (bottom line). 120 ms, n= 7; 300 ms, n=6. (From Aksamaz et al., 2024, EJN, Supplementary Figure S3).

Supplementary Figure 2.



S2. Event-coupling histograms between SO, spindle and SWR events respectively.
A. SO-spindle coupling (left) and SO-SPWR coupling (right) time-locked to the down-state peak of the SO (t = 0, SO trough) for the 120ms stimulation and control conditions.
B. Same as A, but for the 300 ms stimulation condition. Horizontal lines correspond to significant differences between conditions (paired t-tests) for FDRs set to 0.05 (top line) and 0.01 (bottom line). Mean ± SEM for event-event coupling, mean for control SO (gray line). Note, to facilitate comparisons with overlaid event-event coupling, the SO trough is depicted downward. (From Aksamaz et al., 2024, EJN, Supplementary Figure S4).

Supplementary Table 1.

Sleep Events		SOs		Spindles		Ripples	
Statistics		***F (4,29)***	***p***	***F (4,27)***	***p***	***F (4,29)***	***p***
Parameters	**Density**	1.369	0.269	1.077	0.387	0.708	0.593
	Amplitude	0.766	0.556	1.121	0.367	2.036	0.116
	Slope	1.00	0.422	_	_	_	_
	RMS	_	_	1.053	0.399	2.091	1.077
	Duration	0.234	0.916	2.136	0.104	1.719	0.173

Table S1. Mixed –effects analysis statistics of oscillatory sleep event parameters in the 3h sleep interval across sCLAS conditions. The spindle density value of one animal for two delay conditions overreached the acceptable value, therefore these conditions were eliminated from the analysis of spindles. The spindle duration showed a trend therefore this value was indicated in the text. Control: n = 10, 2 ms: n = 10, 120 ms: n = 7, 180 ms: n = 10, 300 ms: n = 6. Mean ± SEM. (From Aksamaz et al., 2024, EJN, Supplementary Table S1).

Supplementary Table 2.

SOs

	Parameter	Density(min^{-1})		Amplitude (mV)		Slope (mV/s)		Duration (s)	
Early	control	11.274 ± 1.104		0.602 ± 0.071		0.807 ± 0.098		0.686 ± 0.009	
	2ms	14.079 ± 1.985		0.612 ± 0.063		0.824 ± 0.084		0.69 ± 0.009	
	120ms	12.308 ± 1.283		0.668 ± 0.078		0.89 ± 0.108		0.684 ± 0.008	
	180ms	15.064 ± 1.775		0.612 ± 0.065		0.821 ± 0.087		0.691 ± 0.007	
	300ms	11.748 ± 1.391		0.667 ± 0.092		0.9 ± 0.131		0.705 ± 0.017	
Late	control	10.571 ± 0.615		0.595 ± 0.068		0.795 ± 0.089		0.718 ± 0.009	
	2ms	12.405 ± 1.98		0.602 ± 0.062		0.797 ± 0.084		0.723 ± 0.013	
	120ms	9.404 ± 1.197		0.658 ± 0.074		0.862 ± 0.103		0.719 ± 0.009	
	180ms	12.754 ± 1.891		0.601 ± 0.063		0.801 ± 0.082		0.726 ± 0.01	
	300ms	10.24 ± 1.721		0.659 ± 0.092		0.882 ± 0.131		0.726 ± 0.017	
	Statistics	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Factors	condition (4,36)	1.378	0.261	0.754	0.562	1.238	0.312	0.534	0.711
	time (1,9)	12.25	0.007**	13.24	0.005**	5.471	0.044*	27.93	0.0005***
	interaction (4,22)	1.146	0.361	0.333	0.853	0.363	0.832	1.724	0.189

Table S2. Slow oscillation event parameters across sCLAS conditions in early and late NREM sleep. SO-event parameters of density, amplitude, slope, and duration within Early and Late NREM sleep periods. *p < 0.05, ** p < 0.01, *** p < 0.001. Control, 2 ms, 180 ms, n = 10; 120 ms, n = 7; 300 ms, n = 6. Mean ± SEM. (From Aksamaz et al., 2024, EJN, Supplementary Table S2).

Supplementary Table 3.

Spindles

	Parameter	Density(min^{-1})		Amplitude (mV)		RMS Mean (mV)		Duration(s)	
Early	sham	1.973 ± 0.094		0.378 ± 0.027		0.088 ± 0.006		0.869 ± 0.018	
	2ms	2.121 ± 0.406		0.414 ± 0.022		0.096 ± 0.005		0.792 ± 0.044	
	120ms	2.194 ± 0.339		0.398 ± 0.024		0.092 ± 0.006		0.832 ± 0.043	
	180ms	2.542 ± 0.378		0.407 ± 0.02		0.094 ± 0.005		0.837 ± 0.025	
	300ms	2.07 ± 0.452		0.399 ± 0.031		0.094 ± 0.008		0.78 ± 0.04	
Late	sham	2.605 ± 0.17		0.384 ± 0.028		0.089 ± 0.007		0.878 ± 0.022	
	2ms	2.407 ± 0.46		0.41 ± 0.02		0.095 ± 0.005		0.835 ± 0.041	
	120ms	2.609 ± 0.376		0.404 ± 0.027		0.093 ± 0.006		0.838 ± 0.022	
	180ms	2.677 ± 0.455		0.411 ± 0.02		0.095 ± 0.005		0.857 ± 0.025	
	300ms	2.483 ± 0.595		0.403 ± 0.03		0.094 ± 0.007		0.845 ± 0.043	
Factors	**Statistics**	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
	condition (4,36)	0.785	0.543	2.163	0.093	1.153	0.347	2.597	0.0525#
	time (1,9)	2.286	0.164	1.521	0.249	0.901	0.367	10.93	0.009**
	interaction (4,18)	0.793	0.545	0.588	0.675	1.47	0.253	1.502	0.244

Table S3. Spindle event parameters across sCLAS conditions in early and late NREM sleep. Spindle event parameters of density, amplitude, RMS, and duration within Early and Late NREM sleep periods. Spindle density values of one animal for two delays were erroneously high and were therefore omitted. #p < 0.1, ** p < 0.01. Control, 2 ms, 180 ms, n = 10; 120 ms, n = 7; 300 ms, n = 6. Mean ± SEM (From Aksamaz et al., 2024, EJN, Supplementary Table S3).

Supplementary Table 4.

Rippl								
	Parameter	**Density(min^{-1})**		**Amplitude (mV)**		**RMS Mean (mV)**		**Duration(s)**	
Early	control	14.478 ± 1.465		0.17 ± 0.023		0.038 ± 0.005		0.071 ± 0.001	
	2ms	17.844 ± 2.183		0.173 ± 0.022		0.038 ± 0.005		0.071 ± 0.001	
	120ms	15.09 ± 2.916		0.189 ± 0.025		0.042 ± 0.005		0.069 ± 0.002	
	180ms	18.162 ± 2.804		0.178 ± 0.024		0.039 ± 0.005		0.071 ± 0.001	
	300ms	17.24 ± 3.108		0.193 ± 0.03		0.043 ± 0.007		0.07 ± 0.002	
Late	control	13.568 ± 1.428		0.158 ± 0.021		0.035 ± 0.005		0.07 ± 0.001	
	2ms	16.767 ± 2.534		0.166 ± 0.022		0.037 ± 0.005		0.07 ± 0.001	
	120ms	14.048 ± 2.992		0.181 ± 0.023		0.04 ± 0.005		0.068 ± 0.002	
	300ms	15.772 ± 2.524		0.185 ± 0.028		0.041 ± 0.006		0.069 ± 0.002	
	Statistics	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Factors	condition F (4,36)	1.513	0.219	1.415	0.249	1.321	0.281	1.441	0.241
	time F (1,9)	6.123	0.035*	7.189	0.025*	6.366	0.033*	66.55	<0.0001****
	interaction F (4,22)	0.089	0.985	0.481	0.749	0.431	0.784	1.433	0.256

Table S4. Ripple event parameters across sCLAS conditions in early and late NREM sleep. Ripple event parameters of density, amplitude, RMS, and duration within early and late NREM sleep periods. *p < 0.05, p < 0.001, **** p < 0.0001. Control, 2 ms, 180 ms, n = 10; 120 ms, n = 7; 300 ms, n = 6. Mean ± SEM (From Aksamaz et al., 2024, EJN, Supplementary Table S4).

5 Discussion

5.1 Precondition for the discussion

The experimental design was newly implemented. Therefore before addressing the main hypotheses it is important to confirm the general effect of the experimental paradigm. sCLAS indeed evoked electrophysiological responses in the cortex and hippocampus (**Figure 3**) as compared to the Pseudo Stimulation. Importantly, timely delivery of sCLAS, namely; at 2 ms, 120 ms, 180 ms and 300 ms stimulation conditions targeted as intended the Down-State, Down-to-Up-Transition-State, Up-State and Up-to-Down-Transition-State, respectively (**Figure 3.B**).

Confirmation that the delivery of sCLAS targeted the intended SO-states, enables a straightforward discussion of the main hypotheses.

5.2 Discussion of the electrophysiological results

Here in this section, the results will first be summarized with respect to the hypotheses, followed by a more in-depth discussion.

Hypothesis I. Stated that the sCLAS delivery at different SO-states will affect electrophysiological responses differently. Firstly, to contribute any differential effects of the Acoustic Stimulation conditions to targeting the different SO-states, it was crucial to verify that sCLAS did not affect the sleep structure. Any modification in sleep structure would influence the interpretation of sCLAS results. The results confirm that there was no effect of condition on the duration of NREM sleep, REM sleep and Wake (**Table 1.A**). The results validate **Hypothesis I.a** in regard to these sleep-stages. However, the duration of PreREM sleep differed between the Up-State Stimulation and the Up-to-Down-State Transition Stimulation conditions. Notably, the latter condition included only six animals; therefore, these findings require validation in future studies.

Secondly, the impact of Up-State Stimulation was investigated. In sleep literature, Up-State sCLAS has been associated with an increase in mean SO amplitude and enhancement of SO-coupled fast spindle activity (Ngo et al., 2013; Navarrete et al., 2020) as well as increase in mean SO slope and acute entrainment of SOs (Ngo et al., 2013). Moreover, it has been reported to lead to increased slow-wave and sleep spindle power (Leminen et al., 2017). Therefore it was conceptualized in **Hypothesis I.b**, that several measures of these sleep rhythms including the coupled hippocampal ripple activity will be increased by Up-State Stimulation. However our results have shown that although an acute increase in **iii)** hippocampal ripples was observed under the 180 ms stimulation condition compared to the Pseudo Stimulation (**Figure 4.C, stimulus-event correlation: ripple activity**), this increase was followed by a sustained suppression. The same suppression effect was also observed on **ii)** cortical spindles following the 180 ms stimulation, although there was not an increase in spindles (measured by the cumulative number of peaks and troughs) at the time of the stimulation. Furthermore in our study, continuous responses such as **iv)** SO density, **v)** SO slope, **vi)** SO amplitude and **vii)** spindle density (**Table S1.**), as well as **viii)** SO-spindle-coupling and **ix)** SO-ripple coupling (**Figure 5.B, event-coupling: 180 ms**) did not show any positive changes under 180 ms stimulation condition compared to the control session as had been hypothesized. However, the 180 ms condition significantly modulated SO-ripple coupling, specifically suppressing it closely following the SO trough compared to the control condition. This significant suppression was preceded by a non-significant increase in the mean SO-ripple coupling value. Therefore the results do not validate **Hypothesis I.b** .

In **Hypothesis I.c**, the results of Down-State Stimulation was investigated which was previously associated with disruption of SO activity (Ngo et al., 2013) and decrease in slow wave and spindle power (Moreira et al., 2021). Thus, similar changes were expected under 2 ms stimulation condition. Our results have shown that acutely, indeed the **i)** cortical SOs decreased at the time of the stimulus delivery (**Figure 4.A, stimulus-event correlation: SO activity**) which could indicate SO disruption. However, the **ii)** cortical spindles and **iii)** hippocampal ripples did

not show a specific decrease in comparison to Pseudo Stimulation (**Figure 4.B,C; stimulus-event correlation: spindle and ripple activity**). Here the activity change patterns following the 2ms stimulation showed high resemblance to the changes seen after 180 ms stimulation. Continuously **iv)** SO-spindle-coupling did not show specific changes under 2 ms stimulation condition in comparison to the control session (**Figure 5.A, event coupling: 2 ms**). However, the 2 ms condition significantly suppressed **v)** SO-ripple coupling immediately following the SO trough, unlike the 180 ms condition, which was preceded by a mean increase in SO-ripple coupling. Based on these findings, only **Hypothesis I.c v)** can be validated, specifically, that SO-ripple coupling was reduced under Down-State Stimulation in comparison to the control session.

Additionally, it was conceptualized that changes in each stimulation condition will be investigated in **Hypothesis I.d**, if Up-State Stimulation could not improve the investigated sleep rhythms. Since **Hypothesis I.b** cannot be validated, the investigation was extended to other stimulation conditions. However, acute changes of **i)** cortical SOs, **ii)** cortical spindles and **iii)** hippocampal ripples following sCLAS delivery were mostly similar for all stimulation conditions in comparison to Pseudo Stimulation (**Figure 4.** and **Supplementary Figure S1., stimulus-event correlation**). Furthermore, **iv)** SO density, **v)** SO slope, **vi)** SO amplitude, **vii)** spindle density did not show any effect of condition (**Table S1**) and **viii)** SO-spindle-coupling and **ix)** SO-ripple coupling only revealed modest pattern changes relative to the control session (**Figure 5 and Supplementary Figure S2., event coupling)**.

There could be many reasons why sCLAS delivery did not result in continuous electrophysiological changes during NREM sleep. In controlled experiments the effect of timing at distinct delays after SO down-state peak detection was tested only, but it is possible that other parameters which were introduced could interfere with the optimal application of sCLAS. Although our protocol is very similar to Ngo and colleagues (2013), considerable differences can be listed as follow; introduction of white noise instead of pink noise, single and alternating

stimuli (acoustic and pseudo) instead of double acoustic stimuli, 10ms stimulus duration instead of 50 ms stimulus duration, over 180 minutes (3 hours) instead of 210 minutes of NREM sleep. Additionally, the individual differences in SO duration was not taken into account in this study as they reported (Ngo et al., 2013). Furthermore, lack of a sound-proof recording box and the delivery of sCLAS also during the Wake stages could be counted as the limitations of this study.

The results of this study have shown an acute increase in ripple activity after the Acoustic Stimulation proceeded with suppression of spindles and ripple activity at all stimulation conditions (**Figure 4.** and **Supplementary Figure S1., stimulus-event correlation**). This pattern mirrors a dynamic reported in a study where interruptions in hippocampal-cortical communication resulted in impairment in learning through a similar suppression of spindles and ripples following an acute increase in rats (Novitskaya et al., 2016). This could be the main reason why there aren't significant changes for mean sleep-event parameters across all conditions (**Table S1.**) and the lack of expected specific changes for the mean sleep event-coupling between control condition and any stimulation condition (**Figure 5.** and **Supplementary Figure S2., event-coupling**): The suppression of the spindles lasted at least 2 seconds (**Figure 4.B,** and **Supplementary Figure S1.B, stimulus-event correlation: spindle activity**). Investigation of spindle rhythmicity in humans showed that spindles are more likely to occur 3 - 6 seconds following the prior spindle and presenting cues just after this presumptive refractory period improved memory. Functionally, the spindle refractory period was stated to be a crucial element which protects memory processing from interference memory processing from interference (Antony et al., 2019). For this reason, it is very important to investigate whether it is possible to adapt the sCLAS application parameters so that the suppression of spindles would be reduced. Similarly ripple activity was suppressed for approximately 1 second following the acoustic stimuli (**Figure 4.C** and **Supplementary Figure S1.C, stimulus-event correlation: ripple activity**). Although the ripple refractory period is not pointed to as a crucial factor as spindle refractory in sleep literature yet, the duration of the ripples was (Fernández-Ruiz et al., 2019). Since the ripple activity was detected through the

number of ripples through and peaks, it's plausible that the suppression could also be an indicator of reduction in ripple duration at a critical time for memory consolidation. Therefore reducing the ripple suppression under sCLAS should be also targeted in the following studies.

Although it was captured that similar temporal-coupling patterns of sleep-events as Binder et al., (2019) in **Figure 5.** and in **Supplementary Figure S2., event-coupling**, it cannot be excluded that the placement of the frontal electrode in cingulate cortex could be suboptimal to detect the modulation hippocampocortical activity, as temporal associations are very sensitive to local positioning (Oyanedel et al., 2020). There are other studies investigating sleep-dependent memory consolidation in mice preferred to place the electrodes in medial prefrontal cortex (Binder et al., 2019; Maingret et al., 2016).

A further note should be made for the following experiments utilizing the same experimental conditions. Delivery at Down-State (2 ms) is the only condition where deviations from the endogenous SO activity are observed right after (< 100 ms) the acoustic stimuli delivery (**Figure 4.A, stimulus-event correlation: SO activity**). This could indicate that sCLAS application under this condition acutely disturbed the SO activity. Combined with the below chance level preference index (**Figure 6**), it is plausible to use this condition as Down-State stimulation for further sCLAS application in mice.

Acute enhancement of ripple activity at Up-State (180 ms, **Figure 4.C, stimulus-event correlation: ripple activity**) coincides with the endogenous increase of ripple activity which supports that the timing is preferable for testing Up-State Stimulation. Furthermore, PreREM duration is statistically longer in 180 ms condition than 300 ms (**Table 1.A**). A recent study has reported that the spindle count during the NREM/REM sleep transitions (corresponds to PreREM in this study) is a good predictor of behavioral performance in mice during post learning sleep (Yuan et al., 2021). Taken together with the exploratory analysis of behavioral results

where the performance at 180 ms is higher than 300 ms (**Results 3.6**), it is plausible to use this condition as Up-State stimulation for further sCLAS application in mice.

5.3 Discussion of the behavioral results

Here a closer look at behavioral changes was taken and investigated the memory performance at different SO-states in **Hypothesis II.**. Although it is not the case in all applications, Up-State sCLAS application was reported to lead to an increase in memory retention in several cases (Leminen et al., 2017; Ngo et al., 2013). Therefore it was hypothesized that Up-State Stimulation will increase the Preference Index in this study, which was used to measure memory retention (**Hypothesis II.a**). Accordingly, the PI in the 180 ms condition was expected to be above chance level and higher than the PI under the control condition. The one sample t-test revealed that PI in 180 ms condition was indeed higher than chance level. Subsequently, this condition was compared to the control condition with a paired t-test however no difference was found (**Results 3.1**, **Figure 3**). Thus, **Hypothesis II.a** could not be validated. Another point of focus was the memory performance under Down-State stimulation. Until now, sCLAS application was associated with either no changes (Ngo et al., 2013) or with a negative change (Moreira et al., 2021) in behavior. Here, it was conceptualized in **Hypothesis II.b.** that under Down-State Stimulation the PI will not differ from the PI for the control condition. Therefore an effect on memory was not expected. Please note, if active forgetting was initiated then such a condition may have differed significantly from control. Here, although the mean PI value in 2ms stimulation condition was lower than chance level, it was not lower than control condition (**Results 3.6**, **Figure 6**). Therefore, the results validate the **Hypothesis II.b**.

The same principle in **Hypothesis I.d.** was followed for the memory retention since Up-State sCLAS stimulation did not increase the PI in **Hypothesis II.a.** The preference index was compared to the control for all stimulation conditions in **Hypothesis II.c.**, in order to determine the sCLAS condition which would increase the PI. The analysis did not reveal improvement of

PI under any stimulation condition in comparison to the control **(Results 3.6**, **Figure 6)**. Therefore, the sCLAS condition to increase memory retention cannot be detected in this study.

When the behavioral performance is considered, the number of animals could be a reason behind our results where an effect of condition is lacked. Since the exploratory analysis without the control group gave positive results (**Results 3.6**) and fewer animals were recruited in the study as it was planned due to technical reasons, this is plausible. Another point is the duration of the memory retention period (3 hours) which was expected to provide clear distinction across stimulation conditions. It was expected to have chance level behavioral performance for the control condition, which could be improved with a specific (most likely Up-State) stimulation. Please note that two mice studies reported that the exploration time of familiar and unfamiliar objects for OPR task differed at 2 hours intertrial interval but not at 4 hours (Belblidia et al., 2015; Murai et al., 2007). Additionally, recent studies in humans have demonstrated that memory for semantically incongruent associations is not enhanced by (CLAS) (Ngo et al., 2015; Prehn-Kristensen et al., 2020). In this context, an alternative behavioral test that provides shelter, such as the Barnes Maze (Binder et al., 2019), may be more suitable for investigating memory consolidation under CLAS.

Moreover, as discussed in Section **5.2 Discussion of the electrophysiological results**, the acute suppression of spindles and ripples may offer an explanation for the lack of improvement in memory performance following Up-State Stimulation. The intricate triple coupling of slow oscillations, spindles, and ripples plays a pivotal role in hippocampus-dependent memory consolidation, as evidenced by experiments in rodents. A seminal study by Maingret et al. (2016) provided direct evidence of a causal relationship between increased hippocampo-cortical coupling and memory consolidation in rats. By inducing delta-spindle sequences upon the online detection of hippocampal ripples, researchers observed enhanced temporal coordination between sleep rhythms, resulting in improved memory performance in a classical spatial task, such as the object place recognition task. Similarly, a study in mice demonstrated the efficacy

of optogenetic induction of thalamic spindles, which naturally coincide with hippocampal ripples, in enhancing memory consolidation. However, this enhancement was observed only when the induction was phase-locked to the SO Up-State, as shown by Latchoumane et al. (2017). Therefore, the suppression of ripples and spindles may disrupt their coupling, consequently affecting memory retention.

It is important to note that, the one sample t-test results, where PI value of each condition was compared to chance level, were in parallel with the results of a recent application of CLAS in rats which stated that Up-State application of CLAS does not induce a significant change in behavioral performance but down-state application exerted a detrimental effect in the behavioral test (Moreira et al., 2021). If these results could be replicated in mice, this could also indicate that the behavioral performance can easily be disturbed by Down-State CLAS in rodents whereas it is harder to improve it with the Up-State application.

5.4 Discussion of the exploratory hypothesis

Followingly, sleep rhythms in early- and late-NREM sleep were investigated separately in order to reveal the modulatory effect of sCLAS as stated in the exploratory **Hypothesis III.**. However, an effect of condition could not be found for the investigated continuous changes on; **i)** SO density, **ii)** SO slope, **iii)** SO amplitude, **iv)** spindle density (**Supplementary Table 2 and 3)** and **v)** SO-spindle-coupling and **vii)** SO- ripple coupling (not shown) in comparison to the control session in early- and late-NREM sleep. Therefore, the modulatory effect of sCLAS cannot be revealed in more detail through analyzing early- and late- NREM sleep separately.

Nevertheless, a consistent effect of time was present for most of the sleep- event parameters (**Figure 7.**). Previously such changes were observed in mice, for example, spindle density and SO power were specifically increased in the 1 h – 4 h of NREM sleep following a motor learning task in mice (Kam et al., 2019). Interestingly, another study revealed that sleep deprivation of 3 h can be recovered when it is applied right after the OPR task but not when

1h of sleep introduced in between (T.-M. Prince et al., 2014). It´s plausible that such an intervention of sleep deprivation could interrupt the development of individual sleep rhythms and their interactions to a degree that recovery through sleep will not be possible at a later time point. Investigating the sleep-event parameter dynamics in short time intervals within NREM sleep could support to elucidate in more detail that how these sleep-events are involved in memory consolidation.

5.5 Further Notes

The electrophysiological changes monitored in this study were expected to be a correlate of memory performance. In sleep literature, increase in memory performance could be traced back to the modulations in sleep rhythms such as acute entraining of SOs, increase in SO slope and amplitude, increase in SO-coupled fast spindle activity (Ngo et al., 2013) and increase in slow-wave and spindle power (Moreira et al., 2021). Potentially, not yet distinguished sleep-oscillatory patterns which would predict stronger memory consolidation could be identified in this study. However, the lack of significant behavioral differences between the sCLAS and control sessions made it challenging to directly associate hypothesized retention performance changes with electrophysiological measures. Consequently, the identification of potential new electrophysiological biomarkers for memory retention was also not feasible.

6 Conclusion

In this study it was aimed to establish a mouse-model to investigate the effects of a typical CLAS application which was previously introduced in humans. For this purpose, acoustic-evoked changes on endogenous sleep rhythms and memory performance following a 3 hours sleep interval were taken under scope. Four states of the SO-cycle were successively targeted, without disturbing the NREM sleep which resulted in acute modulatory changes on cortical SO, cortical spindle and hippocampal ripple activity. Furthermore, memory retention differed from the chance level for control condition, for the Up-State Stimulation and Down-to-Up-State-Transition stimulation but not for the Down-State Stimulation and Up-to-Down-State-Transition which are in parallel with the behavioral results of a recent application of CLAS in rats. Although a direct correlation between sleep oscillatory activity patterns and memory retention was not found due to the lack of significant results lasting over NREM sleep, (s)CLAS application in mice has great potential to reveal the neurophysiological mechanisms underlying memory consolidation in NREM sleep.

For future experiments with this tool in mice, several considerations could enhance the outcomes:

- Introducing a sound-proof recording box to prevent interference from other acoustic stimuli, as implemented by Moreira et al.
- Increasing the accuracy of (s)CLAS delivery to ensure it is applied exclusively during NREM sleep, as planned.
- Adapting (s)CLAS delivery parameters to potentially reduce suppression in spindle-ripple activity.
- Measuring brain activity from the medial prefrontal cortex to more effectively detect modulation of hippocampocortical activity, as implemented by Binder et al. (2019) and Maingret et al. (2016).

- Introducing a longer inter-trial interval for the OPR task to capture potential differences in memory performance due to Up-State stimulation compared to the control condition.
- Changing the behavioral task to introduce a reward or shelter association, making tasks semantically congruent, as only these associations were enhanced by CLAS in humans (Ngo et al., 2015; Prehn-Kristensen et al., 2020).
- Employing different offline analyses to capture potential modulatory effects on electrophysiology.
- Adapting the delay of (s)CLAS application for each individual mouse, following the method of Ngo et al. (2013), to ensure the timing of auditory stimulus delivery is more precise relative to the targeted SO state.

7 References

Aksamaz, S., Mölle, M., Akinola, E. O., Gromodka, E., Bazhenov, M., & Marshall, L. (2024). Single closed-loop acoustic stimulation targeting memory consolidation suppressed hippocampal ripple and thalamo-cortical spindle activity in mice. *European Journal of Neuroscience*, *59*(4), 595–612. https://doi.org/10.1111/ejn.16116

Antony, J. W., & Paller, K. A. (2017). Using Oscillating Sounds to Manipulate Sleep Spindles. *Sleep*, *40*(3). https://doi.org/10.1093/SLEEP/ZSW068

Antony, J. W., Schönauer, M., Staresina, B. P., & Cairney, S. A. (2019). Sleep Spindles and Memory Reprocessing. *Trends in Neurosciences*, *42*(1), 1–3. https://doi.org/10.1016/j.tins.2018.09.012

Barker, G. R. I., & Warburton, E. C. (2011). When is the hippocampus involved in recognition memory? *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(29), 10721–10731. https://doi.org/10.1523/JNEUROSCI.6413-10.2011

Bartos, M., Vida, I., & Jonas, P. (2007). Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nature Reviews Neuroscience*, *8*(1), 45–56. https://doi.org/10.1038/nrn2044

Bartos, M., Xia, F., Richards, B. A., Tran, M. M., Josselyn, S. A., Takehara-Nishiuchi, K., & Frankland, P. W. (2017). *Parvalbumin-positive interneurons mediate neocortical-hippocampal interactions that are necessary for memory consolidation*. https://doi.org/10.7554/eLife.27868.001

Bazhenov, M., Timofeev, I., Steriade, M., & Sejnowski, T. J. (2002). Model of Thalamocortical Slow-Wave Sleep Oscillations and Transitions to Activated States. *The Journal of Neuroscience*, *22*(19), 8691. https://doi.org/10.1523/JNEUROSCI.22-19-08691.2002

Binder, S., Baier, P. C., Mölle, M., Inostroza, M., Born, J., & Marshall, L. (2012). Sleep enhances memory consolidation in the hippocampus-dependent object-place recognition task in rats. *Neurobiology of Learning and Memory*, *97*(2), 213–219. https://doi.org/10.1016/j.nlm.2011.12.004

Binder, S., Mölle, M., Lippert, M., Bruder, R., Aksamaz, S., Ohl, F., Wiegert, J. S., & Marshall, L. (2019). Monosynaptic Hippocampal-Prefrontal Projections Contribute to Spatial Memory Consolidation in Mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *39*(35), 6978–6991. https://doi.org/10.1523/JNEUROSCI.2158-18.2019

Brem, A. katharine, Ran, K., & Pascual-leone, A. (2013). Learning and memory. *Handbook of Clinical Neurology*, *116*, 693–737. https://doi.org/10.1016/B978-0-444-53497-2.00055-3

Buzsáki, G. (2015). Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus*, *25*(10), 1073–1188. https://doi.org/10.1002/HIPO.22488

Buzsáki, G., & Draguhn, A. (2004). Neuronal Oscillations in Cortical Networks. *Science*, *304*(5679), 1926–1929. https://doi.org/10.1126/science.1099745

Cai, D. J., Shuman, T., Gorman, M. R., Sage, J. R., & Anagnostaras, S. G. (2009). Sleep selectively enhances hippocampus-dependent memory in mice. *Behavioral Neuroscience*, *123*(4), 713–719. https://doi.org/10.1037/a0016415

Cairney, S. A., Ashton, J. E., Roshchupkina, A. A., & Sobczak, J. M. (2015). A Dual Role for Sleep Spindles in Sleep-Dependent Memory Consolidation? *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *35*(36), 12328–12330. https://doi.org/10.1523/JNEUROSCI.2463-15.2015

Chambers, A. M. (2017). The role of sleep in cognitive processing: focusing on memory consolidation. *WIREs Cognitive Science*, *8*(3). https://doi.org/10.1002/wcs.1433

Cirelli, C., & Tononi, G. (2008). Is Sleep Essential? *PLoS Biology*, *6*(8), e216. https://doi.org/10.1371/journal.pbio.0060216

Cohen, N. J., & Squire, L. R. (1980). Preserved learning and retention of pattern-analyzing skill in amnesia: dissociation of knowing how and knowing that. *Science (New York, N.Y.)*, *210*(4466), 207–210. https://doi.org/10.1126/SCIENCE.7414331

Cox, R., Korjoukov, I., De Boer, M., & Talamini, L. M. (2014). Sound asleep: processing and retention of slow oscillation phase-targeted stimuli. *PloS One*, *9*(7). https://doi.org/10.1371/JOURNAL.PONE.0101567

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neuroscience & Biobehavioral Reviews*, *31*(5), 673–704. https://doi.org/10.1016/j.neubiorev.2007.01.005

Eschenko, O., Mölle, M., Born, J., & Sara, S. J. (2006). Elevated Sleep Spindle Density after Learning or after Retrieval in Rats. *The Journal of Neuroscience*, *26*(50), 12914–12920. https://doi.org/10.1523/JNEUROSCI.3175-06.2006

Feld, G. B., & Diekelmann, S. (2015). Sleep smart-optimizing sleep for declarative learning and memory. *Frontiers in Psychology*, *6*(MAY). https://doi.org/10.3389/FPSYG.2015.00622

Fernandez, L. M. J., & Lüthi, A. (2020). Sleep Spindles: Mechanisms and Functions. *Physiological Reviews*, *100*(2), 805–868. https://doi.org/10.1152/physrev.00042.2018

Fernández-Ruiz, A., Oliva, A., de Oliveira, E. F., Rocha-Almeida, F., Tingley, D., & Buzsáki, G. (2019). Long-duration hippocampal sharp wave ripples improve memory. *Science*, *364*(6445), 1082–1086. https://doi.org/10.1126/SCIENCE.AAX0758/SUPPL_FILE/AAX0758_FERNANDEZ-RUIZ_SM.PDF

Girardeau, G., & Lopes-dos-Santos, V. (2021). Brain neural patterns and the memory function of sleep. *Science*, *374*(6567), 560–564. https://doi.org/10.1126/science.abi8370

Girardeau, G., & Zugaro, M. (2011). Hippocampal ripples and memory consolidation. *Current Opinion in Neurobiology*, *21*(3), 452–459. https://doi.org/10.1016/j.conb.2011.02.005

Grimaldi, D., Papalambros, N. A., Zee, P. C., & Malkani, R. G. (2020). Neurostimulation techniques to enhance sleep and improve cognition in aging. *Neurobiology of Disease*, *141*, 104865. https://doi.org/10.1016/j.nbd.2020.104865

Harrington, M. O., & Cairney, S. A. (2021). Sounding It Out: Auditory Stimulation and Overnight Memory Processing. *Current Sleep Medicine Reports*, *7*(3), 112–119. https://doi.org/10.1007/S40675-021-00207-0

Henin, S., Borges, H., Shankar, A., Sarac, C., Melloni, L., Friedman, D., Flinker, A., Parra, L. C., Buzsaki, G., Devinsky, O., & Liu, A. (2019). Closed-Loop Acoustic Stimulation Enhances Sleep Oscillations But Not Memory Performance. *ENeuro*, *6*(6). https://doi.org/10.1523/ENEURO.0306-19.2019

Kam, K., Pettibone, W. D., Shim, K., Chen, R. K., & Varga, A. W. (2019). Dynamics of sleep spindles and coupling to slow oscillations following motor learning in adult mice. *Neurobiology of Learning and Memory*, *166*. https://doi.org/10.1016/J.NLM.2019.107100

Killgore, W. D. S. (2010). *Effects of sleep deprivation on cognition* (pp. 105–129). https://doi.org/10.1016/B978-0-444-53702-7.00007-5

Klinzing, J. G., Niethard, N., & Born, J. (2019). Mechanisms of systems memory consolidation during sleep. *Nature Neuroscience 2019 22:10*, *22*(10), 1598–1610. https://doi.org/10.1038/s41593-019-0467-3

Krueger, J. M., Frank, M. G., Wisor, J. P., & Roy, S. (2016). Sleep function: Toward elucidating an enigma. *Sleep Medicine Reviews*, *28*, 46–54. https://doi.org/10.1016/j.smrv.2015.08.005

Krugliakova, E., Volk, C., Jaramillo, V., Sousouri, G., & Huber, R. (2020). Changes in cross-frequency coupling following closed-loop auditory stimulation in non-rapid eye movement sleep. *Scientific Reports*, *10*(1). https://doi.org/10.1038/S41598-020-67392-W

Latchoumane, C. F. V., Ngo, H. V. V., Born, J., & Shin, H. S. (2017). Thalamic Spindles Promote Memory Formation during Sleep through Triple Phase-Locking of Cortical, Thalamic, and Hippocampal Rhythms. *Neuron*, *95*(2), 424-435.e6. https://doi.org/10.1016/J.NEURON.2017.06.025

Leminen, M. M., Virkkala, J., Saure, E., Paajanen, T., Zee, P. C., Santostasi, G., Hublin, C., Müller, K., Porkka-Heiskanen, T., Huotilainen, M., & Paunio, T. (2017). Enhanced Memory Consolidation Via Automatic Sound Stimulation During Non-REM Sleep. *Sleep*, *40*(3). https://doi.org/10.1093/SLEEP/ZSX003

Lewis, P. A., & Bendor, D. (2019). How Targeted Memory Reactivation Promotes the Selective Strengthening of Memories in Sleep. *Current Biology : CB*, *29*(18), R906–R912. https://doi.org/10.1016/J.CUB.2019.08.019

Lo, J. C., Chong, P. L. H., Ganesan, S., Leong, R. L. F., & Chee, M. W. L. (2016). Sleep deprivation increases formation of false memory. *Journal of Sleep Research*, *25*(6), 673–682. https://doi.org/10.1111/JSR.12436

Maingret, N., Girardeau, G., Todorova, R., Goutierre, M., & Zugaro, M. (2016). Hippocampo-cortical coupling mediates memory consolidation during sleep. *Nature Neuroscience*, *19*(7), 959–964. https://doi.org/10.1038/NN.4304

Malkani, R. G., & Zee, P. C. (2020). Brain Stimulation for Improving Sleep and Memory. *Sleep Medicine Clinics*, *15*(1), 101–115. https://doi.org/10.1016/j.jsmc.2019.11.002

Marshall, L., Helgadóttir, H., Mölle, M., & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature*, *444*(7119), 610–613. https://doi.org/10.1038/nature05278

Mölle, M., & Born, J. (2011). Slow oscillations orchestrating fast oscillations and memory consolidation. *Progress in Brain Research*, *193*, 93–110. https://doi.org/10.1016/B978-0-444-53839-0.00007-7

Mölle, M., Eschenko, O., Gais, S., Sara, S. J., & Born, J. (2009). The influence of learning on sleep slow oscillations and associated spindles and ripples in humans and rats. *European Journal of Neuroscience*, *29*(5), 1071–1081. https://doi.org/10.1111/j.1460-9568.2009.06654.x

Moreira, C. G., Baumann, C. R., Scandella, M., Nemirovsky, S. I., Leach, S., Huber, R., & Noain, D. (2021). Closed-loop auditory stimulation method to modulate sleep slow waves and motor learning performance in rats. *ELife*, *10*. https://doi.org/10.7554/ELIFE.68043

Mumby, D. G., Gaskin, S., Glenn, M. J., Schramek, T. E., & Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *9*(2), 49–57. https://doi.org/10.1101/LM.41302

Navarrete, M., Schneider, J., Ngo, H. V. V., Valderrama, M., Casson, A. J., & Lewis, P. A. (2020). Examining the optimal timing for closed-loop auditory stimulation of slow-wave sleep in young and older adults. *Sleep*, *43*(6). https://doi.org/10.1093/SLEEP/ZSZ315

Neske, G. T. (2016). The Slow Oscillation in Cortical and Thalamic Networks: Mechanisms and Functions. *Frontiers in Neural Circuits*, *9*. https://doi.org/10.3389/fncir.2015.00088

Ngo, H. V. V., Martinetz, T., Born, J., & Mölle, M. (2013). Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. *Neuron*, *78*(3), 545–553. https://doi.org/10.1016/J.NEURON.2013.03.006

Ngo, H. V. V., Miedema, A., Faude, I., Martinetz, T., Mölle, M., & Born, J. (2015). Driving sleep slow oscillations by auditory closed-loop stimulation-a self-limiting process. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *35*(17), 6630–6638. https://doi.org/10.1523/JNEUROSCI.3133-14.2015

Novitskaya, Y., Sara, S. J., Logothetis, N. K., & Eschenko, O. (2016). Ripple-triggered stimulation of the locus coeruleus during post-learning sleep disrupts ripple/spindle coupling and impairs memory consolidation. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *23*(5), 238–248. https://doi.org/10.1101/LM.040923.115

Ong, J. L., Lo, J. C., Chee, N. I. Y. N., Santostasi, G., Paller, K. A., Zee, P. C., & Chee, M. W. L. (2016). Effects of phase-locked acoustic stimulation during a nap on EEG spectra and declarative memory consolidation. *Sleep Medicine*, *20*, 88–97. https://doi.org/10.1016/J.SLEEP.2015.10.016

Oyanedel, C. N., Durán, E., Niethard, N., Inostroza, M., & Born, J. (2020). Temporal associations between sleep slow oscillations, spindles and ripples. *The European Journal of Neuroscience*, *52*(12), 4762–4778. https://doi.org/10.1111/EJN.14906

Papalambros, N. A., Santostasi, G., Malkani, R. G., Braun, R., Weintraub, S., Paller, K. A., & Zee, P. C. (2017). Acoustic Enhancement of Sleep Slow Oscillations and Concomitant Memory Improvement in Older Adults. *Frontiers in Human Neuroscience*, *11*. https://doi.org/10.3389/FNHUM.2017.00109

Perrault, A. A., Khani, A., Quairiaux, C., Kompotis, K., Franken, P., Muhlethaler, M., Schwartz, S., & Bayer, L. (2019). Whole-Night Continuous Rocking Entrains Spontaneous Neural Oscillations with Benefits for Sleep and Memory. *Current Biology*, *29*(3), 402-411.e3. https://doi.org/10.1016/j.cub.2018.12.028

Petersen, R. C., Jack, C. R., Xu, Y.-C., Waring, S. C., O'Brien, P. C., Smith, G. E., Ivnik, R. J., Tangalos, E. G., Boeve, B. F., & Kokmen, E. (2000). Memory and MRI-based hippocampal volumes in aging and AD. *Neurology*, *54*(3), 581–581. https://doi.org/10.1212/WNL.54.3.581

Prehn-Kristensen, A., Ngo, H. V. V., Lentfer, L., Berghäuser, J., Brandes, L., Schulze, L., Göder, R., Mölle, M., & Baving, L. (2020). Acoustic closed-loop stimulation during sleep improves consolidation of reward-related memory information in healthy children but not in children with attention-deficit hyperactivity disorder. *Sleep*, *43*(8). https://doi.org/10.1093/SLEEP/ZSAA017

Prince, T. M., Wimmer, M., Choi, J., Havekes, R., Aton, S., & Abel, T. (2014). Sleep deprivation during a specific 3-hour time window post-training impairs hippocampal synaptic plasticity and memory. *Neurobiology of Learning and Memory*, *109*, 122–130. https://doi.org/10.1016/J.NLM.2013.11.021

Prince, T.-M., Wimmer, M., Choi, J., Havekes, R., Aton, S., & Abel, T. (2014). Sleep deprivation during a specific 3-hour time window post-training impairs hippocampal synaptic plasticity and memory. *Neurobiology of Learning and Memory*, *109*, 122–130. https://doi.org/10.1016/j.nlm.2013.11.021

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological Reviews*, *93*(2), 681–766. https://doi.org/10.1152/PHYSREV.00032.2012

Rayan, A., Agarwal, A., Samanta, A., Severijnen, E., van der Meij, J., & Genzel, L. (2024). Sleep scoring in rodents: Criteria, automatic approaches and outstanding issues. *European Journal of Neuroscience*, *59*(4), 526–553. https://doi.org/10.1111/ejn.15884

Rothschild, G., Eban, E., & Frank, L. M. (2017). A cortical–hippocampal–cortical loop of information processing during memory consolidation. *Nature Neuroscience*, *20*(2), 251–259. https://doi.org/10.1038/nn.4457

Ruch, S., Schmidig, F. J., Knüsel, L., & Henke, K. (2022). Closed-loop modulation of local slow oscillations in human NREM sleep. *NeuroImage*, *264*. https://doi.org/10.1016/J.NEUROIMAGE.2022.119682

Save, E., Poucet, B., Foreman, N., & Buhot, M.-C. (1992). Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. *Behavioral Neuroscience*, *106*(3), 447–456. https://doi.org/10.1037/0735-7044.106.3.447

Schönauer, M., & Pöhlchen, D. (2018). Current Biology Sleep spindles. *Current Biology*, *28*, 1121–1142.

Slotnick, S. D. (2022). The hippocampus and long-term memory. *Cognitive Neuroscience*, *13*(3–4), 113–114. https://doi.org/10.1080/17588928.2022.2128736

Squire, L. R. (2009). Memory and Brain Systems: 1969–2009. *The Journal of Neuroscience*, *29*(41), 12711. https://doi.org/10.1523/JNEUROSCI.3575-09.2009

Squire, L. R., Genzel, L., Wixted, J. T., & Morris, R. G. (2015). Memory consolidation. *Cold Spring Harbor Perspectives in Biology*, *7*(8). https://doi.org/10.1101/CSHPERSPECT.A021766

Squire, L. R., & Zola-Morgan, S. (1991). The Medial Temporal Lobe Memory System. *Science*, *253*(5026), 1380–1386. https://doi.org/10.1126/science.1896849

Steriade, M., Contreras, D., Dossi, R. C., & Nunez, A. (1993). The slow (. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *13*(8), 3284–3299. https://doi.org/10.1523/JNEUROSCI.13-08-03284.1993

Steriade, M., Nunez, A., & Amzica, F. (1993). A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *The Journal of Neuroscience*, *13*(8), 3252. https://doi.org/10.1523/JNEUROSCI.13-08-03252.1993

Timofeev, I., Grenier, F., Bazhenov, M., Sejnowski, T. J., & Steriade, M. (2000). Origin of slow cortical oscillations in deafferented cortical slabs. *Cerebral Cortex (New York, N.Y. : 1991)*, *10*(12), 1185–1199. https://doi.org/10.1093/CERCOR/10.12.1185

Todorova, R., & Zugaro, M. (2020). Hippocampal ripples as a mode of communication with cortical and subcortical areas. *Hippocampus*, *30*(1), 39–49. https://doi.org/10.1002/HIPO.22997

van de Ven, G. M., Trouche, S., McNamara, C. G., Allen, K., & Dupret, D. (2016). Hippocampal Offline Reactivation Consolidates Recently Formed Cell Assembly Patterns during Sharp Wave-Ripples. *Neuron*, *92*(5), 968–974. https://doi.org/10.1016/j.neuron.2016.10.020

Wang, G., Grone, B., Colas, D., Appelbaum, L., & Mourrain, P. (2011). Synaptic plasticity in sleep: learning, homeostasis and disease. *Trends in Neurosciences*, *34*(9), 452–463. https://doi.org/10.1016/j.tins.2011.07.005

Weigenand, A., Mölle, M., Werner, F., Martinetz, T., & Marshall, L. (2016). Timing matters: open-loop stimulation does not improve overnight consolidation of word pairs in humans. *European Journal of Neuroscience*, *44*(6), 2357–2368. https://doi.org/10.1111/EJN.13334

Wilson, M. A., & McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science (New York, N.Y.)*, *265*(5172), 676–679. https://doi.org/10.1126/SCIENCE.8036517

Wiltgen, B. J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M. G., Parivash, S. N., Li, W., & Silva, A. J. (2010). The hippocampus plays a selective role in the retrieval of detailed contextual

memories. *Current Biology : CB*, *20*(15), 1336–1344. https://doi.org/10.1016/J.CUB.2010.06.068

Yuan, R. K., Lopez, M. R., Ramos-Alvarez, M. M., Normandin, M. E., Thomas, A. S., Uygun, D. S., Cerda, V. R., Grenier, A. E., Wood, M. T., Gagliardi, C. M., Guajardo, H., & Muzzio, I. A. (2021). Differential effect of sleep deprivation on place cell representations, sleep architecture, and memory in young and old mice. *Cell Reports*, *35*(11). https://doi.org/10.1016/J.CELREP.2021.109234

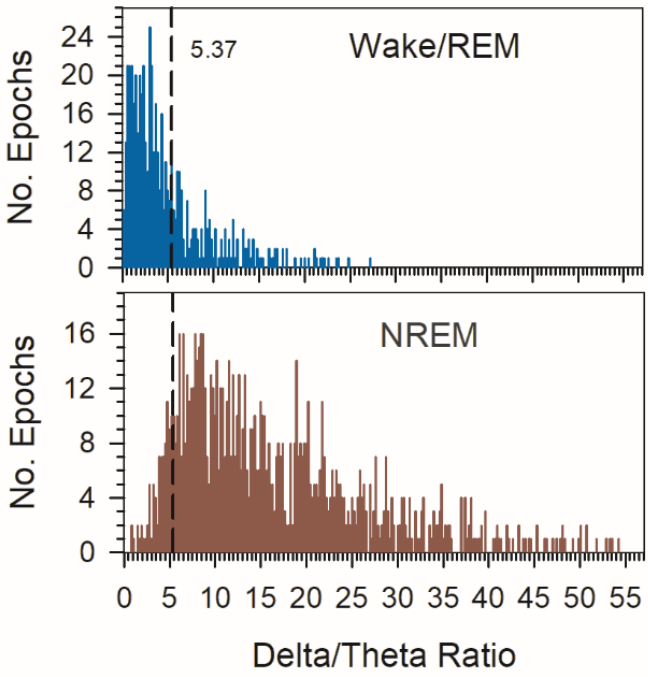
Zielinski, M., T. McKenna, J., & W. McCarley, R. (2016). Functions and Mechanisms of Sleep. AIMS Neuroscience, 3(1), 67–104. https://doi.org/10.3934/Neuroscience.2016.1.67

Zlotnik, G., & Vansintjan, A. (2019). Memory: An Extended Definition. *Frontiers in Psychology*, *10*. https://doi.org/10.3389/FPSYG.2019.02523

8 Attachments

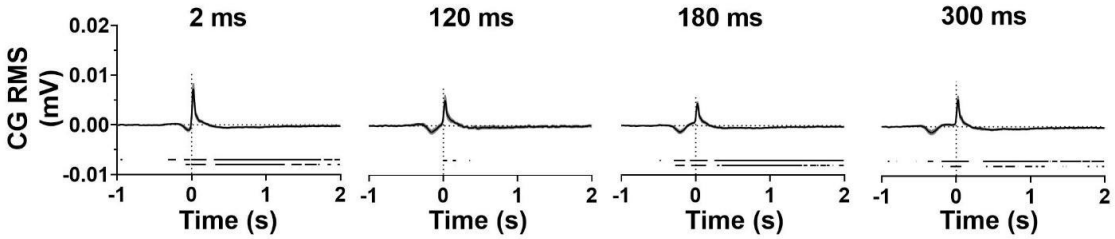
8.1 Supplementary Data

Supplementary Data 1.



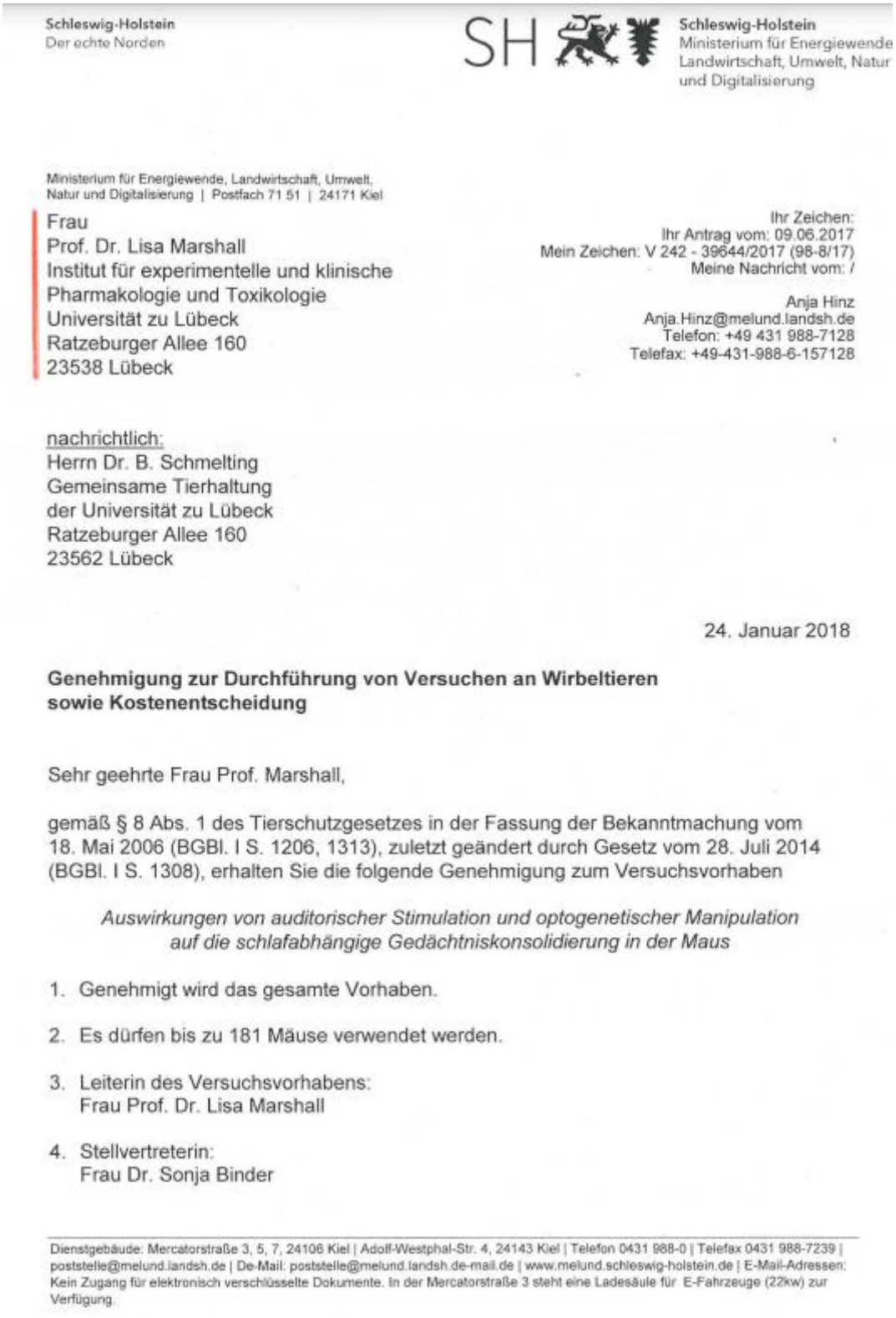
D1. Histograms to determine the delta/theta ratio from a hippocampal LFP recording. The top histogram gives the number of 5-sec epochs scored as Wake or REM-sleep for each delta/theta ratio given on the x-axis. The corresponding bottom histogram is for NREM sleep epochs. The threshold of 5.37 (vertical black bar) indicates the value after which most NREM sleep epochs, but minority of Wake and REM sleep epochs were scored. (From Aksamaz et al., 2024, EJN, Supplementary Figure S1).

Supplementary Data 2.



D2. sCLAS delivery time-locked to the differential SO-states modulates cortical activity in the ripple frequency band. For Acoustic Stimulation, baseline normalized (mean ± SEM) cortical RMS for the four stimulation conditions. Horizontal lines correspond to significant differences from the baseline level (−1 to −0.5 s, paired t-tests) for false discovery rate set to 0.05 (top line) and 0.01 (bottom line). T = 0 s corresponds to the time of stimulation. 2 ms, 180 ms, n = 10; 120 ms, n = 7; 300 ms, n = 6. (From Aksamaz et al., 2024, EJN, Supplementary Figure 3).

8.2 Animal experiment approval by the ethics committee



Schleswig-Holstein
Der echte Norden

SH Schleswig-Holstein
Ministerium für Energiewende
Landwirtschaft, Umwelt, Natur
und Digitalisierung

Ministerium für Energiewende, Landwirtschaft, Umwelt,
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Frau
Prof. Dr. Lisa Marshall
Institut für experimentelle und klinische
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Universität zu Lübeck
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Ihr Zeichen:
Ihr Antrag vom: 09.06.2017
Mein Zeichen: V 242 - 39644/2017 (98-8/17)
Meine Nachricht vom: /

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Gemeinsame Tierhaltung
der Universität zu Lübeck
Ratzeburger Allee 160
23562 Lübeck

24. Januar 2018

Genehmigung zur Durchführung von Versuchen an Wirbeltieren sowie Kostenentscheidung

Sehr geehrte Frau Prof. Marshall,

gemäß § 8 Abs. 1 des Tierschutzgesetzes in der Fassung der Bekanntmachung vom 18. Mai 2006 (BGBl. I S. 1206, 1313), zuletzt geändert durch Gesetz vom 28. Juli 2014 (BGBl. I S. 1308), erhalten Sie die folgende Genehmigung zum Versuchsvorhaben

Auswirkungen von auditorischer Stimulation und optogenetischer Manipulation auf die schlafabhängige Gedächtniskonsolidierung in der Maus

1. Genehmigt wird das gesamte Vorhaben.
2. Es dürfen bis zu 181 Mäuse verwendet werden.
3. Leiterin des Versuchsvorhabens:
   Frau Prof. Dr. Lisa Marshall
4. Stellvertreterin:
   Frau Dr. Sonja Binder

Dienstgebäude: Mercatorstraße 3, 5, 7, 24106 Kiel | Adolf-Westphal-Str. 4, 24143 Kiel | Telefon 0431 988-0 | Telefax 0431 988-7239 | poststelle@melund.landsh.de | De-Mail: poststelle@melund.landsh.de-mail.de | www.melund.schleswig-holstein.de | E-Mail-Adressen: Kein Zugang für elektronisch verschlüsselte Dokumente. In der Mercatorstraße 3 steht eine Ladesäule für E-Fahrzeuge (22kw) zur Verfügung.

5. Wechselt die Leiterin des Versuchsvorhabens oder ihre Stellvertreterin, so ist diese Änderung unverzüglich anzuzeigen; die Genehmigung gilt weiter, wenn sie nicht innerhalb eines Monats widerrufen wird.

6. Ort der Durchführung: Universität zu Lübeck, CBBM

7. Das Versuchsvorhaben ist gem. § 35 Tierschutz-Versuchstierverordnung nicht rückblickend zu bewerten.
8. Die im Antrag gemachten Angaben sind im übrigen Bestandteil dieser Genehmigung.

Die Genehmigung ist mit folgenden Nebenbestimmungen verbunden:

9. Die Genehmigung ist bis zum 31. Januar 2022 befristet.

Zur Genehmigung ergeht folgende Kostenentscheidung:

10. Es werden keine Kosten erhoben.

Begründung:

A. Zur Hauptsache:
Die Voraussetzungen für die Genehmigung nach § 8 Abs. 1 TierSchG sind erfüllt. Insbesondere wurde hinreichend dargelegt, dass das Vorhaben unerlässlich und die ethische Vertretbarkeit gegeben ist, die Einhaltung der gesetzlichen Vorschriften insbesondere nach dem Tierschutzgesetz und der Tierschutz-Versuchstierverordnung erwartet werden kann und durch die/den Tierschutzbeauftragte/n mit sichergestellt wird.

B. Zu den Nebenbestimmungen.
Vom beantragten Zeitraum wurde nicht abgewichen.

C. Zur Kostenentscheidung
Ihre Einrichtung ist gem. § 8 Abs. 1 Nr. 6 Verwaltungskostengesetz des Landes Schleswig-Holstein als öffentlich-rechtliche Stiftung , die gemeinnützigen Zwecken dient, von Verwaltungsgebühren befreit, da die der Kostenentscheidung zugrunde liegende Angelegenheit nicht einen steuerpflichtigen wirtschaftlichen Geschäftsbetrieb betrifft.

Rechtsbehelfsbelehrung
Gegen diesen Bescheid kann innerhalb eines Monats nach Bekanntgabe Klage bei dem Schleswig-Holsteinischen Verwaltungsgericht, Brockdorff-Rantzau-Straße 13, 24837 Schleswig, schriftlich, in elektronischer Form oder zur Niederschrift der/des Urkundsbeamtin/Urkundsbeamten der Geschäftsstelle erhoben werden.

Hinweis:
Bei der Verwendung der elektronischen Form sind besondere Voraussetzungen zu beachten (vgl. Landesverordnung über den elektronischen Rechtsverkehr mit den Gerichten und Staatsanwaltschaften vom 12.12.2006 (GVOBl. 2006, 361) in der z. Zt. geltenden Fassung. *Hiernach wird die elektronische Form insbesondere durch eine qualifiziert signierte Datei gewahrt, die nach den Maßgaben der genannten Landesverordnung übermittelt wird. Weitere Informationen zum elektronischen Rechtsverkehr mit den Gerichten und Staatsanwaltschaften sind auf der Internetseite www.justizpoststelle.schleswig-holstein.de abrufbar.*

Mit freundlichen Grüßen

Dr. Michaele Sekulla

8.3 List of first author publications and poster presentations

Publication:

Aksamaz, S., Mölle, M., Akinola, E. O., Gromodka, E., Bazhenov, M., & Marshall, L. (2024). Single closed-loop acoustic stimulation targeting memory consolidation suppressed hippocampal ripple and thalamo-cortical spindle activity in mice. The European journal of neuroscience, 59(4), 595–612. https://doi.org/10.1111/ejn.16116

Poster Presentations:

Aksamaz, S., Binder, S., Schumann, M., Mölle, M., & Marshall, L. (2019). Auditory closed-loop stimulation during sleep in mice modulates hippocampal sharp wave ripples and thalamo-cortical activity. Bernstein Conference. Bernstein Conference 2019 (g-node.org)

Aksamaz, S., Mölle, M., Golden, R., Bazhenov, M., & Marshall, L. (2021) Auditory closed-loop stimulation during sleep in mice modulates the retention of spatial memory. SfN Global Connectome: A Virtual Event. https://www.sfn.org/-/media/SfN/Documents/NEW-SfN/Meetings/SfN-Global-Connectome/SFN21_Connectome_Abstracts_PDFs.pdf

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