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Use of Non-Invasive Brain Stimulation to Modulate Endogenous Cortical Activity Across Brain States – a glimpse into a secret garden

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"If the human brain were so simple that we could understand it, we would be so simple that we couldn't"

—EMERSON M. PUGH

CONTENTS

Abstract

Non-invasive brain stimulation (NIBS) comprises various techniques to modulate brain activity such as (anodal) slow oscillatory transcranial direct current stimulation (so-tDCS). Studies using so-tDCS during sleep demonstrated an enhanced power of endogenous electroencephalography (EEG) for slow oscillations (SO) and overnight memory consolidation, albeit some studies also reported no effect on electrophysiology and behaviour. One reason for the discrepant results may be differences in the underlying neural network at the time of stimulation. Learning can induce modifications in EEG activity during post-learning sleep. Inter-individual differences in intelligence can also be reflected in sleep spindle activity. Thus, it was hypothesized that the network modifications induced by learning and inter-individual differences in intelligence scores may potentially be confounds for so-tDCS efficacy (Study I). In addition, so-tDCS applied during wakefulness with eyes open can induce SO, theta, and beta power. This led to another hypothesis that so-tDCS induces SO activity during quiet wakefulness (Study II). Auditory closed-loop stimulation (ACLS) is another method to target individual electrophysiological events (e.g., SO) in which it evokes an immediate response. SO is a low frequency component of slow wave activity (SWA). It has been linked to the process of reduction in synaptic strength that facilitates subsequent memory formation. Hence, it was hypothesized that enhancing SO would improve subsequent encoding (Study III). The overarching aim of this dissertation is two-fold: 1) to employ NIBS to investigate the interactions between rhythms of brain electric activity and behaviour and 2) to analyse potential confounds affecting the efficiency of NIBS in modulating human electrophysiology and behaviour.

To test these hypotheses, three separate studies using within-subject design took place. Study I entailed three separate conditions: non-learning (CTRL), learning without so-tDCS (SHAM), learning with so-tDCS (STIM). The results of Study I showed that only participants with high memory quotient showed increased fast spindle power in the SHAM as compared to the CTRL group. These individuals also improved significantly on figural paired-associate task in STIM as compared to SHAM. Although slow spindle density

was enhanced in SHAM compared to CTRL and fast spindle parameters were increased in STIM compared to SHAM in total participants, no significant change in behaviour was found. These results suggest that inter-individual differences play a role in mediating differential task-induced spindle modifications that ultimately lead to distinct so-tDCS efficacy. Study II comprised two conditions: so-tDCS (STIM) and control (SHAM), and revealed that so-tDCS applied during quiet wakefulness with eyes closed did not induce SO and further suppressed ongoing alpha power, reflecting the dependency of so-tDCS on the brain state. Study III also comprised comparison of two conditions: application of ACLS (STIM) and without auditory stimulation (SHAM). Results in Study III depicted the SO and fast spindles specifically during slow wave sleep, indicating that these oscillations may be relevant to reducing synaptic strength that is responsible for the subsequent encoding performance.

In conclusion, NIBS showed efficacy in modulating brain rhythms but its effect is subjected to post-learning modifications on underlying neural oscillations (micro-state) as well as ongoing brain states (macro-state). These results shed light on future studies aiming to use NIBS to modulate various brain rhythms, learning and memory. However, the underlying mechanisms of so-tDCS and ACLS is yet to be elucidated in further studies.

Zusammenfassung

Die nicht-invasive Hirnstimulation (engl. non-invasive brain stimulation, NIBS) umfasst verschiedene Techniken zur Modulation der Hirnaktivität wie die (anodale) langsam oszillierende transkranielle Gleichstromstimulation (engl., slow oscillatory transcranial direct current stimulation, so-tDCS). Studien, die so-tDCS während des Schlafs anwendeten, zeigten eine Verbesserung der endogenen Elektroenzephalografie (EEG)-Leistung bei langsamen Oszillationen (engl. Slow oscillation, SO) und der nächtlichen Gedächtniskonsolidierung, obwohl einige Studien auch keinen Einfluss auf die Elektrophysiologie und das Verhalten berichteten. Ein Grund zur Erklärung der diskrepanten Ergebnisse könnten Unterschiede im zugrunde liegenden neuronalen Netzwerk zum Zeitpunkt der Stimulation sein. Lernen kann Veränderungen der EEG-Aktivität während des darauffolgenden Schlafs hervorrufen. Inter-individuelle Unterschiede in der Intelligenz können sich auch in der Aktivität von Schlafspindeln widerspiegeln. Daher wurde die Hypothese aufgestellt, dass die durch das Lernen induzierten Netzwerkveränderungen und die inter-individuellen Unterschiede in der Intelligenz ein Störfaktor für die Wirksamkeit von so-tDCS darstellen (Studie I). Darüber hinaus kann so-tDCS bei der Anwendung während des Wachzustandes und mit offenen Augen SO, Theta- und Beta-Leistung induzieren. Dies führte zu einer weiteren Hypothese, dass so-tDCS während des wachen Ruhezustands SO-Aktivität induzieren kann (Studie II). Die auditorische Closed-Loop-Stimulation (ACLS) ist eine weitere Methode zur Beeinflussung einzelner elektrophysiologischer Ereignisse (z.B. SO), welche eine sofortige Reaktion hervorruft. SO ist eine niederfrequente Komponente der sogenannten Slow Wave Aktivität (SWA), welche mit Schwächung von Synapsen in Verbindung gebracht wird, was die nachfolgende Gedächtnisbildung erleichtert. Daher wurde die Hypothese aufgestellt, dass die Verstärkung der SO, die nachfolgende Enkodierung verbessern würde (Studie III). Diese Dissertation verfolgt zwei übergeordnete Ziele: zum einen die Verwendung von NIBS zur Untersuchung der Wechselwirkungen zwischen den Rhythmen der elektrischen Aktivität des Gehirns und des Verhaltens und zum anderen die Analyse möglicher Störfaktor, die die Effizienz von NIBS bei der Modulation der menschlichen Elektrophysiologie und des Verhaltens beeinflussen.

Um diese Hypothesen zu testen, wurden drei separate Studien mit einem Inner-Subjekt-Design durchgeführt. Studie I beinhaltete drei separate Bedingungen: Nicht-Lernen (CTRL), Lernen ohne so-tDCS (SHAM). Lernen mit so-tDCS (STIM). Die Ergebnisse von Studie I zeigten, dass nur Teilnehmer mit hohem Gedächtnisquotienten eine erhöhte Leistung der schnellen Spindeln in der SHAM im Vergleich zur CTRL Gruppe zeigten. Diese Personen verbesserten sich auch bei dem Bilder-Gedächtnistest bei STIM im Vergleich zu SHAM signifikant. Obwohl die Dichte der langsamen Spindeln bei SHAM im Vergleich zu CTRL sowie die Parameter der schnellen Spindeln bei STIM im Vergleich zu SHAM bei den Gesamtteilnehmern erhöht waren, wurde keine signifikante Änderung des Verhaltens festgestellt. Diese Ergebnisse deuten darauf hin, dass inter-individuelle Unterschiede eine Rolle in der Vermittlung der durch differentielles Lernen induzierten Spindelmodifikationen spielen, die letztlich zu einer unterschiedlichen so-tDCS-Wirksamkeit führen. Studie II umfasste zwei Bedingungen: so-tDCS (STIM) und Kontrolle (SHAM). Es zeigte sich, dass so-tDCS, welche während des wachen Ruhezustands mit geschlossenen Augen angewendet wurde, keine SO induzierte und die Alpha-Power weiter unterdrückte, was die Abhängigkeit der so-tDCS vom Gehirnzustand widerspiegelt. Studie III umfasste ebenfalls den Vergleich von zwei Bedingungen: die Anwendung von ACLS (STIM) im Vergleich mit keiner auditorischen Stimulation (SHAM). ACLS verstärkte SO und schnelle Spindeln speziell während des Tiefschlafs, was daraufhin deutet, dass diese Oszillationen für die Abschwächung der Synapsen relevant sein könnten, welche für die spätere Enkodierungsleistung verantwortlich ist.

Zusammenfassend lässt sich sagen, dass NIBS eine Wirksamkeit bei der Modulation von Hirnrhythmen zeigte. Jedoch ist die Wirkung nach dem Lernen sowohl den zugrunde liegenden neuronalen Oszillationen (Mikrozustand) als auch den laufenden Hirnzuständen (Makrozustand) unterworfen. Diese Ergebnisse werfen ein Licht auf zukünftige Studien, die darauf abzielen, NIBS zur Modulation verschiedener Hirnrhythmen, des Lernens und des Gedächtnisses zu verwenden. Der zugrundeliegende Mechanismus von so-tDCS und ACLS muss jedoch in weiteren Studien noch aufgeklärt werden.

1. Introduction

1.1 Non-invasive brain stimulation

Non-invasive brain stimulation (NIBS) is a broad term that describes the use of techniques to modulate brain rhythms or their relevant functions externally. The history of NIBS can be traced as far back as 43 AD using electrical shocks in animals and humans to modulate neural firing (Pascual-Leone & Wagner, 2007). The electrical stimulation was later developed slowly to a more applicable version, which is weak transcranial direct current stimulation (termed tDCS; Priori, 2003). Weak tDCS uses currents traveling in a constant manner and has shown efficiency in modulating the excitability of neural membrane potential (Nitsche & Paulus, 2000) with little to no side effects reported (Poreisz *et al.*, 2007). Depending on the polarity used, an anodal (positive) tDCS is thought to increase the resting membrane potential whereas cathodal (negative) tDCS would lead to an inhibition of excitability (Jamil & Nitsche, 2017). Another more recent approach is weak transcranial alternating current stimulation (tACS) that involves currents alternating between positive and negative polarities (Antal *et al.*, 2008). Such alternating operation has indicated the ability to synchronize or desynchronize ongoing rhythms in the brain and could potentially modify plasticity (Antal & Paulus, 2013). About a decade ago, a novel technique that combined the oscillating pattern and yet restricted the currents travelling within a single polarity has been employed in an attempt to synchronize the endogenous oscillation of brain activity while modulating the neural membrane potential (Marshall *et al.*, 2006) (see **section 1.1.1**). In contrast to injecting currents through the scalp, another approach employed auditory stimulation to affect the peripheral sensory system and thereby modified the neural activity (Davis *et al.*, 1939) (see **section 1.1.2**).

1.1.1 Slow oscillatory-transcranial direct current stimulation

Slow oscillatory-transcranial direct current stimulation (so-tDCS) originated from sleep. It is an approach to combine the oscillating pattern and limit the currents flowing within a polarity (i.e. anodal). Initially, the direct current (DC) potential recordings during sleep were conducted to measure changes in cortical excitability, similar to event-related negative potential shifts (e.g. Bereitschaftspotential) (Marshall *et al.*, 1996; Hallschmid *et*

al., 2004). The authors found a negative-going DC potential shift that corresponded to the slow wave sleep (SWS). The negative slope is thought to reflect a gradual increase in cortical excitability resulted from the widespread changes in depolarised apical dendrites (Marshall *et al.*, 1998). Evidence in favour of this hypothesis was provided by a study that used intermittent anodal tDCS during SWS to increase the slow oscillatory electroencephalography (EEG) activity. Here, an upsurge of slow wave activity (SWA; see **section 1.3.1.1**) concomitantly with an improvement in memory consolidation was shown (see **section 1.3.2**) (Marshall *et al.*, 2004). Subsequently, another study based on the concept that aside from the polarity, the change in polarity may be essential in inducing electrophysiological response; thus the introduction of so-tDCS, in the aim to induce plasticity changes by implementing the oscillating operation while preserving the DC component was put forth (Marshall *et al.*, 2006). While using so-tDCS at a slow frequency (0.75 Hz) that coincided with the slow oscillation (SO) in SWS located at the bilateral dorsal lateral prefrontal cortex (DLPFC), Marshall *et al.* (2006) found an increase of SO power and improved memory consolidation. The authors thus suggested such an increase may reflect the applied exogenous currents resonated with ongoing cortical rhythm and is functionally beneficial. Subsequent studies using this approach in sleep have shown a similar effect on SO activity (Prehn-Kristensen *et al.*, 2014; Munz *et al.*, 2015; Westerberg *et al.*, 2015; Ladenbauer *et al.*, 2016; Ladenbauer *et al.*, 2017), slow spindle activity (Del Felice *et al.*, 2015), fast spindle activity (Ladenbauer *et al.*, 2016) as well as on behaviour (Floel *et al.*, 2012; Antonenko *et al.*, 2013; Göder *et al.*, 2013; Prehn-Kristensen *et al.*, 2014; Del Felice *et al.*, 2015; Munz *et al.*, 2015; Westerberg *et al.*, 2015; Ladenbauer *et al.*, 2016; Ladenbauer *et al.*, 2017). However, other studies have failed to find behavioural effects (Eggert *et al.*, 2013; Sahlem *et al.*, 2015; Paßmann *et al.*, 2016; Bueno-Lopez *et al.*, 2019).

Furthermore, anodal so-tDCS applied over the motor cortex during wakefulness modified cortical excitability as probed with transcranial magnetic stimulation (TMS) (Bergmann *et al.*, 2009; Groppa *et al.*, 2010), suggesting so-tDCS was also effective during wakefulness. Similarly, so-tDCS applied during declarative encoding enhanced learning

performance and increased SO and theta power (Kirov *et al.*, 2009). On the other hand, the same study applied so-tDCS during relaxed wakefulness after learning showed no impact on memory consolidation during a subsequent of 7 hours of wakefulness, despite an increase in SO power (Kirov *et al.*, 2009). Another study that employed so-tDCS as well as theta-tDCS during the eyes-closed resting state, however, found no increase of SO induced by so-tDCS. Instead, an increase of delta activity associated with increased sleepiness after theta-tDCS was observed (D'Atri *et al.*, 2016).

Taken together, these findings suggest that so-tDCS can modulate the cortical activity and brain rhythms aside from the SO frequency (see **Hypothesis 2a**). Nevertheless, a number of underlying factors such as stimulation parameters, cognitive tasks, and unidentified inter-individual factors may have influenced the stimulation efficacy and thus lead to inconsistent outcomes (Berryhill, 2012; Marshall *et al.*, 2020)(see **Hypothesis 1b** and **Hypothesis 1c**).

1.1.2 Auditory closed-loop stimulation (ACLS) during sleep

The use of short duration auditory stimulation to investigate sensory processing during sleep was already reported since the beginning of the 1930s. Using a 500 Hz tone, it was observed that following the stimulation, K-complexes (KCs) and/or spindles (see **section 1.3.1**) were induced (Davis *et al.*, 1939). Furthermore, Gao *et al.* (2009) observed that sound stimuli led to entrainment of SO in the auditory thalamus in the guinea pig, suggested the involvement of thalamocortical (TC) activity in auditory stimulation. Numerous studies illustrated auditory stimulation evoked KCs that consist of an early negative event-related potential (ERP[1]) component peaking at approximately 350 ms (N350) followed by larger negative wave amplitude peaking at around 500 ms (N550) (Bastien & Campbell, 1992; Plihal *et al.*, 1996; Ngo *et al.*, 2013b). Moreover, the amplitude of auditory evoked potentials (AEPs) was found to increase in sleep stage 2

[1] An ERP is an EEG measurement to assess the brain's response time-locked to specific external stimuli like a sensory, cognitive, or motor event. The ERP waveforms are composed of a series of positive and negative deflections, in which they reflect different psychophysiological processes. The letter "N" stands for negative polarity and "P" for positive polarity, and the number corresponds to the peak latencies, for instance 1=100 ms. Luck, S. (2005) An introduction to event related potentials and their neural origins. *An introduction to the event related potential technique*, 11.

whereas sustained negativity of evoked potential occurred in sleep stage 3, indicating disparate sensory processing in different sleep stages (see **section 1.3**) (Nielsen-Bohlman *et al.*, 1991). Recently, a more sophisticated closed-loop approach to modulate neural activity was established. This approach endorsed real-time detection of ongoing neural oscillation and thereby enables precise manipulation of the functionally relevant activity (Chen *et al.*, 2011). Taken together, these results formed a foundation of a pioneer study that utilized the closed-loop system with auditory stimuli upon the detection of the SO. Specifically, two pink noise (1/*f*) bursts lasting 50 ms each timed to the SO depolarisation state (active up-state) were delivered during sleep. This stimulation regime was effective in increasing the SO amplitude as well as fast spindle activity (see **section 1.3.1.2**), and as a result, enhanced memory consolidation (Ngo *et al.*, 2013a). Following studies have uniformly pointed towards the direction that ACLS is effective in enhancing SOs and spindle activity (Ngo *et al.*, 2013a; Ngo *et al.*, 2015; Ong *et al.*, 2016; Santostasi *et al.*, 2016; Besedovsky *et al.*, 2017; Leminen *et al.*, 2017; Papalambros *et al.*, 2017; Debellemaniere *et al.*, 2018; Garcia-Molina *et al.*, 2018; Ong *et al.*, 2018; Patanaik *et al.*, 2018; Henin *et al.*, 2019) as well as memory performance in humans (Ngo *et al.*, 2013a; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017; Ong *et al.*, 2018), although no improvement on memory consolidation was recently reported (Henin *et al.*, 2019). Along the same line, a study employing the stimulation in an open-loop fashion reported a failure to improve memory consolidation, despite enhancement of SO and spindle activity (Weigenand *et al.*, 2016), thus underscoring the relevant temporal stimulation parameters. On the whole, ACLS that targets SO in sleep is efficient in augmenting SO amplitude and associated spindle activity. This method therefore allows us to explore the function of SO and spindles (see **Hypothesis 3a**) and the effect of ACLS at different sleep stages (see **Hypothesis 3b**).

1.2 Learning and memory

Behaviourally, Ebbinghaus, a German psychologist, was the first to experimentally study learning and memory (Ebbinghaus, 2013). After extensively establishing a list of non-word syllables, Ebbinghaus reported an exponential forgetting curve, where sharp decay of remembering was observed at the beginning of the learning and the decrement evens out gradually, reflecting less information is forgotten after each repetition. Likewise, he

also observed a learning curve (although this term was not employed then), an inversed exponential curve where more information was learned at the beginning upon acquisition and the learning capacity slowly reaches a plateau (Ebbinghaus, 2013).

At the cellular and molecular level, the term plasticity is used to refer to the change of neuronal properties as a consequence of experience. Such change can develop into permanent modification of new synaptic connections (Steriade & Timofeev, 2003). Long term potentiation (LTP) refers to the increased efficiency of synaptic transmission between neurons after stimulation (e.g. high-frequency stimulation) (Bliss & Collingridge, 1993). On the other hand, long term depression (LTD), as the name suggests, refers to long-lasting depression in synaptic strength (Ito, 1989). From a top-down perspective, motor learning followed by the induction of paired associative stimulation[2] led to an increase of motor-evoked potential that lasted even after the cessation of stimulation, suggesting the involvement of LTP on learning and memory (Muellbacher *et al.*, 2001; Ziemann *et al.*, 2004). A large body of work focused on the role of LTP and LTD in learning and memory consolidation has established a consensus that behavioural learning can induce LTP (Maren, 1999; Moser & Moser, 1999; Nishiyama, 2014).

Memory formation is a process that involves encoding (acquisition of information), consolidation (strengthening of memory traces) and retrieval (recall of information) and is very crucial for engaging in daily life activity in humans and animals (Diekelmann & Born, 2010). A multi-store model proposed that memory systems are complex processes that constitute different components rather than being a unitary system: sensory memory, short-term memory, and long-term memory (Atkinson & Shiffrin, 1968). In short, when one exposed to a new environment or receiving new information for the first time, the external input is first registered by the corresponding sensory domain, such as visual or spatial systems (sensory memory). Subsequently, the acquired information is stored temporarily (short-term memory). This latter type of memory is subjected to decay within minutes and has limited capacity. On the other hand, a large amount of information can

[2] In this specific study, 200 pairs of electrical stimulation over right median nerve followed by focal transcranial magnetic stimulation over left M1 was used to elicit right abductor pollicis brevis muscle.

be stored in long term memory almost permanently (Cowan, 2008). As the research in memory matured, more types of memory are specified like working memory (Baddeley & Hitch, 1974), autobiography memory (Conway, 2005) and prospective memory (Einstein & McDaniel, 2005). However, this dissertation focuses on declarative and procedural memories and only these will be discussed in depth.

1.2.1 Long term memory

Long term memory can hold information from hours to years and is further elaborated into the two generally accepted domains termed declarative or explicit and nondeclarative or implicit memories (see **Figure 1**). Declarative memory refers to remembering specific events (episodic) or facts and information about the world (semantic) whereas nondeclarative memory refers to other forms of learning such as conditioned response, skills, and priming (Squire & Zola, 1996). Tulving (1985a) advocated that semantic, episodic and procedural memories differed in the acquisition, representation, and expression of knowledge. For example, while procedural memory is associated with anoetic consciousness (non-knowing), semantic memory is associated with noetic consciousness (knowing) and episodic with autonoetic consciousness (self-knowing) upon acquisition (Tulving, 1985b). It must, however, be borne in mind that the memory systems and their associated brain functions are seemingly more complex and interactive than previously assumed (Henke, 2010). Specifically, declarative and procedural memory will be discussed in the following sections.

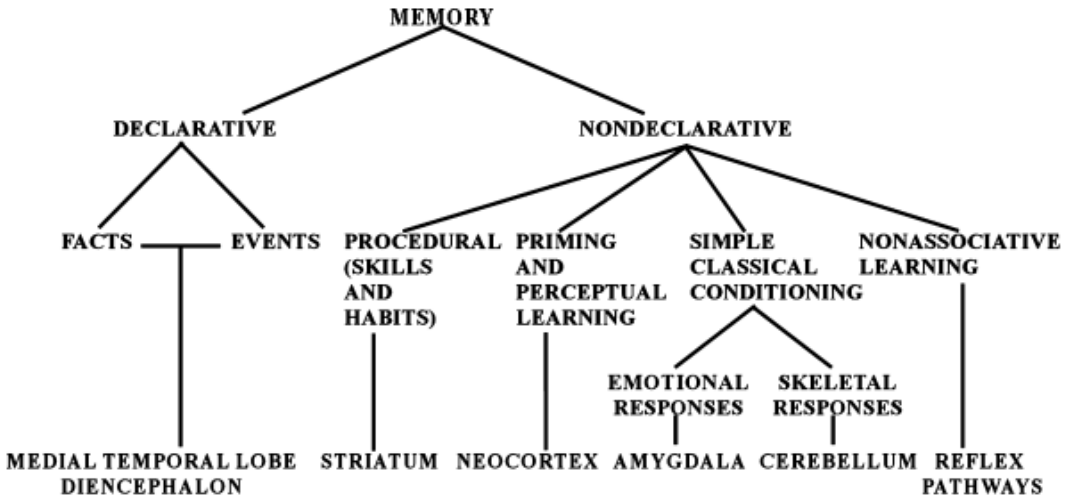

Figure 1. A taxonomy of long-term memory system in mammals and their associated brain regions. (reprinted from Squire, 2004, with permission from Elsevier).

1.2.1.1 Declarative memory

Declarative memory is the composition of semantic and episodic memory. As above mentioned, semantic memory refers to factual information about the world that is explicitly acquired whereas episodic memory underpins personal past experiences or episodes, where one can relive the past and use the information for future planning (Tulving, 1985). Nevertheless, the process of semantic and episodic memories is not exclusively independent of each other.

Neuroanatomically, the medial temporal lobe is profoundly involved in the formation of declarative memory. This was revealed by studies on patients with bilateral medial temporal lobe resections (known as H.M. and D.C.), where these patients could no longer form new memory despite their intelligence and intact remote memory (Scoville & Milner, 2000). Subsequent neuroimaging studies have likewise indicated that medial temporal lobe, including the hippocampus and parahippocampal gyrus, is essential for declarative memory (Eichenbaum, 2000; Murty *et al.*, 2010).

1.2.1.2 Procedural memory

The key feature of procedural memory is the lack of awareness while acquiring information, in which one is not consciously aware of the formation of memory (Squire & Zola, 1996). A good example of procedural memory is knowing how to ride a bike, one can easily perform the task without being able to explicitly elaborate on the detailed procedure. Explicit learning will, however, be partially involved in acquiring a procedural memory (Diekelmann *et al.*, 2009). It was proposed that motor skill learning is based on three stages: cognitive (skill acquisition), associative (repeat practice), and autonomous (automated execution of a skill) (Saywell & Taylor, 2008). Furthermore, procedural memory entails different components within the same hierarchy such as priming, classical conditioning, skills and habit and non-associative learning (Squire, 2004). These components also possess different neurobiological properties. For instance, conditioning learning was associated with the amygdala (Bechara *et al.*, 1995); motor learning involves motor, supplementary motor, prefrontal, and premotor cortex as well as the striatum (Grafton *et al.*, 1995; Vahdat *et al.*, 2017; Bönstrup *et al.*, 2019).

1.3 Sleep

Sleep is a consistently reoccurring behaviour within the 24 hour activity cycle (Saper *et al.*, 2005) and it encompasses a stereotype position upon its occurrence, is characterized as a reversible state (unlike coma) and is accompanied by a loss of consciousness along with reduced responsiveness to external stimuli (Kleitman, 1963; Berry *et al.*, 2017). Sleep has been observed in species from taxonomy ranging from invertebrates to mammals (Kushida, 2012). This phenomenon reflects a fundamental and yet vital function of sleep in the light of evolution. To engage in sleep aquatic mammals as the dolphin have evolved "uni-hemisphere sleep" wherein one hemisphere reveals SWS while the other remains in wakefulness (Mukhametov *et al.*, 1977; Oleksenko *et al.*, 1992). Indeed, prolonged sleep deprivation or perturbation leads to a number of cognitive impairments (Nebes *et al.*, 2009) and increases the risk factor of cardiovascular, inflammatory and metabolic consequences (Mullington *et al.*, 2009). In addition, studies point toward sleep as relevant to consolidation of memory, a crucial function for adapting behaviour to future needs for survival (see **section 1.3.2**).

1.3.1 Sleep brain rhythms (Polysomnography)

Electrophysiologically, sleep is categorized into different sleep stages based on the expression of different brain rhythms, muscle activity, and eye movements, also widely known as polysomnography (Berry *et al.*, 2012). According to the American Academic of Sleep Medicine (AASM), sleep is divided into three non-rapid eye movement (NREM) stages: stage 1 (N1), stage 2 (N2), stage 3 (N3), and rapid-eye-movement (REM) sleep (Berry *et al.*, 2017) (see **Figure 2**). Stage N2 is characterised by the emergence of sleep spindles, while commencing high amplitude slow frequency oscillations are characteristic of N3, but occur already in N2. REM sleep is characterized by low muscle tone and is dominated by lower amplitude desynchronized oscillations as well as phasically occurring rapid eye movements (Iber & Iber, 2007). Human sleep usually undergoes the transitions Wake, N1, N2, N3, REM sleep and fluctuates again between NREM and REM sleep. This dissertation focuses on stages N2 and N3 of NREM sleep, hence only brain rhythms during these stages will be subsequently discussed.

1.3.1.1 *Slow wave activity*

Slow wave activity (SWA) is comprised of two main components: SO and delta activity. During the SO (~ 1 Hz), the membrane potential of cortical neurons alternate between hyperpolarisation (neural silence) and depolarisation (when firing can occur) at approximately 1 Hz (Steriade *et al.*, 1993) (see **Figure 2**). Neuroanatomically, the origin of SO is located in the cortex, evidenced by the absence of SO in a decorticated cat (Timofeev & Steriade, 1996). Furthermore, it was found to appear more pronounced at the insula and cingulate regions in humans (Murphy *et al.*, 2009). In addition, not only the SO was found to behave as a travelling wave that propagates in an anteroposterior pattern (Massimini *et al.*, 2004) it also travels preferentially in the mesial part of the brain at a speed of around 2.2 m/s (Murphy *et al.*, 2009).

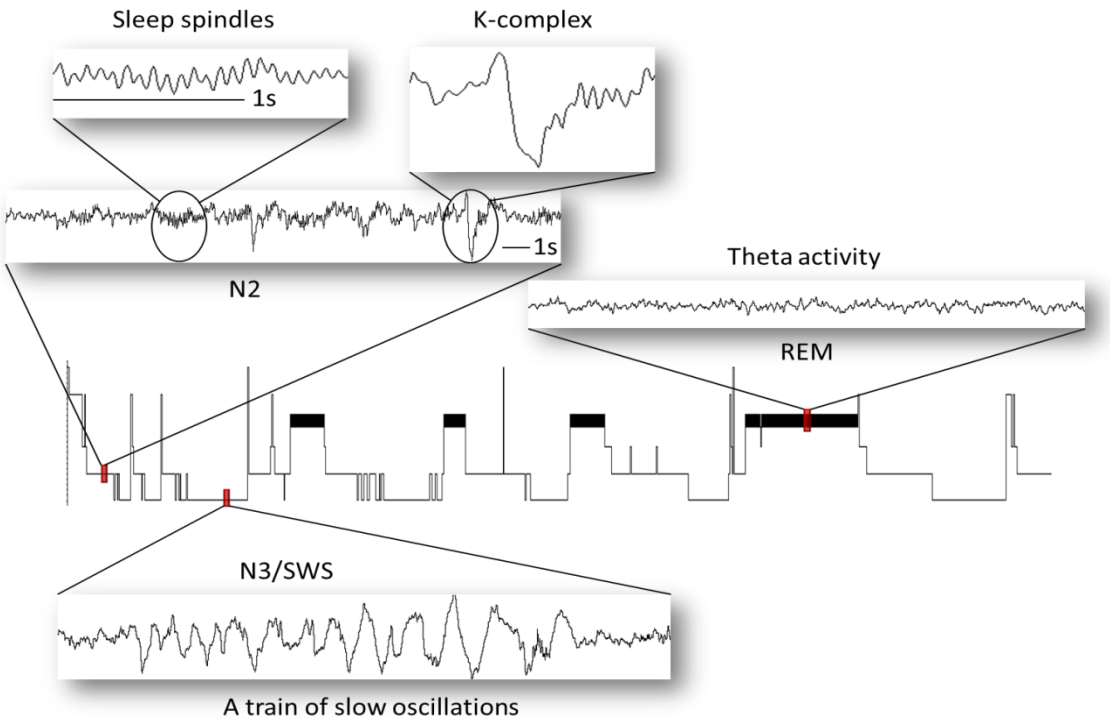

Figure 2. A hypnogram of a healthy young adult during nocturnal sleep. The red rectangles indicate N2, N3(SWS) and REM sleep characteristic in 30 sec time window, respectively.

The KC occurs mainly during N2 and lies within the SO frequency range. It has a unique waveform, namely, a sharp negative deflection that is usually larger than 100 µV, followed by a slower positive wave (Cash *et al.*, 2009) (see **Figure 2**). The KC was found to associate with various brain regions including the thalami, temporal frontal, parietal, and occipital lobes (Caporro *et al.*, 2012). KC is claimed to represent an isolated cortical down-

state (Cash *et al.*, 2009) and was recently found to be generated at the anterior cingulate cortex (Ioannides *et al.*, 2019). It has reached a consensus that KC´s amplitude peaks over the prefrontal and frontal area (McCormick *et al.*, 1997; Bellesi *et al.*, 2014). Moreover, KCs are also found to be inducible by external stimuli of different modalities with responses largest for auditory stimuli (Colrain, 2005).

Delta activity occurs within the frequency range of 1-4 Hz (Rasch & Born, 2013). A simultaneous EEG and fMRI study has demonstrated whereas SO was associated with parahippocampal gyrus, cerebellum, and brainstem, delta wave was related to frontal responses (Dang-Vu *et al.*, 2008). A recent animal study has just revealed a competing role between SO and delta in memory consolidation, suggesting that delta promotes forgetting, and reactivation during SO leads to memory consolidation in rats (Kim *et al.*, 2019). However, the ultimate function of delta remains elusive.

1.3.1.2 Sleep spindles

Sleep spindles are uniquely found only during sleep and are one of the hallmarks of N2 (see **Figure 2**) but also occur during N3. They have a waxing and waning pattern (De Gennaro & Ferrara, 2003) and last about 0.5-3 s (Lüthi, 2014). The generation of a spindle is the result of an interplay between GABAergic reticular neurons and excitatory TC cells (Steriade *et al.*, 1985). Sleep spindles could appear either as an isolated discrete spindle or are grouped by KC or SO (Steriade, 1999). One key role of spindles attributes to the sensory gating, in which spindles reduce the sensory transmission during sleep to protect sleeping individual from disruptive environmental noise (Dang-Vu *et al.*, 2010).

Formerly, it was observed that the frequency of spindles ranged from 12-14 Hz but it was later reported that another slower frequency oscillation (9-12 Hz) exists (De Gennaro & Ferrara, 2003). The two spindle types differ in several aspects such as topography, temporal grouping with SO or KC, and functionality. On the topographical aspect, slow spindles were found to occur preferentially over the frontal region whereas fast spindles prevail over centro-parietal regions (Mölle *et al.*, 2011). On the temporal grouping of SO, slow spindles couple favourably at the transition from up to down-state of a SO cycle

whereas fast spindles occur predominantly at the transition from down to up-state (Mölle *et al.*, 2011).

While fast spindles were observed to be prevailing during sleep stage N2, slow spindles occurred more frequently during SWS (Tamaki *et al.*, 2009). Schabus *et al.* (2007) used the fMRI technique to detect sleep spindle activity and found that slow spindles were exclusively associated with increased cortical activity over the frontal gyrus while fast spindles are associated with the sensorimotor regions, mesial frontal and hippocampus activity. One study administered carbamazepine (antagonist of voltage-dependent Na^+ channels) prior to sleep and revealed an enhanced slow spindle activity conjoint with increased SO power during post-medication sleep whereas fast spindle activity was decreased, underlining a distinct generating mechanism of the two types of spindles (Ayoub *et al.*, 2013). On the other hand, the relevant functions of fast spindles not only have been found to associate with motor memory consolidation (Barakat *et al.*, 2011; Lustenberger *et al.*, 2016) but are also linked to intelligence (Bódizs *et al.*, 2005; Schabus *et al.*, 2006; Fogel & Smith, 2011). Nevertheless, findings and concepts on fast and slow spindles are not cohesive and are open for debate (Gonzalez *et al.*, 2018) and the general function of slow spindles remains elusive.

Despite the potential distinction between spindle types and their functions, a handful of studies on spindle activity have indicated that spindles are closely associated with general mental and cognitive ability in adults (Bódizs *et al.*, 2005; Schabus *et al.*, 2006; Fogel & Smith, 2011) as well as in children (Hoddes *et al.*, 1972; Chatburn *et al.*, 2013; Hoedlmoser *et al.*, 2014). This suggests an underlying inter-individual difference in spindle activity that is dependent on cognitive ability (see **Hypothesis 1b**) may be a biomarker for intelligence.

1.3.2 Sleep and memory consolidation

Without much understanding of the electrophysiological mechanism behind sleep and memory, sleep has already been shown to be preventive to memory decay in the 1920s. This result was reported in a study by Jenkins and Dallenbach (1924), where they instructed two participants to alternatively stay awake and sleep on two sessions

subsequent to Ebbinghaus's learning method. They showed that after sleep, the decay of remembering was reduced compared to staying awake after learning. However, in this study, sleep was regarded as having a passive function on memory (i.e. preventing memory decay).

Some decades later, as the understanding of sleep (e.g. different stages of sleep) and of memory (e.g. distinct types of memory) advanced on their own extent, it emerged that different sleep stages and brain rhythms contribute to the consolidation of distinct types of memory (Diekelmann & Born, 2010). For instance, visual discrimination skills improved specifically after SWS-rich early sleep whereas no improvement was found when sleep was only limited to late sleep, where REM sleep is dominant (Gais *et al.*, 2000). Performance on declarative memory tasks (i.e. word paired list) was improved after early sleep while procedural memory (mirror tracing) was improved after late night sleep (Plihal & Born, 1997). Further studies have then explored the role of sleep stages and their associated electrophysiology for specific types of memory (Walker & Stickgold, 2006).

In particular, sleep and memory consolidation have been extensively studied. Memory consolidation refers to a process that strengthens a memory trace after its initial acquisition and integrates such new information into the pre-existing knowledge (Diekelmann & Born, 2010). Marr (1971) first proposed a two-stage model in which the newly learned information is stored parallel into a temporary store in the hippocampus and into a long-term store in the neocortex, and that sleep plays a role in redistributing those memory traces.

Explicitly for declarative memory, active system consolidation was proposed as such that three cardinal neural oscillations occurring during SWS facilitate the consolidation process - SO in the neocortex, which is the long term store, drive the TC spindles, while the hippocampal sharp-wave ripple nests synchronously at the troughs of spindles (Born & Wilhelm, 2012) (see **Figure 3**).

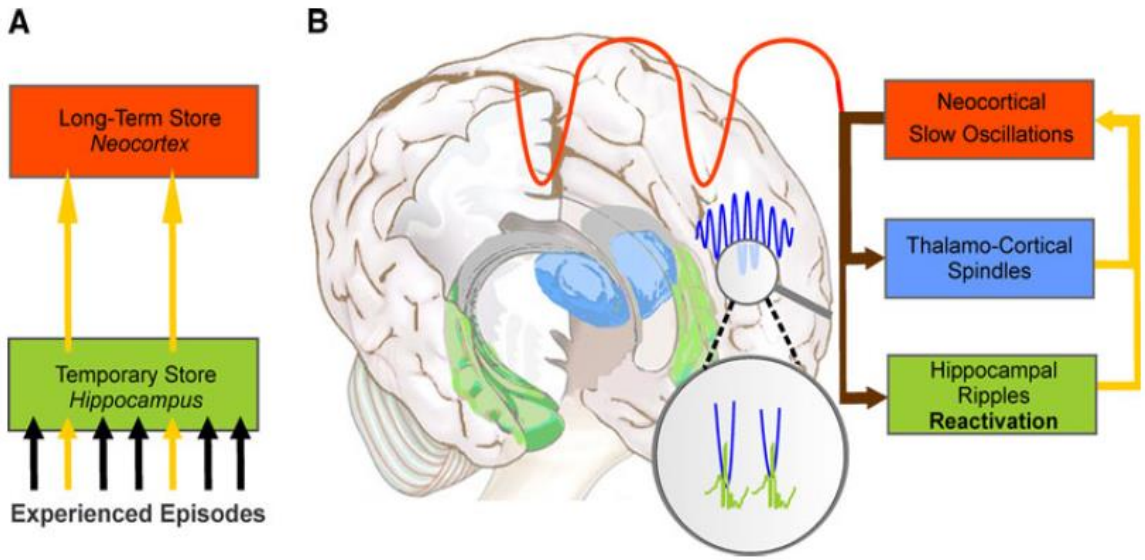

Figure 3. Active memory consolidation system. A. Information is encoded into the temporary store in hippocampus and is redistributed to long-term store in neocortex. B. Different oscillations that occur in distinct brain regions and their temporal relationship with each other (reprinted from Born & Wilhelm, 2012; under the permission of Creative Commons Attribution Noncommercial License (https://creativecommons.org/licenses/by-nc/2.0)).

Several studies consistently showed SO, as well as spindle activity is associated with memory consolidation (Rasch & Born, 2013). A conceivably direct evidence of this causal relationship is implied by studies that experimentally induced SO or spindles, and in turn improved the consolidation process reflected by enhanced recall performance (Ngo *et al.*, 2013a; Ngo *et al.*, 2015; Lustenberger *et al.*, 2016; Ong *et al.*, 2016; Ong *et al.*, 2018). A recent meta-analysis investigating the effect of transcranial electrical stimulation application during sleep and its impact on memory showed that so-tDCS applied during SWS had a beneficial effect on declarative memory consolidation but not on procedural memory (Barham *et al.*, 2016). A correlation between the length of SO up-state and the retention performance in declarative tasks further confirms the role of SO in memory consolidation (Heib *et al.*, 2013). Additionally, many studies also placed the emphasis on the importance of spindles by indicating a positive correlation between the retention of declarative memories and spindle activity (Schabus *et al.*, 2004; Clemens *et al.*, 2005; 2006; Schmidt *et al.*, 2006). Besides the apparent function of spindles in declarative memory, ample evidence also indicated that spindles are correlated with procedural

memory (Walker *et al.*, 2002; Walker *et al.*, 2003; Nishida & Walker, 2007; Tucker & Fishbein, 2009).

Another fundamental aspect of the electrophysiology of sleep and memory is the grouping of brain rhythms (Steriade, 2006). A substantial amount of studies have focused on the temporal coordination between these oscillations, further supporting their role in information transfer from the hippocampus into the neocortex (Siapas & Wilson, 1998; Clemens *et al.*, 2007; Wierzynski *et al.*, 2009; Rasch & Born, 2013; Niknazar *et al.*, 2015). Along this line, a number of studies have demonstrated a direct linkage between the coupling of SO and TC sleep spindles with memory consolidation (Clemens *et al.*, 2007; Mölle & Born, 2011; Cox *et al.*, 2012a; Maingret *et al.*, 2016; Muehlroth *et al.*, 2019) as well as a correlation between grouping hippocampal sharp-wave ripples and spindles with successful memory consolidation (Clemens *et al.*, 2011).

1.3.3 Sleep and learning

In early research of sleep and learning, researchers attempted to induce learning during sleep but to no avail (Simon & Emmons, 1956; Bruce *et al.*, 1970), suggesting learning should only take place during wakefulness. Numerous studies later focused on sleep preceding or subsequent sleep to wakeful learning and showed a profound mutual relationship between sleep and learning. For instance, several studies illustrated that learning led to subsequent modifications of brain rhythms during post-learning sleep (Huber *et al.*, 2004; Mölle *et al.*, 2004; Fogel & Smith, 2006; Schmidt *et al.*, 2006; Fogel *et al.*, 2007b) and some studies additionally indicated positive correlations between learning intensity and SWA or spindles (Schmidt *et al.*, 2006; Mölle *et al.*, 2009).

According to the synaptic homeostasis hypothesis, sleep also benefits subsequent learning. The hypothesis put forth by Tononi and Cirelli suggests that wakefulness leads to an increase in synaptic weight in the cortical circuits and this synaptic potentiation will result in an upsurge of homeostatic regulation, expressed as an increase in SWA, thereby promoting a downscaling of synapses and their depotentiation to a baseline level (Tononi & Cirelli, 2003; Tononi & Cirelli, 2006) (see **Figure 4**).

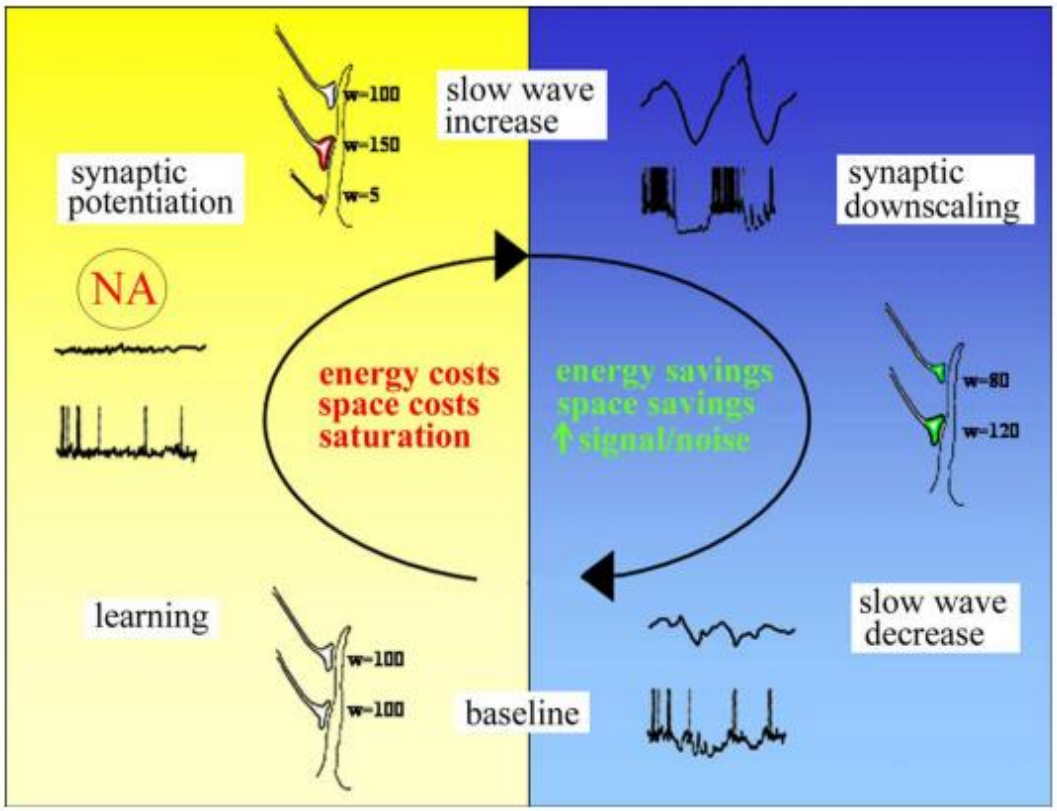

Figure 4. The synaptic homeostasis hypothesis. The left panel represents daytime wakefulness where learning takes place and in turn results in synaptic potentiation, evidenced by the increase of noradrenaline (NA) that is associated with LTP (marked yellow); this increase of synaptic potentiation further leads to the upsurge of SWA. On the right panel, synaptic downscaling take place as SWA occurs (marked blue). As the synapses downscaled and return to baseline, SWA decreases (reprinted from Tononi & Cirelli, 2006, with permission from Elsevier).

This theory can be supported parsimoniously by the shreds of evidence in which prior learning increased SWA (Huber *et al.*, 2004) and enhanced coherence between SO and higher frequency band activity during post-learning sleep (Mölle *et al.*, 2004). Analogously, depression of synaptic strength induced experimentally during learning led to a decrement of SWA in following sleep (Huber *et al.*, 2006). Meanwhile, a few studies, albeit scarce, have reinforced this hypothesis by showing sleep prior to learning is essential to form new memories. Particularly, when Yoo *et al.* (2007) compared the episodic encoding between a sleep-deprived group and a sleep control group, they showed a significantly worse performance in the sleep-deprived group along with a decrease in hippocampal activation upon encoding as compared to the sleep control group. Similarly, a study introduced sleep perturbation during SWS to simulate shallow

sleep in the elderly found reduced encoding performance. This impairment was associated with reduced hippocampal activation compared to a normal sleep session (Van Der Werf *et al.*, 2009). Furthermore, in a study comparing conditions with or without a nap prior to learning, the group that was given nap opportunity showed better learning ability than the group without a nap. Moreover, the learning ability was positively correlated with sleep spindles activity (Mander *et al.*, 2011). Remarkably, interventions that enhanced SWA using so-tDCS during SWS similarly revealed better learning capacity (Antonenko *et al.*, 2013) and enhanced SO by ACLS was positively correlated with hippocampal activity (Ong *et al.*, 2018). Nevertheless, the manipulation of SO and spindles as an event and its relationship with declarative memory encoding were not addressed, leaving a gap of understanding how augmenting SO and spindles could lead to enhancement of learning (see **Hypothesis 3c**).

1.4 Quiet Waking state

The quiet waking state as referred to in this dissertation is equivalent to resting state by reason of separating the utilization of EEG from functional magnetic resonance imaging (fMRI). Quiet waking state alludes to a state of wakeful relaxation with the absence of external distraction and involvement in any particular task (Vago & Zeidan, 2016). Since the invention of EEG by Berger (1929), it was then reported that the brain does not halt upon resting. Clearly, wakeful relaxation does not mean the mind ceases to wander. Instead, it involves various thoughts jumping from one to another including self-reflection, evaluation, and mood shifting (Smallwood *et al.*, 2009; Killingsworth & Gilbert, 2010; Vago & Zeidan, 2016). All these thought processing have been reflected in fMRI studies that showed activation of the associated functional network during resting state, termed default mode network (DMN) (Gusnard *et al.*, 2001; Raichle *et al.*, 2001; Beer, 2007; Mason *et al.*, 2007).

1.4.1 Quiet Waking brain rhythms

Dependent on the brain state and cognitive load, delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (>30 Hz) can be observed during wakefulness (Buzsaki, 2006). Specifically, these oscillations do not occur in an isolated fashion, instead, they

appear in an amalgamated manner within a dynamical large-scale network (Mantini et al., 2007). Similar to sleep brain rhythms, the coupling between oscillations in distinct frequencies as well as in different brain regions was also observed during wakefulness and such coupling has been shown to serve useful information transformation (Mölle et al., 2002a; Chik, 2013). For instance, the coupling between theta and gamma oscillations was stronger when successful encoding took place compared to a weaker coupling for forgotten items (Friese *et al.*, 2013). Conceivably, the intriguing rhythms among these oscillations are the alpha and theta bands, where oscillations in these frequency ranges occur during wakefulness as well as during sleep. For example, activity in the alpha frequency range dominates during the wakeful eyes closed state but it is also prominent during N2 during sleep (Cantero *et al.*, 2002). Similarly, theta dominates during REM sleep but it is also present over anterior midline during waking state (Smith et al., 2019) and during post-learning waking state (Moisello et al., 2013). Though, despite the coincidental frequencies, studies have shown the derivation of such oscillations differed greatly. Specifically, spindles are a consequence of the interplay between GABAergic reticular neurons and excitatory TC cells (see **section 1.3.1.2**) whereas wakeful alpha is largely the contribution of cortical activity (Da Silva & Van Leeuwen, 1977; Cantero et al., 2002).

1.4.1.1 Alpha rhythm

As mentioned above, alpha activity is tightly associated with resting EEG, given that it was the first human brain rhythm disclosed by Hans Berger. Berger (1929) reported the EEG he recorded on humans having around 10-12 waves within 1 s during an eyes-closed state and the amplitude of such oscillations attenuated following eye-opening. The terms event-related synchronization (ERS) and event-related desynchronization (ERD) were later introduced to describe alpha synchronization, where large population of cortical neurons synchronizes and thus contribute to alpha maximal amplitude upon eyes-closed while desynchronization was found to be a consequence of disparate cortical neuronal activation that temper the amplitude as a result of eyes opening (Pfurtscheller, 1997). It was first assumed that alpha was a mere idle state of the brain; however, it emerged that alpha is essential for a functioning brain (Başar, 2012). One aspect is that alpha

oscillations consist of two components: induced vs. spontaneous; whereby induced alpha by eyes state (i.e. eyes closed or open) is associated with activation across anterior superior temporal sulcus, supplementary motor area, and hippocampus; spontaneous alpha resembles the activation of DMN (Ben-Simon *et al.*, 2008). Another aspect proposed is that alpha is a rather global distributed dynamic system that plays a role in integrating diverse brain functions (Başar *et al.*, 1997; Nunez *et al.*, 2001). In short, alpha is a dominant widespread rhythm that serves multiple functions during the quiet waking state.

1.4.2 The quiet waking state and its function

As quiet as it sounds, a quiet waking state may have just implied an idle or a standby mode that does not serve a purposeful function. However, as already introduced in the sections above, the rhythms that occur during quiet waking state seemingly serve an indispensable purpose. One essential function is memory consolidation, akin to the function of sleep, evidenced by improved retention after a short period of wakeful eyes-closed period (Albert *et al.*, 2009a). Despite the little attention paid to the role of quiet waking state for memory consolidation, studies using fMRI have unfalteringly shown the relevant function of memory consolidation during resting state (Rosazza & Minati, 2011). For instance, Sami *et al.* (2014) showed an increase of task-specific sensory-motor loop connectivity accompanying improved serial reaction time task performance. They could additionally see a shift of network activity across time, suggesting relevant functional memory consolidation took place over time. Furthermore, Brokaw *et al.* (2016) recorded EEG from participants after verbal learning and found an increase in SO activity concomitant to decreased alpha activity during the eyes-closed resting state, and memory performance was improved afterward. On the other hand, one study employed so-tDCS during post-learning quiet wakefulness, however with eyes open, did not find an impact on memory consolidation despite induced SO activity (Kirov *et al.*, 2009). Similarly, fMRI obtained during post-motor learning resting state with eyes open failed to observe memory consolidation despite increased connectivity (Gregory *et al.*, 2014). Thus, these findings suggest that the presence of relevant brain rhythms as well as the associated

functional networks is crucial for memory consolidation during the quiet resting state. Moreover, the closed eyes state which blocks external stimuli seems to play a key role during this process.

1.5 Aims and hypotheses

As outlined in the previous section, NIBS is an ideal method to modulate EEG activity. NIBS can potentially be used to study relationships between putative mechanisms of endogenous electrophysiological activity and behaviour, due to its presumed ability to modulate specific oscillations. However, NIBS has revealed inconsistent outcomes and thus introduces uncertainty in its efficacy. Taken together, the majority of the studies found anodal so-tDCS to be beneficial in modulating EEG and behaviour while some studies found no such effect (see **section 1.1.1**). One plausible explanation could be a different number of tasks used prior to sleep between studies. As already illustrated in section Sleep and learning (**1.3.3**), learning can modify post-learning brain rhythms of sleep. It is hence postulated that learning prior to sleep will significantly modify post-learning network activity (**Hypothesis 1a**) and thus introduce a bias in the efficacy of so-tDCS (**Hypothesis 1b**). Furthermore, as the cognitive ability has shown relations to fast spindle activity (see **section 1.3.1.2**) and so-tDCS modulates fast spindle activity, it could be expected that such inter-individual differences could influence the effect of so-tDCS (**Hypothesis 1c**).

In contrast to the many studies that employed so-tDCS in sleep (Zhang & Gruber, 2019), the attempt to investigate effects of so-tDCS during wakefulness is scarce. Considering SO serves to synchronize ongoing brain rhythms, the application of so-tDCS during wakefulness may induce SO, despite the stimulation frequency deviating from ongoing brain oscillations (**Hypothesis 2a**). Alternatively, resonance of the network to so-tDCS may become expressed in a brain rhythm of different frequency (see **section 1.1.1**). Furthermore, in order to induce comparable brain states during quiet waking between sessions and participants (Zhu *et al.*, 2010), a behavioural task previously demonstrated not to be affected by so-tDCS (Barham *et al.*, 2016) would be employed (**Hypothesis 2b**).

ACLS delivered with high temporal precision in-phase with the SO depolarisation up-state produces an evoked slow wave-like response (**Hypothesis 3a**). According to the synaptic homeostasis theory (see **section 1.3.3**), SWA is essential for synaptic downscaling. Therefore, it is conceivable that the SO and spindle that occur during SWA may to some extent contribute to post-sleep learning (**Hypothesis 3c**). Moreover, among all the ACLS studies conducted so far (see **section 1.1.2**), no study has attempted to disentangle the effect of ACLS during the electrophysiologically different NREM sleep stages of N2 and N3 (i.e. KC vs. SO) (see **section 1.3.1.1**) (**Hypothesis 3b**). Putative modulations of electric brain activity (i.e. ERP) during subsequent learning will be investigated in an exploratory manner.

Taken together, the overarching aim of this dissertation is to study both potential confounds (i.e. network modification by learning and differential sleep stages) affecting efficacy of NIBS, inter-individual differences on NIBS efficacy, and to also use NIBS as a tool to gain further insight into the electrophysiology of sleep and waking brain rhythms and their relevant behavioural functions. This dissertation aims to answer the following questions:

1. Is the efficacy of so-tDCS during sleep dependent upon the susceptibility of the underlying network (or 'network state') and is its efficacy subjected to the influence of inter-individual difference?

Study I

Hypothesis 1a: Learning can induce network modification that is reflected in post-learning EEG during sleep

Presuming learning leads to network modification, then

Hypothesis 1b: So-tDCS efficacy is dependent upon such task-induced network modifications

Hypothesis 1c: So-tDCS efficacy is dependent upon an inter-individual network component

2. Can SO be induced by so-tDCS during eyes-closed wakefulness where endogenous SO activity is absent and does so-tDCS enhance activity in other related frequency bands?

Study II

Hypothesis 2a: So-tDCS can induce EEG frontal SO and theta activity

Hypothesis 2b: So-tDCS has no effect on the procedural behavioural task performance

3. Does enhanced SO activity during NREM sleep improve subsequent encoding performance and does the effect of ACLS differ in stages N2 and N3?

Study III

Hypothesis 3a: Auditory stimulation delivered in-phase with the SO depolarisation up-state (ACLS) will induce SOs, slow and fast spindle activity in NREM sleep

Hypothesis 3b: ACLS will have a unique effect on SO, slow and fast spindles in N2 compared to N3

Hypothesis 3c: If ACLS induces SO and spindles, encoding capacity will be enhanced

1.5.1 Approaches

Study I: In order to check for network modification induced by learning in Hypothesis 1a, sleep EEG parameters (EEG power and discrete spindle analyses) after learning (3 declarative tasks and 2 procedural tasks; "SHAM") and control (watching a documentary; "CTRL") were compared (see **section 2.1.2** and **section 2.1.12**). The declarative tasks (see **section 2.1.4**) were used to cover different declarative mechanisms and procedural tasks (see **section 2.1.5**) were used as control. To test for Hypothesis 1b, the sleep EEG parameters between so-tDCS ("STIM") and no stimulation ("SHAM") after learning were compared (see **section 2.1.12**). Hypothesis 1c was investigated by splitting the total participants into high and low memory quotient groups, according to the general memory quotient medium score and examining EEG parameters and behavioural tasks separately in both groups (see **section 2.1.6** and **section 2.1.12**).

Study II: Hypothesis 2a was tested by comparing EEG power during quiet wakefulness between so-tDCS ("STIM") and no stimulation ("SHAM") (see **section 2.2.6**) and

Hypothesis 2b was examined by comparing performance on the behavioural task (see **section 2.2.3** and **section 2.2.6**).

Study III: To test for Hypothesis 3a, the averaged SO amplitude, slow and fast spindle power in NREM sleep time-locked to the ACLS ("STIM") and virtual markers placed time-locked to the detected SO (SHAM) were compared (see **section 2.3.7** and **section 2.3.9**). Hypothesis 3b was investigated by examining averaged SO, slow and fast spindle activity separately for N2 and N3 between conditions (see **section 2.3.9**). Hypothesis 3c was tested by comparing encoding performances between STIM and SHAM (see **section 2.3.9**).

2. Methods

2.1 Study I

Question: Is the efficacy of so-tDCS during sleep dependent upon the susceptibility of the underlying network (or 'network state') and is its efficacy subjected to the influence of inter-individual difference?[3]

2.1.1 Participants

Participants were recruited through flyers advertised on the university-hospital campus. Included participants were non-smoker, right-handed, had no history of psychopathological disorders, had no metallic device implanted in the body, were free from medication, and had regular sleep rhythm. Due to one of the memory quotient tests involved in learning Turkish, participants who also speak Turkish were excluded. All participants who first met our inclusion criteria that were obtained via telephone and questionnaire went through an adaptation night in the sleep lab. Participants could further proceed to the experimental nights when they (1) had a sleep profile within the norm; (2) had a recordable biological-EEG signal; (3) had at least one cycle of sleep (≥ 30 min NREM sleep). Latency to SWS of less than 20 min and at least one hour of NREM sleep within the first 90 min after lights off during the adaptation night were further prerequisites. Considering hormones play a significant role in sleep as well as in memory consolidation (Genzel et al., 2012), only females on contraceptive pills were included. A total of 25 participants (male: 10; female: 15) entered the final analysis (19 to 26 years, mean ± SEM: 22.4 ± 2.12).

2.1.2 Procedures

Participants visited the laboratory in total five times. The first time served as an adaptation where participants were briefed on the protocol and their sleep architecture under the laboratory condition was evaluated. Three separate experimental sessions took place following the adaptation. One session consisted of memory tasks followed by sleep

[3] This study was published under Koo, P.C., Mölle, M. & Marshall, L. (2018) Efficacy of slow oscillatory-transcranial direct current stimulation on EEG and memory - contribution of an inter-individual factor. *Eur J Neurosci*, **47**, 812-823.

and stimulation ("STIM"), another session consisted of memory tasks followed by sleep only ("SHAM"), and another session served as control sleep ("CTRL"; without both memory tasks and stimulation). All sessions were conducted at least seven days apart. Stimulation and SHAM nights were pseudo-randomly assigned to either the first or last session while control night consistently took place in the second session. This arrangement setup served to prolong the interval between learning sessions in order to decrease potential carry-over learning effect from the memory tasks. The last session consisted of the memory quotient test in the afternoon. All participants signed the consent form prior to the experiment. This study was approved by the local ethics committee of the University of Luebeck, Germany (15-060).

During the experimental sessions, participants arrived in the laboratory at approximately 19:30. EEG application started at around 20:00 and lasted about an hour. Participants were prompted to perform a 5 min psychomotor vigilance test (PVT) (Roach *et al.*, 2006) as well as to fill out the Stanford sleepiness scale (SSS) (Hoddes *et al.*, 1972), positive and negative affect schedule (PANAS) (Watson *et al.*, 1988) (see **section 2.1.3**) prior to and after the memory tasks for psychometric monitoring. Five memory tasks consisted of three declarative tasks (word-paired associate, figural-paired associate, 2D-object location) and two procedural tasks (finger sequence tapping and mirror tracing) took place subsequently (learning phase). Participants were given at least 7 hours of sleep after the memory tasks. Once sufficient sleep was obtained, participants were awakened the next morning and were given 45 min of freshening time to reduce sleep inertia before they proceeded to the memory recall (see **Figure 5**).

2.1.3 Psychometric measurements

The PVT is a customized reaction time task. Participants were to focus on a black screen with a fixation in the middle of the screen and they had to press the spacebar as soon as a stimulus was presented. The stimulus remained on the screen until a response was obtained. Once a response was obtained, the stimulus disappeared. The interval between

stimuli was randomized. SSS is a self-rating scale consisting of eight progressive ordinal numbers where one corresponds to feeling active, vital, alert or wide awake and eight corresponds to feeling asleep. Participants were to choose one item out of these orders that best described their sleepiness at the present moment when they filled out the questionnaire. The raw number that participants rated was taken to analyse their sleepiness, thus the higher the number, the sleepier they are. PANAS scale comprises ten positive and ten negative adjectives. Participants were to rate each item within the range of 1–5 (with 1 indicating very slightly or not at all, and 5 indicating extremely). Examples of adjectives are, *interested*, or *enthusiastic* for positive affection and *nervous*, or *distressed* for negative affection. Participants were again instructed to rate these items based on the present moment when they filled out the questionnaire. The positive and negative items were then averaged separately to obtain single positive and negative values before and after the stimulation for analysis.

2.1.4 Declarative memory tasks

2.1.4.1 Word-paired associate task

The word-paired associate (WPA) task was performed using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). Participants were shown a pair of German words (cue-target), for instance, "*Garten* – *Salat*", for 4 s with 1 s transition to the next pair. The learning consisted of a total of 80 word-pairs with a 2 min break in between the first 40 pairs. After learning, participants were to immediately recall the target word verbally after each cue appeared on the screen (immediate recall) and the same procedure applied during the recall the next morning (delayed recall). Participants received feedback (correct pair) after each response while no feedback was given during the delayed recall. The meaning of the paired words was semantically associated and hence, the participants were instructed to think of a story to connect the two words in order to limit the use of various strategies. There was no time limit during both recall phases. In both recall sessions, only the number of correct words was considered for

analysis. Further analysis of absolute retention was obtained using the formula (delayed recall-immediate recall) (see **Supplementary Figure 1**).

2.1.4.2 Figural-paired associate task

The figural-paired associate (FPA) task consisted of 16 pairs (cue-target) geometric or non-geometric lines in which participants were prompted the cue and had to choose the target in a recognition fashion (blended with 7 other figures). Each pair appeared 5 s on the screen with 1 s inter-stimulus interval. Immediate recall with feedback but without time limit took place after learning. A minimum of 60 % learning criterion (10 pairs) was required during the immediate recall to assure a certain amount of encoding. The number of correct pairs was averaged across trials if participants took more than one trial to reach the criterion. Absolute and relative retention was calculated in the same way as for the WPA (see **Supplementary Figure 2**).

2.1.4.3 2D object location

This task involved 15 pairs of different objects located at different positions on a 6 x 5 array using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). Two identical objects (cue-target) located at separated positions were presented simultaneously for 1 s with an interstimulus interval of 900 ms. Participants had to remember the locations of the paired objects during learning. Immediate recall took place subsequent to the end of learning. Upon immediate recall, participants were prompted the cue object and had to recall the corresponding target location by choosing one of the 30 matrices. The location of each pair was presented again after each response. This feedback was not given during the delayed recall session the next morning. At least 60 % of correct responses were required during the immediate recall (see **Supplementary Figure 3**).

2.1.5 Procedural memory task

2.1.5.1 Finger sequence tapping task

This finger sequence tapping task (FSTT) was adapted from previous work by Walker *et al.* (2002). Participants had to type a sequence of five numbers repeatedly (range from 1-4) shown on a monitor as quickly and accurately as possible using the non-dominant hand (little finger to the index finger (1-4)). For the learning session in the evening, 12 blocks of learning (1 block = 30 s learning + 30 s break) took place whereas during the recall the next morning, participants had to repeat the identical procedure for only 3 blocks. This task was performed using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). The number of the correctly typed sequence was used for analysis (see **Supplementary Figure 4**).

2.1.5.2 Mirror tracing (MT)

The mirror tracing task was performed on an auto-scoring mirror tracer device (Model 58024A*C, Lafayette Instrument Company, Lafayette, IN). The device is made up of a mirror that stands perpendicular to an aluminium plate with a paper-made thin outline figure on top. A conductive stylus is attached to the aluminium plate and both the plate and stylus are connected to an error counter. Participants had to trace a complete figure with the stylus through the reflection of the mirror, in which the coordination of their hand was inversely seen. An error was counted when the stylus contacted the plate (i.e. out of the paper-cut figure). The learning part required participants to trace a simpler figure in less than 1 min under 12 errors. The procedure repeated until these criteria were reached. During the test part, participants had to trace another figure with more diverse angles for four times individually without any criteria. They were however instructed to trace the figure as quickly and accurately as possible. The delayed test during the next morning is identical to the test part. The time of completing the figure and the number of errors were used for analysis (see **Supplementary Figure 5**).

2.1.6 General memory quotient (MQ)

A standard German Learning and Memory Test battery was used to assess a memory quotient (Bäumler, 1974). Raw scores of 6 subtests of learning and recall of verbal and figural content within given time limits were transformed into weighted t-weighted value points to obtain the general MQ (sum of all; Figural quotient = 1st & 6th subtests; Verbal quotient = 2nd, 4th & 5th subtests). Participants were first asked to learn on all tests and subsequent recall took place according to the learning order. The first subtest involved memorizing a given route from location A to location B on a map within 1 min. Upon recall, participants were required to draw the route on the same map within 2 min. The second subtest required participants to learn 20 pairs of German-Turkish words (e.g. *Gehilfe – muavin*) in 1 min and in the recall phase, to choose the correct Turkish word out of five options corresponding to the cue German word within 4 min. In the third subtest, 20 objects in black and white were simultaneously presented and participants had 1 min to memorize as many items as possible. They were later given 2 min to write down as many of these items as possible. In the fourth subtest, participants were given 2 min to remember 13 place-number pairs, (e.g. *Post – 989*). During recall, participants were given 2 min to write down the corresponding number for the given place. The fifth subtest required participants to read a short article and remember as many details as possible within 1 min. At recall, they had 4 min to answer 21 short questions in written form (each answer was either a number or a name). The last subtest consisted of remembering the association between company logos and their frame within 1 min. It comprised 20 black and white logos; each logo was either an object or a big letter in the middle of a frame. At recall, participants had to choose the correct frame among three other frames corresponding to the company logos within 4 min.

2.1.7 EEG data acquisition

EEG was acquired with a DC amplifier SynAmps RT (Compumedics Neuroscan, Charlotte, USA) with a 500 Hz sampling rate, low pass filtered at 200 Hz, a gain of 10 dB, an amplitude resolution of 32-bit float values, and an accuracy of 29.80 nV/LSB. Signals were

recorded from Fp1, Fpz, Fp2, F7, Fz, F8, C3, Cz, C4, P3, Pz, P4, A1 and A2 referenced to the nose (international 10:20 system) using an EASYcap (EASYCAP GmbH, Herrsching, Germany) with Ag/AgCl sintered ring electrodes. A1 and A2 were located slightly above the stimulation return electrodes. The vertical and horizontal electrooculograms (EOG), as well as electromyogram (EMG), were recorded for polysomnographic scoring. A ground electrode was positioned 1-2 cm below Fpz. Impedance was kept below 5 kOhm for all electrodes.

2.1.8 SO-tDCS

First, an individual slow oscillatory frequency that ranges from 0.7 to 1.2 Hz was obtained from the first cycle of NREM during the adaptation night for each participant (mean frequency: 0.84 ± 0.02 Hz). The stimulation consisted of five blocks of five min so-tDCS, each block followed by one min stimulation free interval. Two active Ag/AgCl stimulation electrodes (8 mm diameter) were placed at bilateral frontal – F3, F4 (10/20 international system) and the return electrodes were placed at the bilateral mastoid (impedance < 1 kOhm). The active and return electrodes were ipsilaterally referenced (see **Figure 6C**). A battery-driven constant current stimulator delivered the currents that oscillated between 0 and 300 µA (J = 0.5968 mA/cm²) via two synchronized circuits. The anodal oscillatory currents flowed in a trapezoid shape with equally long plateaus and identical rising and falling slopes. Stimulation started four min (approximately 8 epochs of 30 sec window) after the first sleep spindle or KC occurred, however, if movements or any artefact occurred within these two mins, the count was reset. The same procedure applied to the SHAM session except only two oscillations were given at the end of each five min block (see **Figure 5**).

2.1.9 Polysomnographic analysis

The AASM manual (Berry *et al.*, 2012) was used as a guideline for polysomnographic scoring. Sleep stages wake, stage N1, N2, N3, and REM sleep were determined

accordingly by two independent scorers. The term NREM sleep was used here to describe stages of N2 and N3[4] from analysis.

2.1.10 EEG power analyses

Acquired data were imported into Spike2 (Cambridge Electronic Design, version 8, Cambridge) for analysis. Data were filtered high pass at 0.159 Hz and low pass at 33 Hz, re-referenced to linked-mastoids, and subsequently downsampled to 100 Hz for analysis. Epochs with artefacts were excluded for analysis.

Power spectra were calculated using fast Fourier transformation (FFT) in windows of 1024 points with 50 % overlapping Hanning windows. The following frequency bands were analysed for comparisons between CTRL and SHAM: SO (0.5–1.5 Hz), SWA (0.5–4 Hz), Delta (1.5–4 Hz), Theta (4–8 Hz), slow spindle (9–12 Hz), fast spindle (12–15 Hz) and Beta (15–25 Hz). Comparisons between STIM and SHAM were limited to SO, SWA, fast and slow spindle frequency bands.

Spectral power for STIM and SHAM conditions was assessed at two separate time periods: (1) During the acute period of stimulation, i.e. spectral power was averaged across the five 1-min stimulation free epochs and temporally comparative epochs in SHAM (see **Figure 5**); (2) All NREM sleep epochs in a post-stimulation time period of 150 min, starting immediately after the termination of stimulation or sham-stimulation and ending approximately at the end of the second sleep cycle (147.54 ± 33.05 min). Spectral power for CTRL was obtained from time periods corresponding to the acute and post-stimulation periods.

2.1.11 Discrete spindle analysis

Each discrete spindle was identified automatically throughout NREM sleep within the 150-min period following STIM and SHAM. The detection algorithm was similar to the one employed by Mölle *et al.* (2002b). In general, the detection started with the identification

[4] N3 is interchanged with SWS throughout the results and discussion.

of individual peak frequencies for each participant (average frequency of fast spindle 13.65 ± 0.11 Hz; slow spindle 11.78 ± 0.15 Hz across all participants). Then, the EEG signal was filtered with a bandpass width of 3 Hz centered on the detected individual peak frequency. Subsequently, a root-mean-square (RMS) representing the filtered signal was calculated using a sliding window of 0.2 s with a step size of one sample, corresponding to 10 ms. Additional smoothing was performed with a sliding-window average of the same 0.2 s size. Time frames were considered as spindle intervals if the RMS signal exceeded a threshold of 1.5 standard deviations of the filtered signal that lasted for 0.5-3 s. Individual spindle detection thresholds were derived from the control night (endogenous brain rhythm) for each participant and EEG channel. Statistical analyses on discrete spindles included spindle count, density, mean peak to peak amplitude, mean length, and mean spindle RMS within the post-stimulation period.

2.1.12 Statistical analysis

To examine the susceptibility of the underlying neural network (**Hypothesis 1a**), repeated-measures ANOVAs (rmANOVAs) with the factors condition (COND) with levels SHAM and CTRL, and scalp topography (TOPO) with Fp1, Fpz, Fp2, F7, Fz, F8, C3, Cz, C4, P3, Pz, P4, were employed for each frequency band separately (see **section 2.1.10** for defined bands). The factors COND and TOPO were similarly used to examine the stimulation effect on EEG power (STIM vs. SHAM) (**Hypothesis 1b**). EEG analyses focused on two time periods, the acute period of stimulation (mean of five 1-min stimulation intervals) and the 150-min post-stimulation period described above (see **Figure 5**). Statistical analyses of discrete spindles similarly employed rmANOVAs with the factors condition, COND (STIM and SHAM), and corresponding scalp topography, TOPO. Due to the sparse number of discrete spindles in the 1-min epochs, analyses were conducted for the 150-min time period only.

Behavioural performance was analysed using factors COND (SHAM, STIM) and TIME (immediate recall, delayed recall) in rmANOVAs for all memory tasks separately.

Differences in retention performance (delayed recall - immediate recall) between SHAM and STIM were assessed by post-hoc paired-sample t-tests without multiple comparison correction.

To investigate the effect of inter-individual general MQ on so-tDCS efficacy (**Hypothesis 1c**), a mixed ANOVA including GROUP as between-subject factor (high MQ and low MQ) (median = 118) was applied for both EEG power and behavioural analysis. Relationships between MQ and stimulation efficacy on behaviour were investigated by Pearson's correlation. Stimulation efficacy was calculated for EEG and behaviour parameters as the difference between STIM and SHAM conditions (STIM-SHAM).

All ANOVA results were Greenhouse-Geisser corrected for violation of the sphericity assumption (uncorrected degrees of freedom are given). All values are given in mean ± SEM. A value $p < 0.05$ was considered significant. Moreover, considering that spindle activity may be a potential biomarker for intelligence, an independent sample t-test was employed in the CTRL session to assess if there is a group difference in spindle numbers. Bonferroni correction was used to control for type I error, resulting in an adjusted p-value of 0.004 as a true significant value for the independent sample t-test.

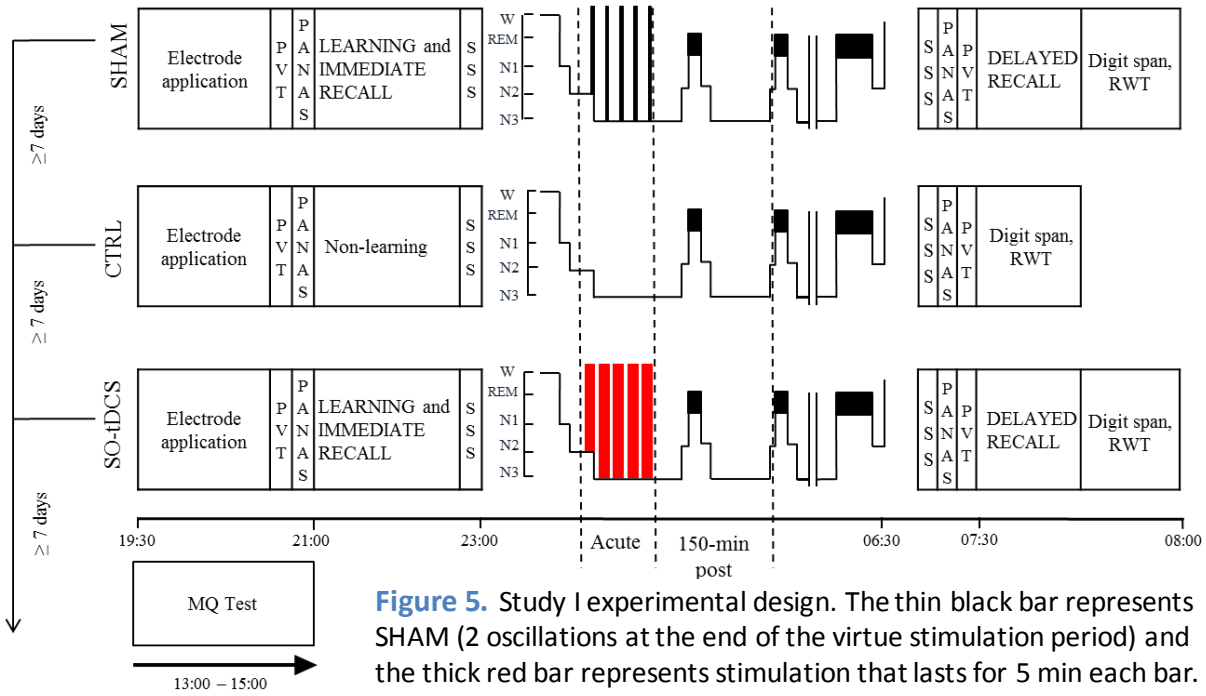

Figure 5. Study I experimental design. The thin black bar represents SHAM (2 oscillations at the end of the virtue stimulation period) and the thick red bar represents stimulation that lasts for 5 min each bar. RWT: Regensburg word fluency test

2.2 Study II

Question: Can SO be induced by so-tDCS during eyes-closed wakefulness where endogenous SO activity is absent and does so-tDCS enhance activity in other related frequency bands?[5]

2.2.1 Participants

The inclusion and exclusion criteria were identical to those in Study I (see **section 2.1.1**) aside from the sleeping parameter, which was not a prerequisite for including the participants.

2.2.2 Procedure

Participants came for an adaptation followed by two experimental sessions. During the adaptation session, participants arrived in the laboratory at 09:00. After EEG application participants were to sit upright in a relaxed position with their eyes closed in a well-lit room and to listen to exerts from two instrumental music pieces during a 30 min EEG recording period. After the session, the participant chose one of the two pieces for both experimental sessions.

The two experimental sessions were at least 7 days apart. On these experimental days, participants arrived in the laboratory at 09:00 and the procedure of applying electrodes for EEG recording and so-tDCS electrodes began. Participants filled out the SSS and PANAS questionnaires (see **section 2.1.3**) prior to and after the EEG recording. To induce comparable vigilance states and cortical activity across sessions and participants (see **section 1.5**), a FSTT (see **section 2.2.3**) was given prior to the EEG recording. The total EEG recording period lasted 60 min. Subsequent to about a 2 min settle-down and 15 min baseline period, a 30 min period followed in which either stimulation ("STIM") or sham-stimulation ("SHAM"; see **section 2.2.4**) was applied followed by another 5 min of EEG recording. The music started at the same time as the EEG recording. The two conditions were pseudo-randomized and counterbalanced across participants and

[5] This study was part of the paper published under Koo-Poeggel, P., Böttger, V. & Marshall, L. (2019) Distinct Montages of Slow Oscillatory Transcranial Direct Current Stimulation (so-tDCS) Constitute Different Mechanisms during Quiet Wakefulness. *Brain Sciences*, **9**, 324.

sessions. EEG, EOG, and EMG were monitored continuously so that at any indication of drowsiness, a soft noise was introduced to maintain wakefulness. After the recording period, participants were retested on the FSTT and again filled out the SSS and PANAS (see **Figure 6**).

After each experimental session, participants were asked: "Did you feel anything on the scalp or elsewhere?", and "Do you think this was a stimulation session?" to assess blinding of the stimulation. All participants signed a consent form prior to participation. The study was approved by the local ethics committee of the University of Luebeck, Germany (14-057).

2.2.3 Finger sequence tapping task

The FSTT was identical to the one described in Study I (see **section 2.1.4.1**). In short, training consisted of 12 blocks of learning each lasting 30 s and followed by a 30 s break interval. The retest, after the stimulation period, consisted of another three blocks of retrieval with the same sequence (see **Supplementary Figure 4**). The mean of the last 3 blocks of learning and the mean of the 3 blocks at retest was used for ANOVA analysis (see **section 2.2.7**).

2.2.4 EEG data acquisition

The electrophysiological activity was acquired using a DC amplifier SynAmps RT (Compumedics Neuroscan, Charlotte, USA) with a 2 kHz sampling rate, low pass filtered at 800 Hz, with a gain of 10 dB, an amplitude resolution of 32-bit float values, and accuracy of 29.80 nV/LSB. EEG was recorded using 12 Ag/AgCl sintered electrodes from F7, Fz, F8, FCz, C3, Cz, C4, P3, Pz, P4, T5 and T6 (see **Figure 6B**), referenced to the nose and ground on the Fpz (International 10:20 system). To detect muscle and eye movement artefacts and to assess vigilance, submental EMG, vertical EOG from bipolar electrodes placed supraorbital and infraorbital, and horizontal EOG from the outer canthus of each eye were recorded. Impedance was kept below 5 kOhm for all electrodes.

2.2.5 SO-tDCS

The stimulation parameters employed in this study were similar to the one in Study I (see **section 2.1.7**). In short, sinusoidal shape anodal so-tDCS was delivered bilaterally at dorsolateral frontal locations, F3, F4 of the international 10:20 system, with return electrodes placed at the ipsilateral mastoid, in 5 epochs of 5-min followed by a stimulation free epoch of at least 1 min. The first stimulation epoch began once a 15 min period of quiet wakefulness without any major movement artefacts occurred. In the SHAM session, at the end of a virtual 5 min stimulation block, two subsequent SO cycles were delivered (see **Figure 6A**). The currents were delivered by a customized battery-driven constant current stimulator with two synchronized circuits. Impedance was kept ≤ 1 kOhm. The stimulation electrodes consisted of Ag/AgCl sintered cup electrodes of 8 mm in diameter (see **Figure 6C**). The sinusoidal stimulation current (0.75 Hz) oscillated between 0 µA and a maximum amplitude of 260 µA. Calculated current density of each pair corresponding to 0.5172 mA/cm² and the total maximum current applied was 0.52 mA.

2.2.6 EEG power analyses

The acquired EEG data were pre-processed using Brain Vision Analyser (Gilching, Germany). Data were first low pass filtered using an infinite impulse response (IIR) at 30 Hz and high passed at 0.3 Hz (12 dB/oct), followed by down-sampling to 200 Hz based on spline interpolation. Independent component analysis (ICA) was applied to remove ocular artefacts (Makeig *et al.*, 1996) and corrected data were re-referenced to the average reference. Movement artefacts were excluded by manual visual inspection at 1 s epoch. Following artefact removal, data were extracted into eight 1-min epochs: baseline 1 and baseline 2 (15-14 min and 2-1 min prior to onset of the stimulation period, respectively), 5 stimulation-free 1-min periods, and 1 post-stimulation period (4-5 min after termination of the last stimulation).

Power spectra were obtained for all recording locations using FFT. Each 1-min epoch was segmented into 10 s windows, with 5 s overlap. Segments were zero-padded to the

length of 10.24 s, with a Hanning window used for data tapering. Since previous studies have found changes in theta (Kirov *et al.*, 2009) and alpha power (D'Atri *et al.*, 2016) after the registration of so-tDCS aside from SO power, the following frequency bands were analysed: SO (0.7-1.2 Hz), Theta (4-8 Hz), and Alpha (8-12 Hz).

2.2.7 Statistical analyses

For EEG power analysis, rmANOVAs using the factors: condition (COND) with levels SHAM and STIM; time (TIME) with the baseline-normalized five stimulation-free epochs and the post-stimulation epoch; and scalp topography (TOPO) with F7, Fz, F8, FCz, C3, Cz, C4, P3, Pz, P4, T5, T6 were used for each frequency band separately (**Hypothesis 2a**). For post-hoc tests, comparisons between conditions at pooled topography frontal (F7, Fz, F8, FCz), central (C3, Cz, C4), temporal (T5, T6) and parietal locations (P3, Pz, P4) (see **Figure 6B**) were conducted.

The FSTT, SSS, PANAS-Positive, PANAS-Negative were analysed using factors COND and TIME (pre-stimulation, post-stimulation). Retention of FSTT was obtained from the formular (Retest – Training) (**Hypothesis 2b**).

All ANOVA results were Greenhouse-Geisser corrected for violation of the sphericity assumption (uncorrected degrees of freedom are given). All values are given in mean ± SEM. A value $p < 0.05$ was considered significant.

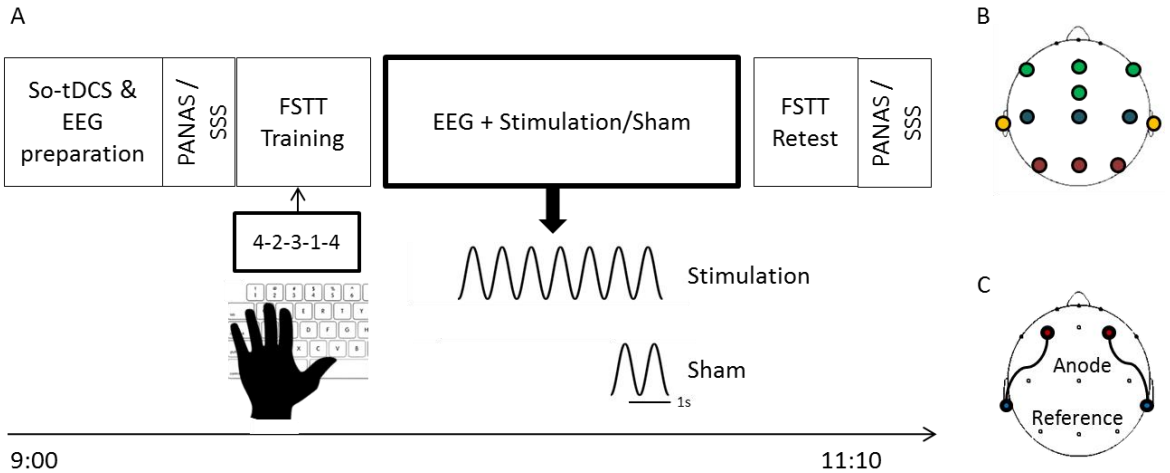

Figure 6. A schematic view of the experimental procedure. (A) Time line of the experimental procedure. (B) Locations of EEG electrodes. Circles with different colour represent pooled electrodes for Frontal (green), Central (blue), Parietal (red) and Temporal (yellow). (C) Stimulation electrodes. Red and blue circles represent the active and return electrodes, respectively.

2.3 Study III

Question: Does enhanced SO activity during NREM sleep improve subsequent encoding performance and does the effect of ACLS differ in stages N2 and N3?

2.3.1 Participants

Young healthy individuals aged between 23 and 30 years were recruited through flyers advertised around the campus. The inclusion and exclusion criteria were similar to the criteria in Study I and II. In short, left-handed, presence or history of any form of sleep disturbance or cognitive impairment, any psychopathological disorder, seizures or brain injury, metal or cardiac pacemaker implantation, pregnancy and intake of medication except for contraceptive pills were exclusion criteria. There were in total 21 participants recruited. Nine of them were excluded due to the following reasons: one could not sleep at all during the adaptation; two because they started night shifts in between sessions; one due to a technical problem; five did not sleep during one of the two sessions. This resulted in a total of 12 participants who completed the study. Three subjects were further excluded for analysis as they did not go into the N3 stage in one of the two experimental sessions. This resulted in 9 participants who went into SWS sleep on both sessions (mean age: 26.58 ± 0.80; male: 4; female: 5) and the data of these 9 participants were used for analysis. Due to a technical failure during one session in one participant, the sleep EEG was excluded for analysis in this session (SHAM) for this particular participant.

2.3.2 Procedure

A single-blind crossed-over design was employed in this study (see **Figure 8**). Participants took part in two experimental sessions, preceded by an adaptation, and followed by a session for assessing intelligent quotient (IQ) score using the Advanced Progressive Matrices (Raven & Raven's Progressive Matrices, 1994) and MQ performance (Bäumler, 1974) (see **section 2.1.6**). Upon arrival on the adaptation and experimental sessions at 12:30, EEG application took place and participants were allowed 90-120 min to sleep starting at 14:00. For the adaptation, participants were shown a simplified version of the

learning tasks after the nap and were then allowed to leave the laboratory. During the experimental sessions, a 30 min interval was given between the nap and learning tasks to avoid sleep inertia. Following learning, a short break filled with a light dinner took place and participants were subsequently prompted to perform a delayed recall on the same tasks. Participants were allowed to leave the lab after the recall (see **Figure 8**). An actiwatch (Resperonic Philips, Massachusetts, USA), a tracker that consisted of accelerometer and light sensor was given to participants at least two days prior to the adaptation as well as to the experimental session to monitor their sleep/wake time. The two experimental sessions were pseudo-randomized and counterbalanced across participants and sessions. All participants were informed about the study and signed a consent form prior to participation. The study was approved by the local ethics committee of the University of Luebeck, Germany (18-176).

2.3.3 Declarative memory tasks

2.3.3.1 Word paired-associate Encoding

The WPA task was performed using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). Participants were shown in total 100 pairs of word, each pair of words consisted of a cue word and a target word and they were semantically not associated, for example, "*Frage – Anfang*". The pair was shown for 3 s with 500 ms inter-stimulus interval. After encoding, participants had to recall the target word when the cue word was presented on the screen without a time limit. This procedure was repeated 5 times resulting in 5 blocks. Feedback was neither given at learning nor at recall. During the delayed recall, participants were again prompted the cue word and they had to verbally recall the target word without time pressure. The percentage of correct words for the immediate and delayed recall was taken for analysis. Absolute retention (delayed recall-immediate recall) was also used for analysis (see **Supplementary Figure 6**).

2.3.3.2 Figural paired-associate Encoding

This task was performed using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). The figural-paired associate task had 16 pairs of figures; each pair consisted of a cue figure and a target figure that was made up of geometric or non-geometric lines. During the encoding, each pair appeared 3 s on the screen with 500 ms inter-stimulus interval. Following learning, the cue figure was presented and participants had to choose the target figure in a recognition fashion (blended with 7 other figures) without feedback and time pressure. The procedure repeated once with the same pairs which resulted in two learning blocks (see **Supplementary Figure 7**).

2.3.3.3 Verbal learning memory test (VLMT)

The verbal learning memory test is the German version of the Rey Auditory Verbal Learning Test. It was likewise performed using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). A standard word list consisting of 15 words (nouns) was orally presented three times, with each run followed immediately by a free recall. Participants had to verbally recall as many of the presented words as possible during each free recall. Words from all lists were presented each for 1 s by a pre-recorded neutral male voice. Participants had unlimited time to recall the words. Immediately after the third run, a second list of 15 semantically unrelated words different from those in the standard word list (i.e., the interference list, IL) was presented in the same way as the standard list, and again the participant had to recall as many of the words as possible. After the IL, participants were requested to recall again the 15 words from the standard list, now without prior presentation of the list words. Performance measures were the number of correctly recalled words.

2.3.4 Procedural memory task

2.3.4.1 Mirror Tracing (MT)

The procedure of the MT was similar to the one described in Study I (see **section 2.1.5.2**) with a slight adjustment of the criteria. In short, to emphasize the learning component,

the participants were required to trace a simpler figure in less than 40 s with less than 6 errors consecutively for four times. The procedure repeated until the criteria were reached. During the test part, participants had to trace another figure with more diverse angles for four times individually without any criteria. They were however instructed to trace the figure as quickly and accurately as possible. During the recall, participants had to trace the figure only once. The time and number of errors were used for analysis (see **Supplementary Figure 5**).

2.3.5 EEG data acquisition

EEG was acquired using a compact wireless EEG amplifier (Brain Products GmbH, Gilching, Germany). An elastic cap with 32 Ag/AgCl sintered-EEG electrodes (see **Figure 7**) mounted on it along with 2 auxiliary bipolar channels (EMG, horizontal EOG) attached to an additional trigger box were used. The signal was recorded with a 500 Hz sampling rate, filtered at 0.03 Hz and 80 Hz along with a notch filter at 50 Hz, and an amplitude resolution of 0.0407 µV. All the unipolar channels were online referenced to FCz and a ground electrode was positioned at Afz. Impedance was kept below 10 kOhm for all electrodes upon acquisition.

2.3.6 Auditory stimulus

The auditory stimulus was the composition of two pink noise bursts ($1/f$, termed “clicks”) that lasted 50 ms with 5 ms rising and falling times each. In general, the stimulus started at the lowest volume at 47 dB and increased every 2 steps (i.e. 49 dB, 51 dB, 53 dB, 55 dB, 57 dB) after 10 stimuli were obtained at each level. The maximal volume was adjusted to each participant´s threshold as such that if participants showed high frequency low amplitude EEG right after the clicks (e.g. 55 dB), the maximal volume was set to two steps lower (e.g. 53 dB). This gradual increase served to reduce the participants´ responsiveness to the stimuli. After each experimental session, participants were asked whether they heard anything throughout the sleep, and whether they thought it was a stimulation or SHAM session.

2.3.7 Online detection of SO and closed-loop stimulation

In order to perform the closed-loop stimulation, a parallel EEG recording system entailing an EEG amplifier (Digitimer, Hertfordshire, United Kingdom) and a high-performance data acquisition interface (Cambridge Electronic Design, Cambridge, England) was used. For real-time SO detection, Fpz electrode referenced to linked mastoids along with a ground placed over the left cheek was recorded with a sampling rate of 200 Hz. Using this configuration, two virtual EEG channels were created: channel 1 consisted of 0.25-4 Hz filter and channel 2 consisted of 0.25-15 Hz filter. Impedance was kept below 5 kOhm. A customized script written in the built-in script language of Spike2 (Cambridge Electronic Design, Cambridge, England) was used to detect real-time occurring SO. Two criteria had to be met in order for this program to trigger the clicks (termed "STIM") or virtual markers (termed "SHAM"): (1) a negative SO surpassing the amplitude of -80 µV at channel 1; (2) reach the pre-defined delta/theta power ratio threshold at channel 2. The delta/theta ratio was obtained from the adaptation session for each participant. During the adaptation, virtual markers were placed online once a negative SO amplitude surpassed -80 µV and the delta/theta ratio reach the default setting of 20. Each stimulus entailed two clicks with a distance of 1075 ms. The next SO detection resumed 2.5 s after the second click (see **Figure 7**).

An average delay time from the SO down-state to SO up-state was then calculated from all the markers obtained from the adaptation for each participant. This customized timing was used to increase the likelihood that the stimulus would occur in-phase with the SO up-state. Furthermore, EEG acquired from the adaptation was sleep scored offline and the ratio between delta and theta (delta power/theta power) during each sleep stage was calculated. The frequency of each ratio occurring during NREM (i.e. N2 and N3) and the remaining stages (Wake, N1 and REM) was then plotted and the interchange point between the NREM and the remaining stages was defined individually per participant as the NREM threshold (see **Supplementary Figure 8**). During the experimental sessions, SO detection was initiated manually after the delta/theta ratio remained constantly beyond

the pre-defined threshold for 4 min. Upon detection of a SO, the first stimulus was delivered after the individualized time delay. The procedure was identical in the SHAM session, apart from only markers but no actual auditory stimuli were delivered. The two conditions were pseudo-randomized and counterbalanced across sessions.

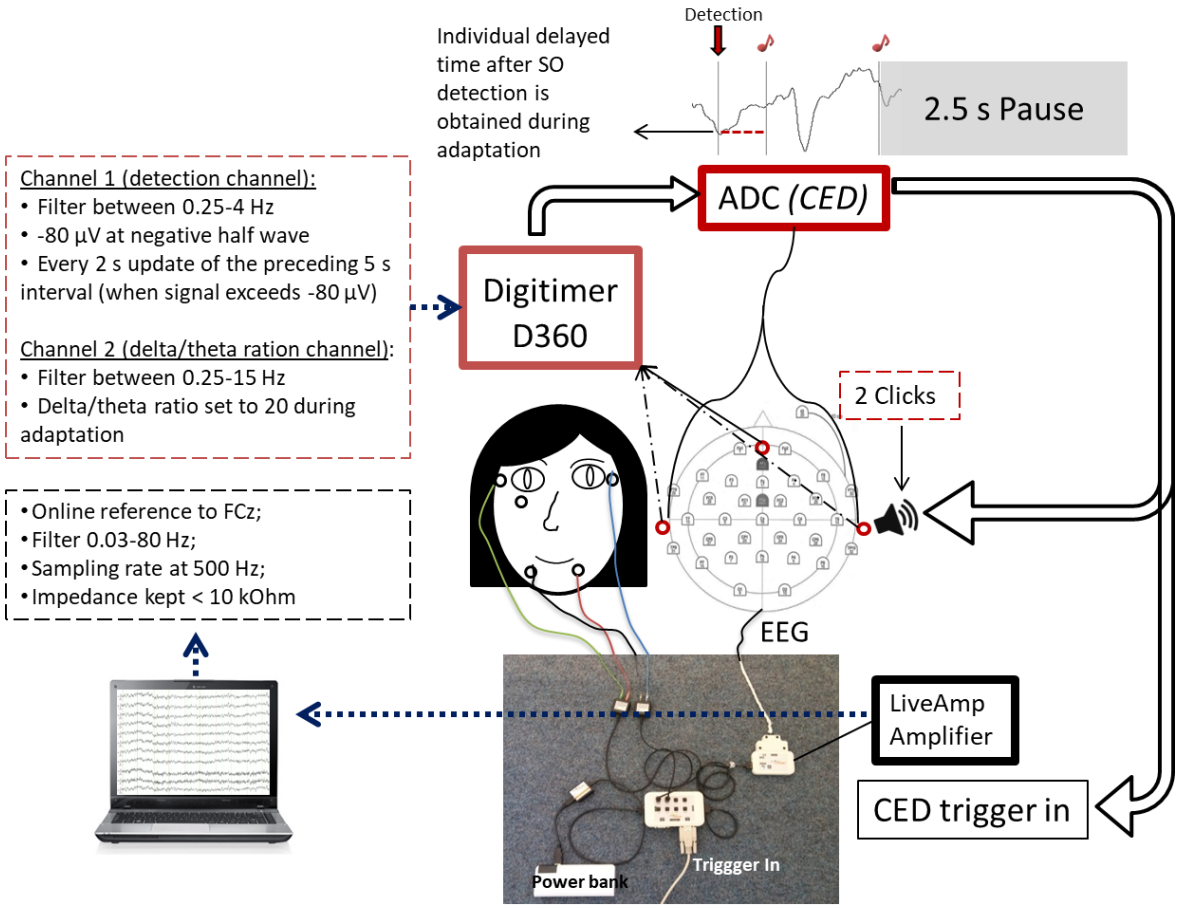

Figure 7. Schematic illustration of the parallel closed-loop and EEG recording setup**.** All red rectangles are part of the closed-loop system while the black rectangles are part of the LiveAmp EEG recording system. The signal of electrode Fpz referenced to the linked-maistoids (open red circles on the scalp topography) was recorded using Digitimer D360 amplifier along with data acquisition interface (CED) for SO detection. Based on the given parameters and pre-defined criteria (left red rectangle), a detected SO would trigger the Digitimer through CED and thus deliver the clicks through in-ear headphone. At the same time, CED sent the trigger markers to LiveAmp so that relevant stimulation epochs could be extracted for analysis. On the EEG recording system, a wireless amplifier was attached on the participants via a breast-belt. The Bluetooth was connected to the one on laptop for visualization.

2.3.8 EEG analyses

The acquired EEG data were offline re-referenced to linked TP9 and TP10 channels and Fp2 channel was referenced to right EOG channel that was built-in on the cap to create a

virtual vertical EOG channel, using Brain Vision Analyser (Brain Products GmbH, Gilching, Germany). Data obtained from sleep were then exported into Spike2 (Cambridge Electronic Design, Cambridge, England) for further analysis as well as for polysomnography evaluation using SleepPilot [6] (https://github.com/xuser/SleepPilot, v0.9.4-beta). Polysomnography was evaluated by two raters who were blinded to the conditions according to the guideline of AASM (see **section 2.1.9**). The learning EEG was then preprocessed in Brain Vision Analyser.

2.3.8.1 SO and spindles ERP analysis

In order to assess the effects of ACLS, ERP analyses in SO, slow and fast spindles similar to the one described in Ngo *et al.* (2013a) was performed. In short, EEG signals were downsampled to 100 Hz and low pass filtered at 35 Hz. Windows time-locked to the first click that comprised of 1 s pre-click and 4 s post-clicks were extracted from artefact-free epochs in N2 and N3. To examine the ACLS effect during NREM, the windows in both N2 and N3 were averaged. To investigate the differential ACLS effect in N2 and N3, windows were averaged separately in N2 and N3. The same procedure applied to SHAM condition, where the EEG was averaged across markers.

Spindles were obtained as following: low pass at 35 Hz; bandpass at 9-12 Hz (slow spindle) and 12-16 Hz (fast spindle) separately; calculated root mean square (RMS) with 0.1 s time constant for both spindles. Identical to the windows in SO, spindles RMS time-locked to the first click were averaged for N2 and N3 separately.

2.3.8.2 EEG power spectral analyses

Power spectra were obtained in artefact-free NREM periods for all recording sites using FFT of 10.24 s with 50 % overlapping Hanning windows, resulted in 0.098 Hz frequency resolution. The following frequency bands were analysed: SWA (0.5-4 Hz), Delta (1-4 Hz), Theta (4-8 Hz), slow spindles (9-12 Hz), and fast spindles (12-16 Hz).

[6] SleepPilot is a java based semi-automatic sleep scoring program written by a former colleague Dr. Arne Weigenand.

2.3.8.3 Event-related potential (ERP) and time-frequency representation (TFR)

As no previous studies investigated the ACLS effect on subsequent learning, an accompanying ERP and wavelet analyses in FPA was performed here. The preprocessing steps were taken as following: re-referenced to mean TP9 and TP10 electrodes, ICA calculation for ocular artefact removal (Makeig *et al.*, 1996), low pass filter at 45 Hz, manual artefact rejection in a 1-s epoch, high pass filter at 0.6 Hz, and lastly rereferenced to average reference. ERP analysis with a window of -100 ms to 1500 ms time-locked to each stimulus was computed. The ERP was further baseline normalized by subtracting the EEG signal 100 ms prior to the stimulus. Additionally, TFR was calculated using the Morlet wavelet transformation. A window of -500 ms to 2500 ms was used to avoid the smearing effects in the time of interest. The number of wavelet cycle used range from 3 Hz to 30 Hz at 1 Hz logarithmic frequency step. Spectral power was computed at each frequency and each time bin. Baseline normalization was not applied in TFR due to the insufficient reference interval (see **Supplementary note – Study III**).

2.3.9 Statistical analysis

The effect of SO and spindles RMS induced by ACLS was directly compared between STIM and SHAM conditions (**Hypothesis 3a**) using a running unequal variance Welch's t-test due to imbalance number of participant between condition (see **section 2.3.1**). The p-value was adjusted by Benjamini-Hochberg correction (p-value rank/number of test*0.05) (Benjamini & Hochberg, 1995). The same test was also performed separately in N2 and N3 to investigate the differential effect of ACLS (**Hypothesis 3b**). Furthermore, the power in each frequency band was also compared between conditions during the NREM using Wilcoxon rank test.

For the learning performance, average learning performance of all learning blocks for each condition was obtained (WPA: mean of 5 learning blocks; FPA: mean of 2 learning blocks; VLMT: mean of 3 learning blocks; MT: mean of 4 learning blocks) and was used to compare between STIM and SHAM (**Hypothesis 3c**). In order to rule out the effect

of psychometric variables before and after each measure (i.e. pre-sleep vs. post-sleep, pre-learning vs. post-learning, pre-recall vs. post-recall), post-pre in SSS, PANAS, and PVT was compared between STIM and SHAM. Despite the Kolmogorov-Smirnov test indicated the data were normally distributed, the outcome is rather unreliable for such a small sample size. Therefore, the Wilcoxon rank test was used to compare all learning performances and psychometric measures between conditions (Bridge & Sawilowsky, 1999). Due to the small sample size in this study, a large variable might lead to a small effect, thus a less conservative Holm-Bonferroni multiple comparison correction (α/number of test-degree of significance rank+1) approach was employed (Holm, 1979).

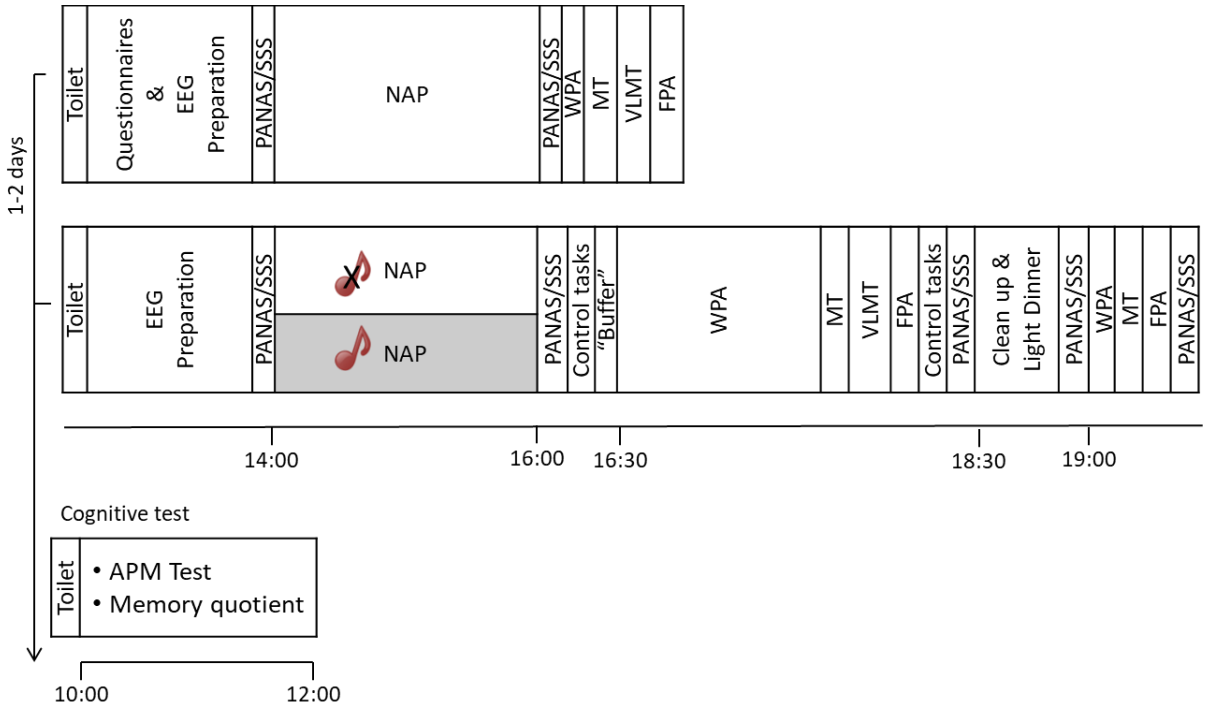

Figure 8. Experimental procedure of Study III. Two experimental sessions took place following the adaptation. Participants were exposed to short versions of all the learning tasks to decrease drastic learning effect on the first experimental session. APM: Advanced Progressive Matrice.

3. Results

3.1 Study I

Question: Is the efficacy of so-tDCS during sleep dependent upon the susceptibility of the underlying network (or 'network state') and is its efficacy subjected to the influence of inter-individual difference?

3.1.1 Blinding of the stimulation

Although two subjects guessed correctly regarding the stimulation session, none of the subjects reported feeling the stimulation during the night, suggesting successful blinding of the conditions.

3.1.2 Psychometric and Polysomnography

There was no significant difference in all the psychometric control tests between conditions. Overall, subjects felt more awake in the morning than in the previous night after learning, regardless of conditions ($p < 0.001$ for TIME). Additionally, they felt more positive and less negative in the morning compared to the evening, which was reflected by the significant TIME x AFFECT interaction in the PANAS questionnaire ($F(1, 23) = 6.10$, $p = 0.021$). No further significant differences in the psychometric measurements were found.

Polysomnographic analyses throughout the sleep did not reveal any significant differences between the three conditions, apart from a slight reduction in sleep onset in CTRL as compared to both STIM and SHAM (see **Table 1**), reflecting equal sleep quality across the sessions besides the participants needed a shorter time to fall asleep in CTRL condition.

Table 1.

Parameters	CTRL	SHAM	STIM
TIB (min)	438.0 ± 3.0	437.2 ± 4.0	443.3 ± 3.2
TST (min)	386.6 ± 5.1	378.5 ± 4.5	382.7 ± 4.0
TWT (min)	18.2 ± 3.1*	26.6 ± 3.2	27.5 ± 3.3*
TMT (min)	2.3 ± 0.6	2.5 ± 0.6	2.5 ± 0.7
N1 (%)	4.0 ± 0.6	3.7 ± 0.4	4.4 ± 0.5
N2 (%)	44.7 ± 2.2	43.0 ± 2.1	44.2 ± 2.2
N3 (%)	30.0 ± 2.5	32.9 ± 2.7	31.3 ± 2.5
REM (%)	21.3 ± 0.9	20.3 ± 1.2	20.1 ± 1.0
NREM (%)	74.7 ± 1.0	76.0 ± 1.3	75.4 ± 1.1
Sleep efficiency (%)	88.2 ± 0.8	86.6 ± 0.80	86.4 ± 0.9
Sleep latency (min)	11.5 ± 1.1	18.7 ± 2.1*	19.6 ± 2.3*
REM latency (min)	111.7 ± 1.0	118.2 ± 8.6	116.1 ± 9.5
1-min post-stimulation epochs			
Wake (s)	0.0	0.9 ± 0.6	0.5 ± 0.5
N1 (s)	6.4 ± 3.8	2.3 ± 1.5	7.3 ± 4.2
N2 (s)	84.1 ± 13.5	98.6 ± 15.2	81.8 ± 11.2
N3 (s)	209.5 ± 13.7	197.7 ± 15.5	209.6 ± 14.2
Movement Time (s)	0.0	0.5 ± 0.5	0.9 ± 0.6
150-min post-stimulation period			
N2 – High MQ (min)	46.6 ± 6.1	50.3± 5.6	52.7 ± 7.4
N2 – Low MQ (min)	52.0 ± 5.8	46.0 ± 4.4	50.2 ± 5.6
N3 – High MQ (min)	73.3 ± 6.9	67.7 ± 7.2	66.7 ± 7.2
N3 – Low MQ (min)	53.1 ± 6.5	68.6 ± 5.4	66.5 ± 7.4

Table 1. Sleep parameters during the three experimental conditions throughout the whole night. Sleep latency is defined as the time from lights-off to the first occurrence of sleep stage 1 followed by sleep stage 2. Participants revealed significantly longer sleep latency in both STIM and SHAM as compared to CTRL. *$p < 0.05$, for comparisons between conditions (N = 25). The groups revealed no significant differences. There were no significant differences in any other sleep parameters between the three conditions. Values are given in mean ± SEM. TIB: Time in Bed; TST: Total Sleep Time; TWT: Total Wake Time; TMT: Total Movement Time

3.1.3 Post-learning EEG modulation

Hypothesis 1a: Learning can induce network modification that is reflected in post-learning EEG during sleep

Analysis for all participants

Task-induced EEG activity was assessed by comparing EEG activity between SHAM (learning 5 tasks) and CTRL (watching a documentary). RmANOVAs analysis revealed that learning induced an enhancement of frontal slow spindle density and count in interaction with topography during the 150-min time period compared to CTRL (density: $F(5, 120) = 3.07$, $p = 0.04$ for COND x TOPO, count: $F(5, 120) = 3.49$, $p = 0.03$ for COND x TOPO; see **Figure 9**). Specifically, the interactions were attributed to increased slow spindle density and count over the left hemisphere (Fp1, F7) as compared to the right hemisphere (Fp2, F8; $p < 0.05$; t-test comparison). Nevertheless, differences between conditions at the single electrode level failed to reach significance (all $p > 0.16$). Additionally, participants reported that CTRL required less concentration (SHAM = 1.76 ± 0.19; CTRL = 3.52 ± 0.23, $T(24) = -5.84$, $p = 0.001$) and was less difficult than SHAM (SHAM = 2.24 ± 0.11; CTRL = 4.24 ± 0.22, $T(24) = -8.17$, $p < 0.001$), confirming intended differential attention required for learning.

Hypothesis 1c: So-tDCS efficacy is dependent upon an inter-individual network component

Analysis for high and low MQ

To further delve into the potential confound of baseline MQ have on learning, participants were split into high and low MQ using the median score. This resulted in 13 participants classified as high MQ (MQ > 118; male: 5; female: 8) and 12 participants as low MQ (MQ < 118; male: 5; female: 7). In order to rule out baseline differences in spindle activity contributed by MQ alone (see **section 1.3.1.2**), an independent sample t-test was employed in CTRL for both fast and slow spindle count and density. The result showed a trend difference between the groups over central region in fast spindle count, but the difference did not reach significant threshold ($p > 0.06$).

When incorporating GROUP into the rmANOVAs slow spindle density analysis, there was a significant COND x GROUP interaction ($F(1, 23) = 5.32$, $p = 0.03$). This result infers slow spindle density in the high-MQ group was significantly increased after learning in SHAM, similar to the average value across participants (see **Figure 9**), whereas learning in the low-MQ group led to a decrease in slow spindle density. The increase in slow spindle density in the high-MQ participants reached significance over the left hemisphere (Fp1, Fp7). The reversed modulation by learning (CTRL > SHAM) in low-MQ participants was significant over F8 (see Figure 9). Slow spindle count tended to be modulated in the same direction ($F(1, 23) = 3.25$, $p = 0.09$; interaction COND x GROUP).

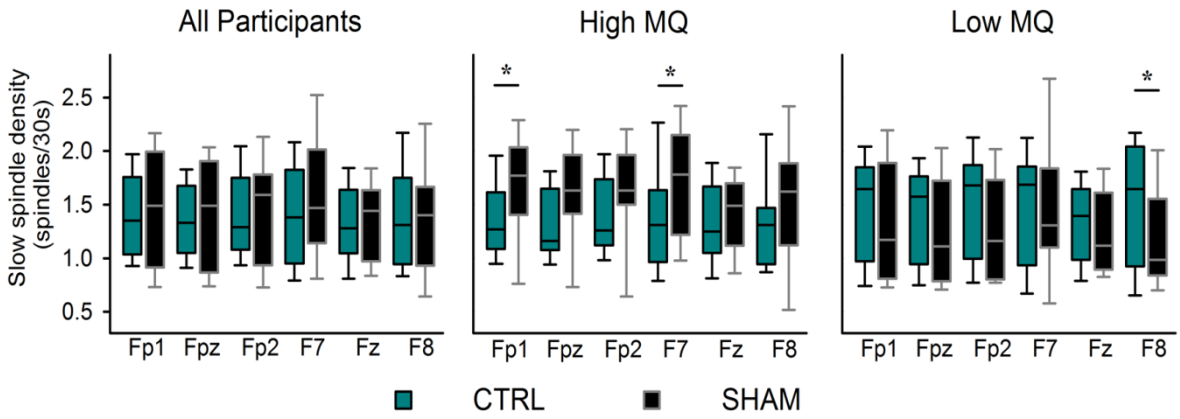

Figure 9. Task-induced learning modified topography of slow spindle density in the 150-min time period. Across all participants there was a significant task-induced effect on slow spindle density (number of spindle per 30s) but failed to reach significance in single electrode level. (B) In participants with high MQ, task-induced slow spindle density was higher than in CTRL over the left hemisphere (Fp1, F7). Participants with low MQ did not show any task-induced increase in slow spindle density. In fact slow spindle density was on average lower after task learning in low MQ. Each box plot shows the median as the horizontal line, with bottom and top whiskers representing 10^{th} and 90^{th} percentiles, respectively. *$p < 0.05$.

Similarly, when examined the acute period of spindles power, rmANOVA showed a significant interaction COND x TOPO x GROUP in fast spindles power ($F(11, 253) = 2.49$, $p = 0.05$). Post-hoc sample t-test revealed the high-MQ group showed learning induced an increase in fast spindle power over the frontal region (Fp1, Fpz, Fp2, F7, $p < 0.03$). Participants of the low-MQ group, in contrast, did not show any increase in fast spindle power after learning ($p = 0.293$) (see **Figure 10**).

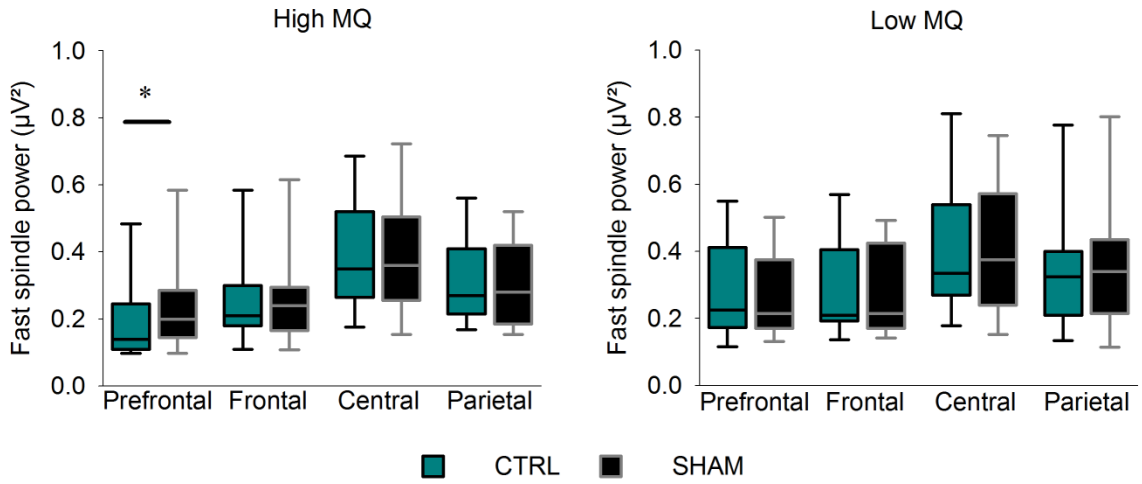

Figure 10 Task-induced fast spindle power during acute period (mean of 5 1-min intervals). Fast spindle power was significantly increased in High MQ after learning over prefrontal region but not low MQ.

In short, all participants showed a weak slow spindle increase after intensive learning. When incorporated the between-subject factor based on MQ, high-MQ group showed a more pronounced increase of acute fast spindle power and slow spindle density over frontal region after learning compared to low-MQ group, suggesting a differential change in task-induced plasticity.

3.1.4 So-tDCS efficacy on EEG activity

Hypothesis 1b: So-tDCS efficacy is dependent upon such task-induced network modifications

Analysis for all participants

The efficacy of so-tDCS on EEG was assessed by comparing STIM with SHAM. So-tDCS successfully modulated fast spindle activity. The acute time period did not reveal a significant difference between conditions in all participants. During the 150-min post-stimulation time period, so-tDCS had a strong impact on fast spindle parameters. Centro-posterior fast spindle count, density, mean length, and power were significantly increased in STIM as compared to SHAM (count: $F(1, 24) = 7.03$, $p = 0.02$; density: $F(1, 24) = 6.58$, $p = 0.02$; mean length; $F(1, 24) = 5.57$, $p = 0.027$; power; $F(1, 24) = 4.41$, $p = 0.05$; main effect of COND (see **Figure 11**).

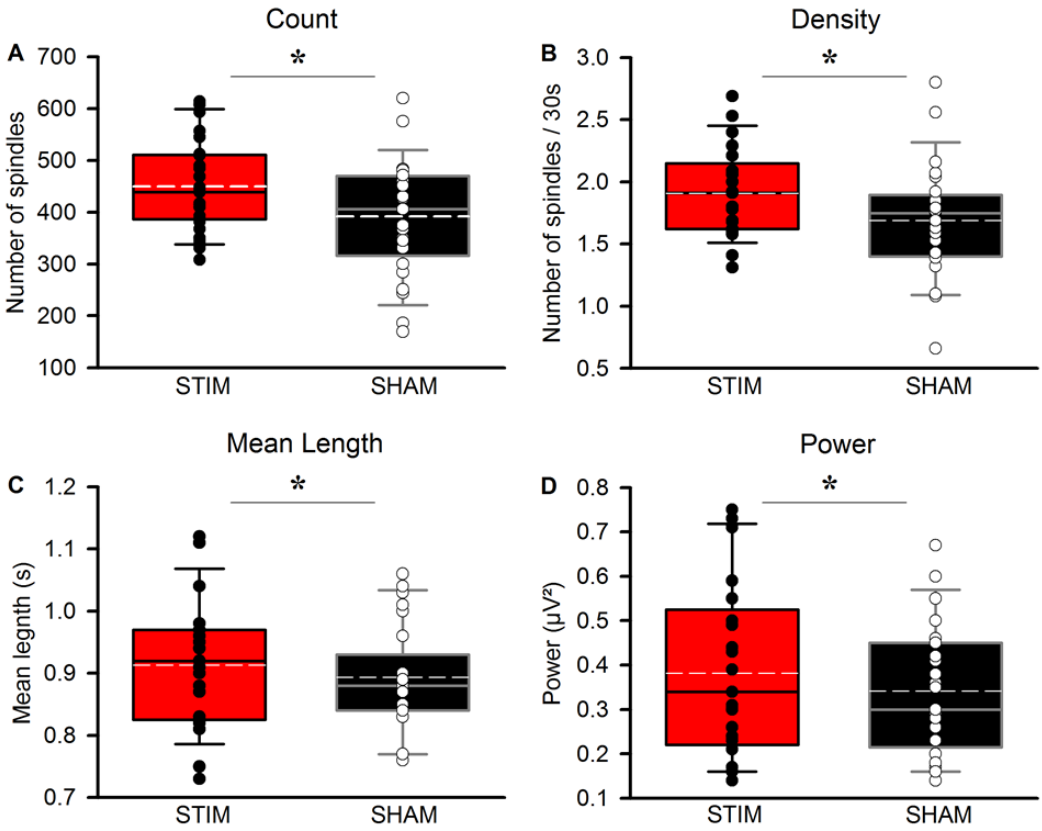

Figure 11. Stimulation during NREM sleep enhanced fast spindle parameters during the 150-min post-stimulation period. (A) Anodal so-tDCS (STIM) enhanced fast spindle (12-15 Hz) count over the centro-parietal location (mean of C3, Cz, C4, P3, Pz, P4). (B, C, D) Same as (A), but for spindle density, mean spindle length, and spindle power. Each box plot shows the median as the horizontal line and mean as the dashed line with the bottom and top whiskers representing 10th and 90th percentiles, respectively. Circles represent individual participants. *$p < 0.05$.

Analysis for high and low MQ

When examining the group separately, there were no significant effects of so-tDCS on EEG activity in either the low- or high-MQ groups. Nevertheless, a tendency for the high-MQ group toward increased fast spindle power over the centro-parietal versus anterior regions during the acute time period after so-tDCS was observed ($F(11, 132) = 2.92$, $p = 0.07$ for the COND x TOPO interaction). The low-MQ participants did not reveal any so-tDCS dependent modulation in topographical spindle distribution ($F(11, 121) = 1.34$, $p = 0.28$). In sum, so-tDCS increase the 150-min post-stimulation fast spindle activity in all participants, but during the acute period, only high MQ showed a tendency of increase in fast spindle power in STIM.

3.1.5 So-tDCS efficacy on behaviour and general memory quotient

Hypothesis 1c: So-tDCS efficacy is dependent upon an inter-individual network component

Analysis for high and low MQ

Using rmANOVA with between-subject factor, there was a significant interaction of COND x TIME x GROUP ($F(1, 23) = 7.98$, $p = 0.01$) for FPA task (see **Figure 12**). Post-hoc t-test revealed so-tDCS significantly increased the retention performance of the high-MQ group in STIM compared to SHAM ($T(12) = -3.49$, $p = 0.004$). Retention performance of the low MQ was not affected by so-tDCS ($T(11) = 1.14$, $p = 0.28$) (see **Figure 12**). There was no significant effect of GROUP on the retention performance of the other two declarative tasks ($p > 0.29$). To investigate if the MQ is associated with FPA retention, the so-tDCS efficacy on the FPA task ($Retention_{STIM} - Retention_{SHAM}$) and MQ score was correlated, in which it revealed a clear positive correlation ($r = 0.55$, $p = 0.005$; $N = 25$), meaning the higher MQ score corresponds to the higher FPA retention in STIM than SHAM. No other significant correlations for the other two declarative memory tasks were found (each $p > 0.30$). The relationship between so-tDCS efficacy on retention most likely did not reflect any difference in learning performance between high- and low-MQ groups since learning performance on FPA was not correlated with MQ ($r = 0.34$, $p > 0.05$). Interestingly, learning performance on the other two declarative tasks was significantly correlated with MQ (WPA: $r = 0.55$, $p = 0.008$; 2DL: $r = 0.54$, $p = 0.007$), which reflected greater learning by the high MQ as compared to the low-MQ group on WPA in SHAM (see **Table 2**).

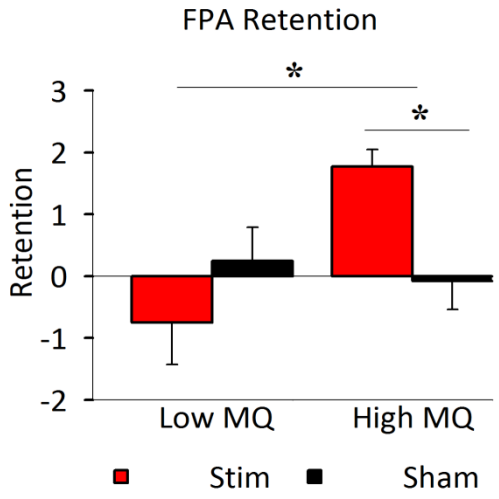

Figure 12. Differential FPA rentention performance between low- and high- MQ groups. Asterisk across the group represents significant ANOVA interaction for COND x TIME x GROUP. Asterisk on high MQ represents significance obtained from t-test (see **Table 2**).

Table 2.

Participants	Task	Learning		Retention	
		SHAM	STIM	SHAM	STIM
Total Subject Sample	FPA	11.4 ± 0.5	11.5 ± 0.5	0.1 ± 0.4	0.6 ± 0.5
	WPA	44.8 ± 1.5	43.4 ± 1.9	10.8 ± 1.0	12.3 ± 1.2
	2D-object location	10.8 ± 0.3	11.6 ± 0.4	-0.7 ± 0.4	-0.7 ± 0.5
	FSTT Accuracy	19.9 ± 1.0	20.4 ± 0.9	3.7 ± 0.7	3.3 ± 0.8
	FSTT Speed	21.1 ± 1.0	21.6 ± 1.0	3.6 ± 0.6	3.4 ± 0.7
	MT	61.4 ± 3.5	61.3 ± 4.0	15 ± 2.4	15.9 ± 2.5
	MT	7.0 ± 1.2	7.8 ± 1.3	-2.7 ± 0.9	-4.2 ± 0.9
High MQ N = 13	FPA	12.0 ± 0.6	11.4 ± 0.5	-0.1 ± 0.5	1.8 ± 0.3**
	WPA, N = 12	47.5 ± 1.8	47.6 ± 2.2	10.5 ± 1.1	11.4 ± 1.6
	2D-object location	11.1 ± 0.5	11.4 ± 0.6	-0.5 ± 0.4	0.1 ± 0.6
	FSTT Accuracy	19.5 ± 0.8	19.8 ± 1.1	3.8 ± 0.8	3.5 ± 0.8
	FSTT Speed	20.8 ± 0.8	21.0 ± 1.2	3.1 ± 0.8	3.7 ± 0.7
	MT	59.4 ± 4.6	63.5 ± 5.6	10.3 ± 1.9	15.4 ± 3.2
	MT	7.0 ± 1.9	7.9 ± 2.0	-3.2 ± 1.4	-4.6 ± 1
Low MQ N = 12	FPA	10.7 ± 0.8	11.7 ± 0.5	0.3 ± 0.6	-0.8 ± 0.7†
	WPA, N = 10	41.6 ± 2.1#	38.2 ± 2.5#	11.2 ± 1.6	13.3 ± 1.8
	2D-object location	10.4 ± 0.5	11.8 ± 0.6§	-0.9 ± 0.7	-1.5 ± 0.6
	FSTT Accuracy	20.4 ± 1.8	21.0 ± 1.5	3.7 ± 1.2	3 ± 1.5
	FSTT Speed	21.5 ± 1.9	22.2 ± 1.5	4.1 ± 0.8	3 ± 1.2
	MT	63.6 ± 5.3	58.8 ± 5.8	20.1 ± 4.2	16.4 ± 3.9
	MT	7.0 ± 1.4	7.7 ± 1.8	-2.1 ± 1.2	-3.8 ± 1.5

Table 2. Immediate recall and retention of five tasks during SHAM and STIM sessions. Mean ± SEM of learning performance (immediate recall) and retention (expressed as delayed recall – immediate recall). The means were the measures of number of correct pairs for FPA, WPA, and 2D-object location; number of correct sequences for FSTT accuracy; total number of sequence for FSTT speed; time in second and number of error for MT, respectively. Asterisks indicate the significant difference in retention between STIM and SHAM on the FPA **$p < 0.005$ (post-hoc paired sample t-test). Participants with high MQ learned significantly more words than low MQ participants on both SHAM and STIM sessions, #$p < 0.05$. Low-MQ participants learned 2DL better in STIM than in SHAM, §$p < 0.05$. Retention of participants with high MQ was greater than that of participants with low MQ on FPA retention in STIM, †$p < 0.005$ (post-hoc independent sample t-tests were used for comparisons between high- and low-MQ groups). The total participant sample was N = 25 for all tasks except for the WPA task where total participant sample was N = 22.

3.2 Study II

Question: Can SO be induced by so-tDCS during eyes-closed wakefulness where endogenous SO activity is absent and does so-tDCS enhance other related frequency bands?

3.2.1 Blinding of the stimulation

In the STIM session, 3 of the 16 participants responded in the affirmative to the question 'Do you think this was a stimulation session?' and 1 of them reported having felt something (i.e. tingling sensation under the eyes). In the SHAM session, 6 participants reported they believed they underwent a stimulation session. Hence, participants were overall successfully blinded to the stimulation.

3.2.2 Psychometric control

The control psychometric analysis revealed that participants felt more positive after the so-tDCS than SHAM session, indicated by significant COND x TIME interaction ($F(1, 15) = 6.86$, $p = 0.02$) for positive affect. Similarly, the SSS revealed that participants felt more awake after so-tDCS compared to SHAM ($F(1, 15) = 10.08$, $p = 0.006$; COND x TIME interaction), although a significant baseline difference might have contributed to this effect (SHAM: 2.38 ± 0.16; STIM: 3.00 ± 0.26, $T(15) = 2.61$, $p = 0.02$).

3.2.3 So-tDCS during wakefulness suppresses alpha power

Hypothesis 2a: So-tDCS can induce EEG frontal SO and theta activity

Contradict to what was hypothesized, so-tDCS applied during eyes-closed failed to induce SO power in any way, revealed by a non-significant main effect of COND ($F(1, 15) = 0.97$, $p = 0.34$), COND x TOPO ($F(11, 165) = 1.02$, $p = 0.37$) or COND x TIME ($F(5, 75) = 1.26$, $p = 0.29$) interaction in the rmANOVA. Likewise, ANOVAs results revealed no significant changes between so-tDCS and SHAM in theta power, suggested theta was not affected by the stimulation (COND main effect, $F(1, 15) = 0.01$, $p = 0.92$; COND x TOPO: $F(11, 165) = 0.69$, $p = 0.57$; COND x TIME: $F(5, 75) = 1.70$, $p = 0.20$). However, alpha power was modulated by so-tDCS, indicated by a significant main effect of COND ($F(1, 15) = 5.56$, $p = 0.03$) and an interaction with topography ($F(11, 165) = 4.68$, $p = 0.04$; COND x TOPO).

Post-hoc analysis revealed that so-tDCS decreased alpha power, in particular over the parietal region (see Figure 13).

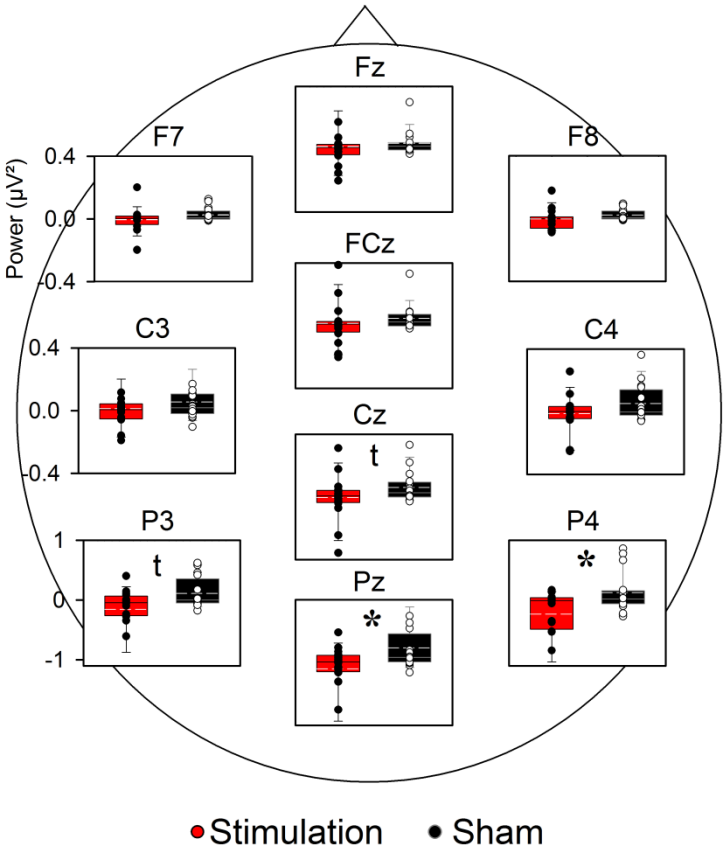

Figure 13. Mean alpha power across five post-stimulation periods. Alpha power was significantly suppressed by so-tDCS as compared to SHAM over the parietal region and trend over Cz. When data P3, Pz, P4 were pooled into parietal and compared between conditions, alpha power in STIM was likewise decreased compared to SHAM ($p = 0.029$). Each box plot shows the median as the horizontal line and mean as the dashed line with the bottom and top whiskers representing 10th and 90th percentiles, respectively. Circles represent the individual participant. $^{*}p < 0.05$; $^{t}p < 0.1$

3.2.4 So-tDCS during wakefulness on behaviour and subjective scales

Hypothesis 2b: So-tDCS has no effect on the procedural behavioural task performance

Although the behaviour and subjective findings were not expected to be modified, performance on FSTT was significantly modified by so-tDCS (COND × TIME: $F(1, 15) = 5.69$, $p = 0.03$; see Figure 14). The post-hoc t-test using retention (Retest-Training) indicated so-tDCS significantly decreased FSTT performance compared to SHAM (STIM: 0.31 ± 0.81; SHAM: 2.13 ± 0.72; $T(15) = -2.39$, $p = 0.03$; see Figure 14). To investigate if the worsening performance on FSTT was due to the suppressed alpha, a correlation between the alpha and FSTT retention was performed but results did not reveal any relationship between the suppressed alpha and detriment FSTT in STIM ($p > 0.10$).

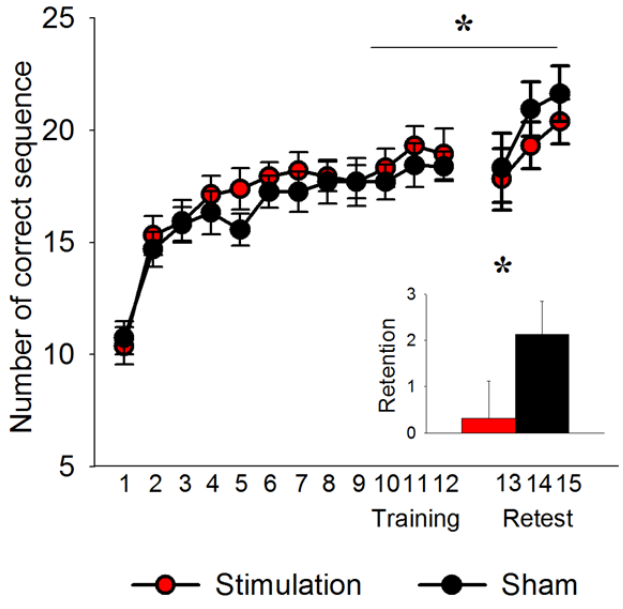

Figure 14. Training and retest performance on the FSTT in the conditions STIM and SHAM. The x-axis represents the number of correct sequences for Training (trials 1-12) and Retest (trials 13-15). In the insets "Training" refers to the average of sequences 10-12 and "Retest" to the average of sequences 13-15. Improvement on FSTT was significantly reduced in STIM compared to SHAM. Retention was calculated as (Retest-Training). Asterisk and vertical line represent the significant COND x TIME interaction; the asterisk in the bar chart depicts the significant difference obtained from the paired-sample t-test. *$p < 0.05$.

3.2.5 Alpha suppression and detrimental effect of FSTT is limited to so-tDCS but not IAF-tDCS

In order to investigate whether the detrimental effect on behaviour and alpha power was specific to so-tDCS, in a supplementary experiment, individual alpha frequency (IAF)-tDCS was applied. The procedure was identical to the main experiment; except that the stimulation frequency was individual alpha frequency instead of SO frequency. Nine out of 16 participants from the main experiment participated in the supplementary experiment and received monetary compensation. Individual alpha frequency within 7-14 Hz (mean frequency = 9 Hz ± 0.6 Hz) was obtained from the average baseline data (prior to stimulation) and the 5 stimulation-free intervals during the SHAM session of the main experiment. Alpha power was not influenced by IAF-tDCS (Cond main effect: $F(1, 8) = 0.06$, $p = 0.82$; COND x TIME: $F(5, 40) = 1.36$, $p = 0.29$). Likewise, performance on the FSTT was not altered by IAF-tDCS as compared to SHAM (COND x TIME: $F(1, 8) = 2.75$, $p = 0.14$), reflecting the detrimental effect on FSTT and alpha power was specific to so-tDCS.

3.3 Study III

Question: Does enhanced SO activity during NREM sleep improved subsequent encoding performance and does the effect of ACLS differ in stages N2 and N3?

3.3.1 Blinding of the stimulation and detected SO

During the STIM session, 5 participants claimed they heard the clicks during sleep and three of them believed it was a STIM session. Two other participants who did not hear the clicks and yet believed it was a STIM session. During the SHAM session, two participants claimed to hear something but did not think they were being stimulated. Three other participants did not hear anything but believe that was a STIM session. Overall, three out of nine participants who heard the clicks claimed that it was a STIM session. In general, there were more SO detected in SHAM than in STIM but such a difference did not reach statistical significance (see Table 3).

Table 3.

	NREM		N3		N2	
Participant	STIM	SHAM	STIM	SHAM	STIM	SHAM
1	37	193	8	72	29	121
2	9	49	5	28	4	21
3	83	124	34	121	49	3
4	95	112	88	68	7	44
5	60	-	30	-	30	-
6	161	68	159	51	2	17
7	83	206	76	202	7	4
8	167	108	164	107	3	1
9	86	181	86	181	0	0
Mean ± sem	86.8± 17.2	130 ± 20.5	72.2 ± 19.8	103 ± 21.8	14.6 ± 5.7	26.4 ± 14.5

Table 3. Number of stimulations / detected SO for STIM and SHAM conditions for each participant. N=9 for NREM and N3. N=8 for N2 for STIM, due to one participant had neither stimulation nor detected SO in N2 on both sessions. N=8 for NREM and N3. Due to a technical issue, the detection of SO was faulty, hence eliminated from analysis. N=7 for N2 in SHAM (same reason as in STIM for N2). Albeit there was more SOs were detected in SHAM, Wilcoxon signed rank test revealed no significant difference between the conditions ($p = 0.2$ in NREM; $p = 0.3$ in N3; $p = 0.4$ in N2).

3.3.2 Psychometric, Polysomnography and sleep EEG power throughout the nap

There was a tendency that participants felt more positive after sleep in STIM compared to SHAM ($p < 0.020$), albeit the significance level was below the adjusted threshold ($p_{adjusted} = 0.008$). There was no other significant difference between STIM and SHAM on psychometric variables. There was also no significant difference in all the polysomnographic parameters (see Table 4). Likewise, the power of SWA, delta, slow and fast spindles did not differ significantly throughout the NREM period (see Table 4).

Table 4.

Parameters	SHAM	STIM	P-value
TIB (min)	118.8 ± 4.7	121.34 ± 3.1	0.77
TST (min)	94.5 ± 4.7	100.23 ± 4.9	0.37
Wake (min)	9.9 ± 3.0	12.67 ± 7.5	0.44
N1 (min)	12.3 ± 1.8	11.23 ± 1.9	0.91
N2 (min)	57.3 ± 4.2	63.39 ± 7.2	0.31
N3 (min)	21.1 ± 4.5	23.78 ± 5.1	0.68
REM (min)	8.6 ± 3.0	7.73 ± 2.7	1.00
NREM (min)	78.4 ± 3.6	87.17 ± 4.1	0.11
N1 latency (min)	7.1 ± 0.8	17.39 ± 10.5	0.86
N2 latency (min)	12.7 ± 1.5	12.23 ± 2.0	0.87
REM latency (min)	49.6 ± 12.5	49.78 ± 12.0	1.00
Arousal (min)	2.8 ± 0.9	13.56 ± 11.3	0.89
Sleep Efficiency (%)	79.1 ± 2.6	74.56 ± 8.8	0.87
Power (μV^2)	**SHAM**	**STIM**	**P-value**
SWA	15.9 ± 2.3	16.7 ± 2.3	0.86
Delta	9.5 ± 1.3	9.9 ± 1.0	0.68
Theta	1.4 ± 0.2	1.5 ± 0.2	0.95
Sspi	0.4 ± 0.1	0.4 ± 0.1	0.21
Fspi	0.4 ± 0.1	0.4 ± 0.1	0.26

Table 4. Sleep parameters throughout the whole nap period. There was no statistical difference between STIM and SHAM throughout the whole nap period. Latency is defined as the time from lights-off to the first occurrence of sleep stage 1 and 2, respectively. Power was calculated from NREM period throughout the nap. TIB: Time in Bed; TST: Total Sleep Time.

3.3.3 Augmented SO, slow spindle, and fast spindle activity by ACLS

Hypothesis 3a: Auditory stimulation delivered in-phase with the SO depolarisation up-state (ACLS) will induce SOs, slow and fast spindle activity in NREM sleep

Hypothesis 3b: ACLS will have a unique effect on SO, slow and fast spindles in N2 compared to N3

During NREM period, ACLS successfully augmented the SO amplitude over Fz and FCz, and Pz. The averaged SO time-locked to the first click was prominent in STIM compared to SHAM condition, revealing a strong EEG response to the in-phase stimulation. Specifically, ACLS evoked an electrophysiological response upon the first click at 140 ms (P100; Fz and FCz; 190 ms at Pz) and the subsequent hyperpolarisation down-state at 360-700 ms (N550; 360-630 ms at FCz; intermittent between 380-700 ms at Pz) as well as following depolarisation up-state at 970-1290 ms (P900; 1100-1260 ms at Fz; 890-1290 ms at FCz; 970-1300 ms at Pz) and subsequent depolarisation at 1820-2340 ms (1860-2230 ms at Fz; 1820-2340 ms at FCz 2040-2290 ms at Pz). When post-hoc examining the sleep stages separately on N3 and N2, SO was only significantly augmented over FCz, Cz, and Pz during N3, despite Fz exhibited a similar pattern. ACLS did not significantly modulate the SO activity in N2, albeit there was a hint of an increase in subsequent SO hyperpolarisation down-state visually (see **Figure 15**). Moreover, the standard error was higher in N2 for both conditions, suggesting the small number of detected SO used for average may contribute to such variability.

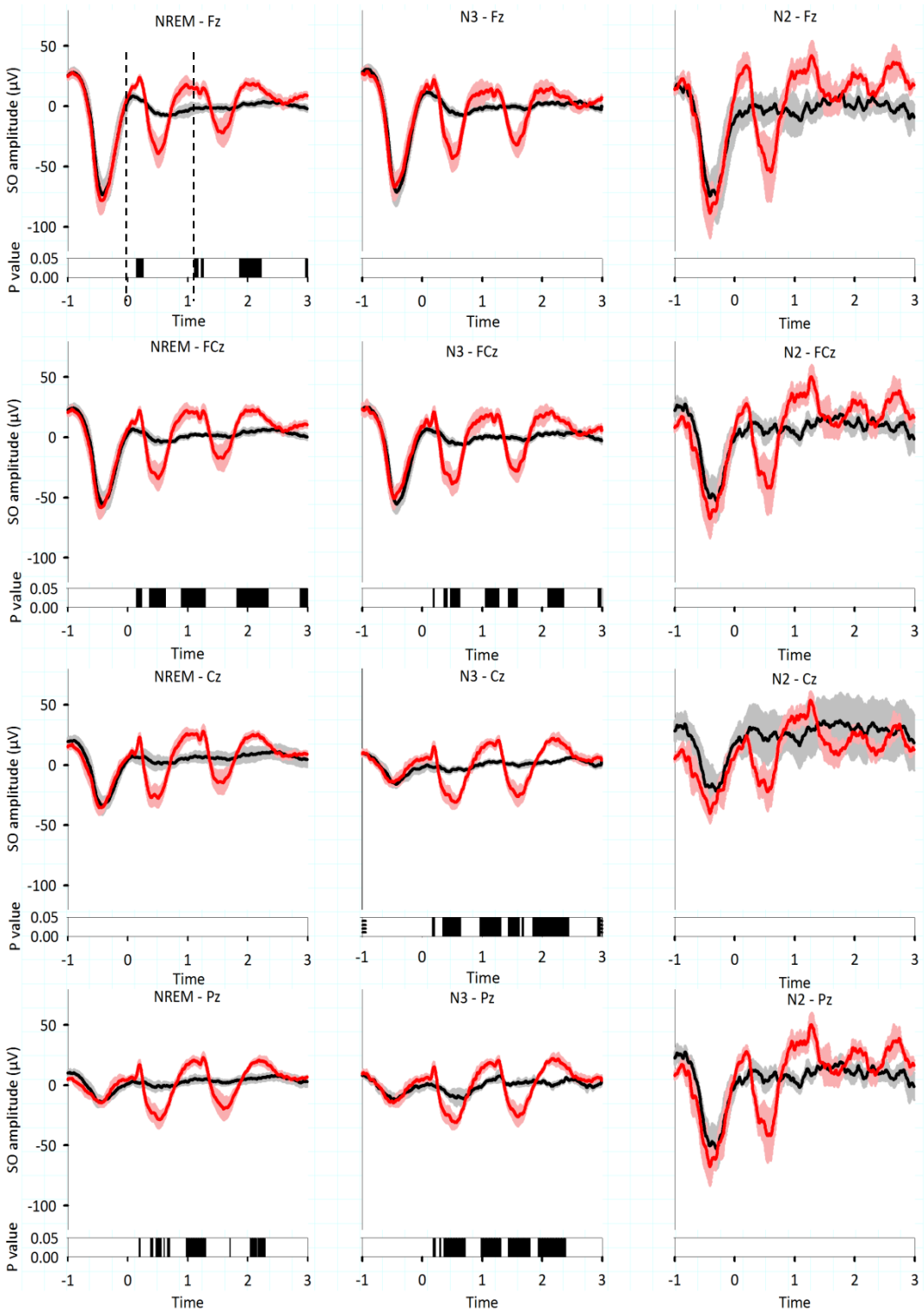

Figure 15. Averaged SOs across all stimuli during STIM (red line) and all detected SOs during SHAM (black line). SO was most prominently increased over central-posterior regions during SWS while no effect of ACLS was found during N2. The three columns consist of all SOs averaged across NREM (N2 & N3), N3 and N2, respectively. The rows represent different topographies - Fz, FCz, Cz, Pz, respectively. The bottom bars on each graph illustrate significant differences between the conditions after Benjamini-Hochberg's correction. First vertical dashed line represents first click while the second dashed line represents the second click.

For fast spindle activity, ACLS increased the power of the fast spindle band concomitantly with the SO up-state during NREM over FCz (760-1250 ms; $p < 0.001$) and Cz (1000-1250 ms; $p < 0.001$). The enhanced fast spindle activity showed more prominent increase in N3 across midlines (Fz: 770-810 ms/1070-1250 ms; $p < 0.003$; FCz: 720-1270 ms; $p < 0.0006$; Cz: 750-1190 ms; $p < 0.0003$; Pz: 780-11600 ms; $p < 0.0001$). On the other hand, N2 showed the first peak ranged 840-1470 ms over Fz (840-1145 ms, $p < 0.0003$) and FCz (860-1470 ms; $p < 0.0004$) and second peak ranged 1660-2190 ms over Fz (1860-2190 ms,; $p < 0.002$) and FCz (1660-2190 ms; $p < 0.0005$) (see **Figure 16**). To note, although the enhancement of fast spindle in NREM and N3 appeared to be almost indistinguishable across topographies, the rank of p-values, as well as the p-values, were taken into account while adjusting the significant threshold, thus, the ultimately significant level may vary considerably.

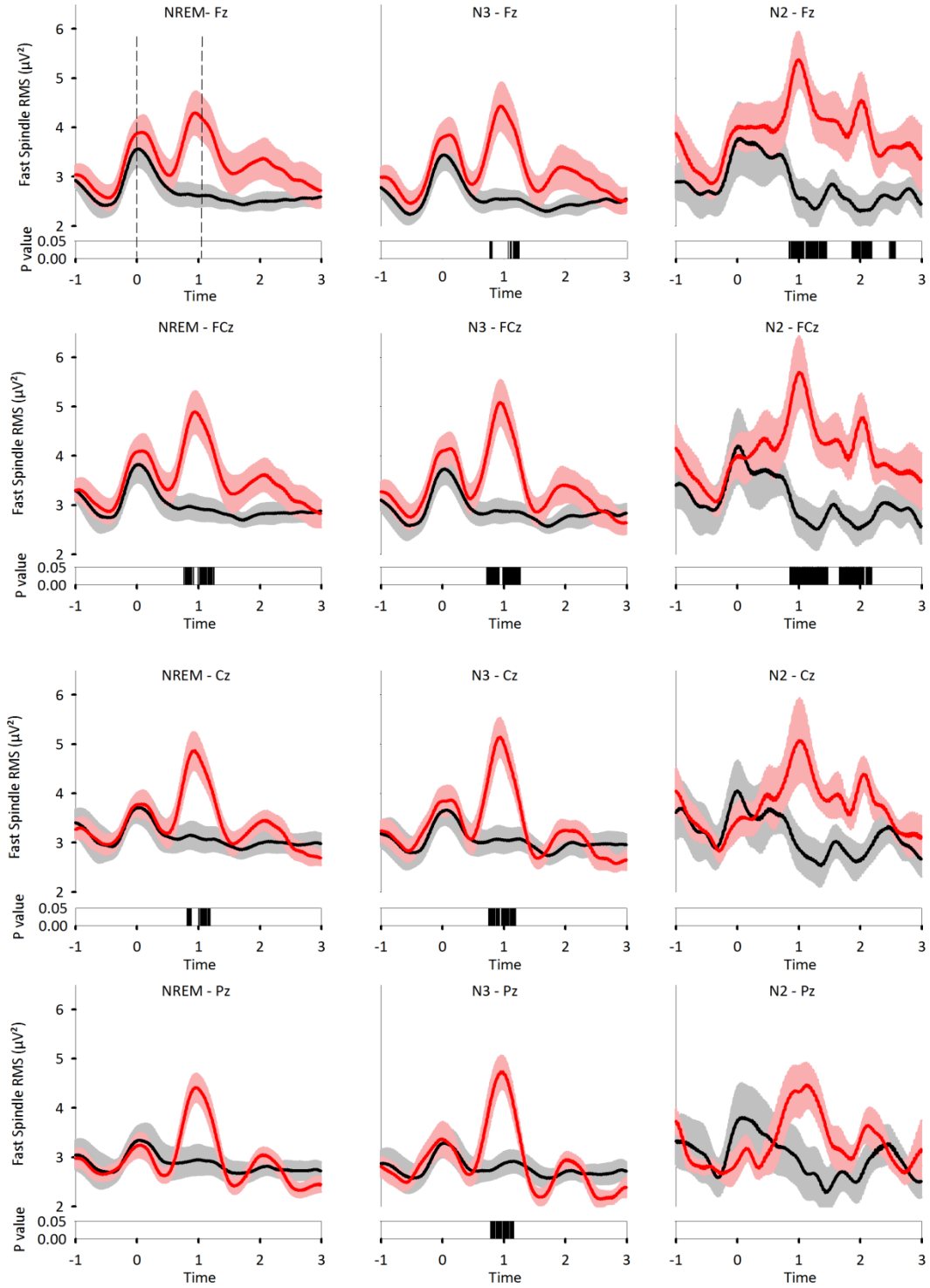

Figure 16. Averaged fast spindles RMS across all stimuli during STIM (red line) and all detected SO during SHAM (black line). Fast spindles were mostly affected by ACLS over frontal regions during N2 and across all regions during SWS (N3). The three columns consist of all SOs averaged across NREM (N2 & N3), N3 and N2, respectively. The rows represent different topographies - Fz, FCz, Cz, Pz, respectively. The bottom bars on each graph illustrate significant differences between the conditions after Benjamini-Hochberg's correction. First vertical dashed line represents first click while the second dashed line represents the second click.

The slow spindle analysis revealed, however, quite a distinct ACLS effect. During NREM sleep, slow spindle activity was not modulated in NREM. N3 closely resembled the pattern observed in NREM, in which no significant increase across all topographies was found. Although there was a slight increase in slow spindle RMS time-locked to the ACLS, this increase did not reach significant level. In contrast, there was a significant increase in slow spindle power during N2 over Fz (1530-1670 ms; $p < 0.001$; 1790-2170 ms; $p < 0.001$; 2430-1760 ms; $p < 0.005$) and FCz (1620-1870 ms; $p < 0.0006$). Furthermore, there was a significant increase in power prior to the stimulation at only Fz (-1000 to -780 ms; $p < 0.009$) (see **Figure 17**).

Taken together, ACLS enhanced the amplitude of the hyperpolarisation down-state and the depolarisation up-state during NREM and N3, and the timings coincide with P100, N550, and P900 of the ERP. Fast spindle activity that corresponded to the SO up-state was similarly enhanced in NREM and N3, suggesting an increase of synchronization between SO and fast spindle. Slow spindle activity was only modulated significantly in N2 at a later time point from the onset of stimulation, proposing a differential effect of ACLS occurred in N2 and N3 as hypothesized.

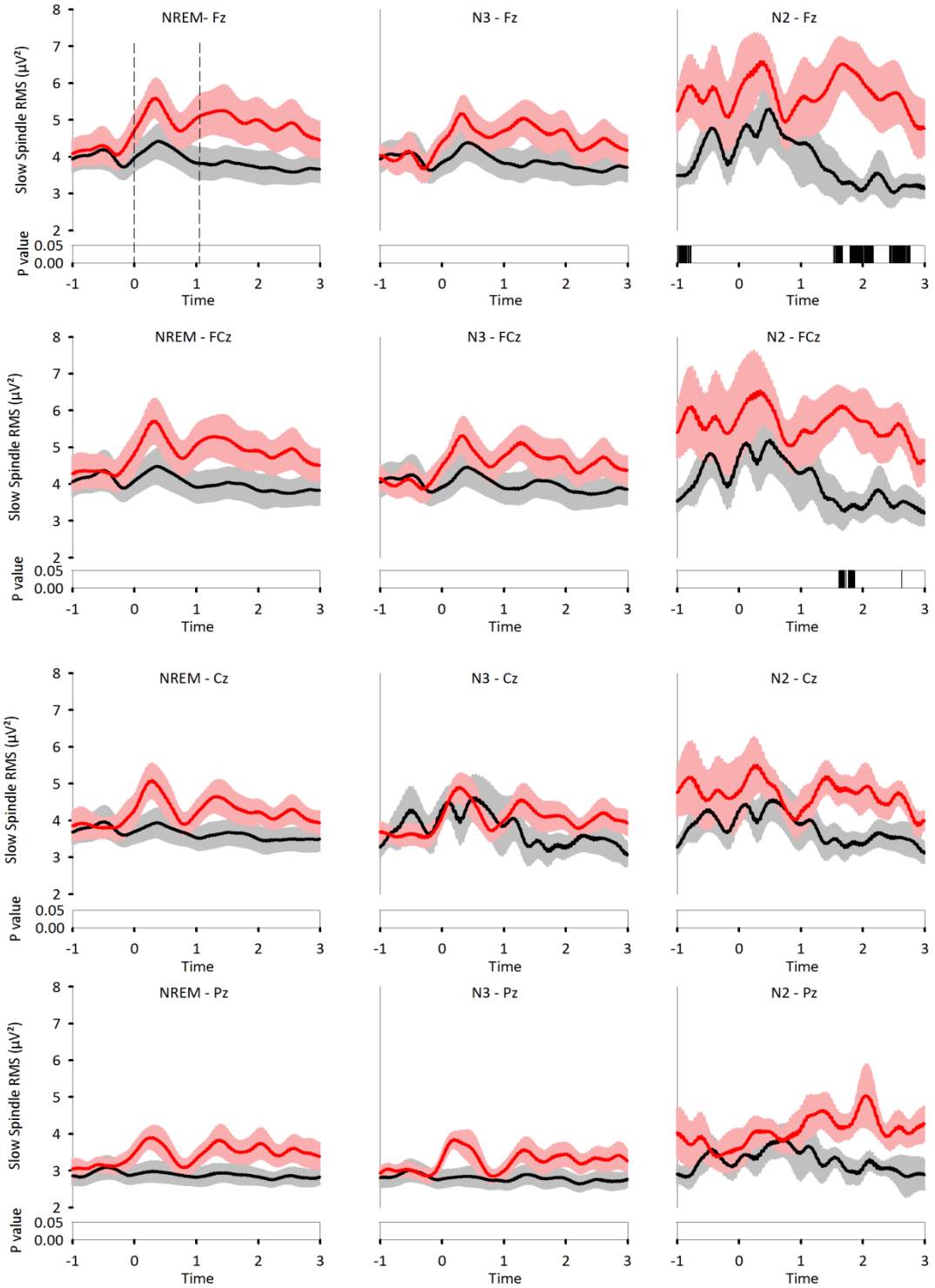

Figure 17. Averaged slow spindles RMS across all stimuli during STIM (red line) and all detected SO during SHAM (black line). ACLS showed no effect on slow spindle during SWS but N2 frontal-central regions. The three columns consist of all SOs averaged across NREM (N2 & N3), N3 and N2, respectively. The rows represent different topographies - Fz, FCz, Cz, Pz, respectively. The bottom bars on each graph illustrate significant differences between the conditions after Benjamini-Hochberg's correction. First vertical dashed line represents first click while the second dashed line represents the second click.

3.3.4 Post-sleep learning performance has a modest effect on only FPA task

Hypothesis 3c: If ACLS induces SO and spindles, encoding capacity will be enhanced

To examine the overall learning performance, the average block of learning in all tasks was used for comparison. ACLS applied during the nap had a modest improvement on subsequent FPA learning (p = 0.027) but it again failed to reach significant level ($P_{adjusted}$ = 0.01). The stimulation also did not impact the other learning tasks (see Table 5).

Table 5.

Tasks	Block	STIM	SHAM	P-value
WPA	L1	10.4 ± 2.5	9.8 ± 2.6	0.69
	L2	26.5 ± 5.2	24.6 ± 5.1	
	L3	39.1 ± 6.6	40.0 ± 6.8	
	L4	51.3 ± 7.8	53.5 ± 7.8	
	L5	59.5 ± 8.6	58.9 ± 8.4	
	Mean Learning	37.3 ± 5.9	37.3 ± 5.9	
	Delayed Recall	56.6 ± 8.4	57.9 ± 8.7	
FPA	L1	51.4 ± 7.4	45.1 ± 6.7	*0.027*
	L2	62.5 ± 6.1	54.9 ± 5.1	
	Mean Learning	57.0 ± 6.1	50.0 ± 5.7	
VLMT	L1	60.8 ± 8.1	57.8 ± 5.7	0.95
	L2	84.5 ± 4.5	86.7 ± 2.9	
	L3	93.3 ± 2.5	90.4 ± 3.7	
	Mean Learning	79.5 ± 4.3	78.3 ± 3.7	
	Interference	54.1 ± 5.6	54.8 ± 5.4	
	L4	88.9 ± 5.1	88.2 ± 4.7	
MT	L1	60.5 ± 3.6	55.3 ± 3.9	0.77
	L2	54.6 ± 1.8	54.1 ± 3.8	
	L3	53.0 ± 3.3	55.2 ± 5.8	
	L4	52.3 ± 2.4	55.1 ± 5.6	
	Mean Learning	55.1 ± 2.5	54.9 ± 4.5	
	Delayed Recall	50.1 ± 2.3	50.8 ± 4.6	
MT error	L1	3.8 ± 1.5	8.0 ± 1.7	0.68
	L2	6.9 ± 2.9	6.9 ± 2.1	
	L3	7.6 ± 3.0	4.3 ± 1.0	
	L4	6.7 ± 4.0	6.6 ± 2.3	
	Mean Learning	6.2 ± 2.4	6.4 ± 1.2	
	Delayed Recall	4.3 ± 2.5	3.6 ± 1.3	

Table 5. Learning performance of each task and each block. Due to a technical issue for FPA during delayed recall time, the data were excluded for analysis. Each p-value corresponds to the mean learning between STIM and SHAM using Wilcoxon rank test. FPA was the only test that was marginally improved in STIM compared to SHAM (p-value in italic). N=9.

3.3.5 ERP and TFR on encoding

To putatively explore the effect of stimulation has on neurophysiological processing of FPA, only later correctly recalled figural pairs were used for the average ERP during encoding. As the posterior regions showed the largest ERP response upon stimuli presentation, P7, P8, O1, and O2 electrodes were selected for analysis. The running t-test showed, however, no significant difference between conditions on these electrodes (see **Figure 18**). Wavelet analysis revealed higher activation for correct items in SHAM compared to correct items in STIM. Thus, the highest activation across the time course at alpha frequency was extracted for statistical comparison (see **Figure 19**). The mean power within the time window of 130-220 ms at a frequency range of 7.4-12.4 Hz was extracted for comparison between conditions. Wilcoxon rank test revealed, however, no significant difference in STIM and SHAM ($p > 0.46$). Supplementary figure 9 illustrated the wavelet transformation for correct and incorrect pairs individually for STIM and SHAM.

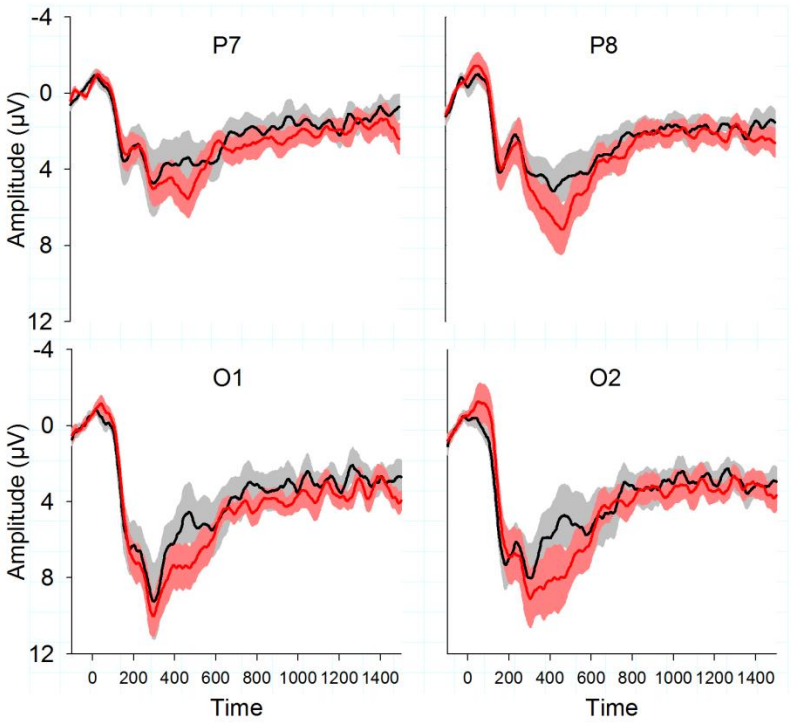

Figure 18. ERP of correct pairs in FPA task. Running t-test showed no significant difference between STIM (red line) and SHAM (black line). Time 0 represents the onset of stimulus' presentation.

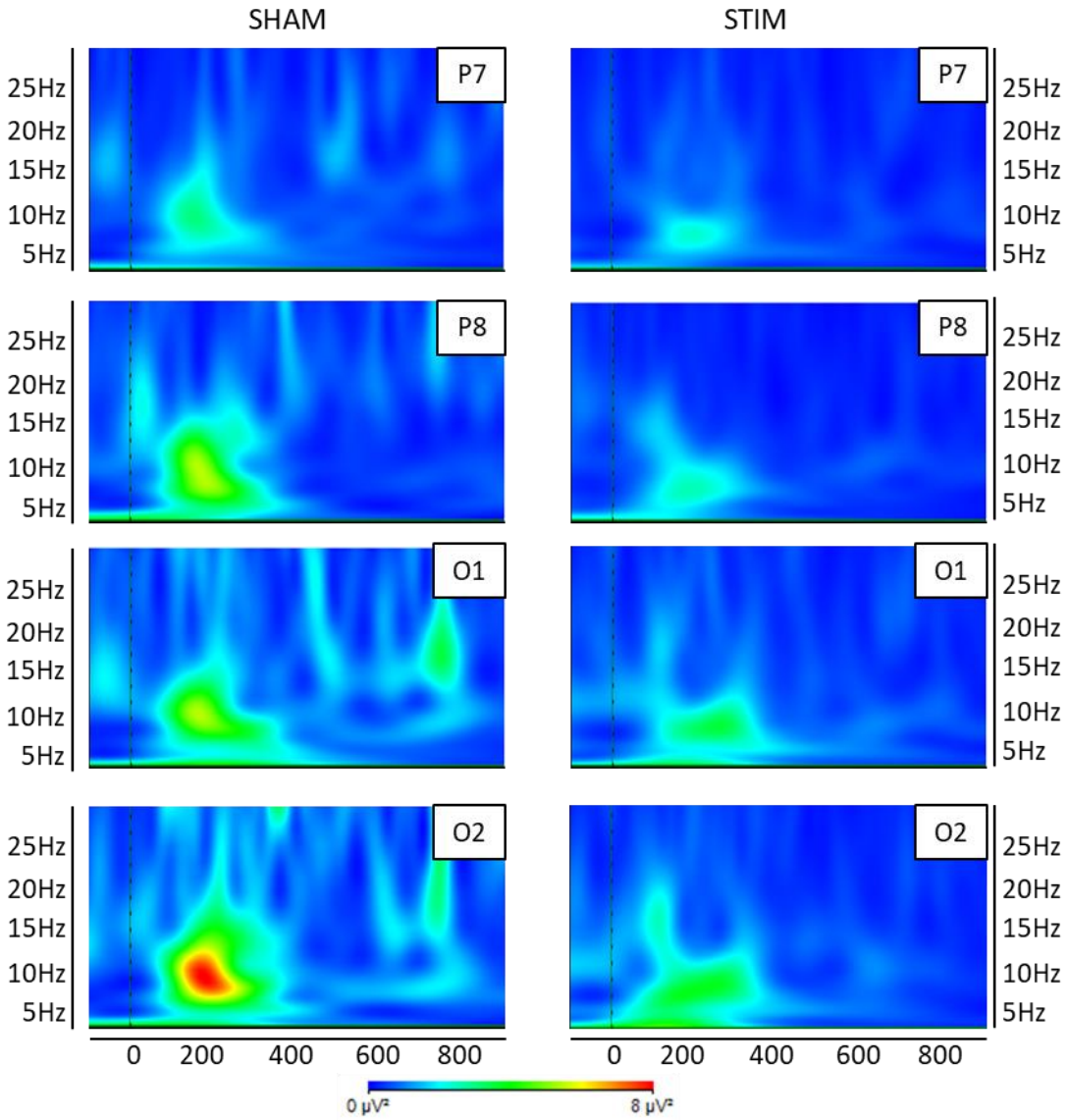

Figure 19. Heat map of the wavelets in STIM and SHAM for correctly recalled pairs. Y-axis is the frequency and x-axis is the time in ms. Time 0 is the onset of stimulus (vertical line).

4. Discussions

4.1 Learning induces spindles activity

Hypothesis 1a: Learning can induce network modification that is reflected in post-learning EEG during sleep

In study I, five intense learning tasks were employed and subsequent sleep EEG (on the same night) was compared with the sleep EEG on the (non-learning) CTRL night, where participants watched a documentary. Hypothesis 1a was that learning could lead to subsequent network modifications compared to control session.

The results showed that after learning the five memory tasks, slow spindle density and count during 150-min post-stimulation period were increased compared to control condition (termed "task-induced slow spindle"). Despite the change being significant across all participants, only the high-MQ group showed significant task-induced slow spindle activity dominant over the left hemisphere whereas the low-MQ group showed a decrease in slow spindle density over the right hemisphere. The absence of a significant difference in spindles between the groups in the control session suggests the increase of slow spindle density was in fact induced by learning. This finding is line with a study reporting an increase of spindles over the left hemisphere during post-learning sleep in individuals with higher cognitive abilities (Schabus *et al.*, 2006). Furthermore, only the high-MQ group showed increased fast spindle power over the prefrontal region during the interval corresponding to the acute stimulation time period in SHAM compared to CTRL night. As sleep spindles were suggested to reflect a more efficient functional TC communication system (Fogel *et al.*, 2007a), the task-induced spindles may thus imply that learning in high-MQ participants was more efficient. The topographic differences between the groups presumably reflect the differential involvement of underlying network activity. Behaviourally, high-MQ participants learned significantly better than low-MQ participants on the WPA, further supporting a greater learning ability.

Despite compelling evidence suggesting that sleep spindles might reflect a physiological index of intellectual ability (Fogel & Smith, 2011), the high-MQ participants

did not show a higher number of spindles in CTRL session. It could be speculated that this outcome is due to differences in the employed intelligence batteries. The battery used in Study I was notably dominant in requiring memory capacity, meaning all the subtests involved memorizing information rather than components of reasoning or logical thinking. In fact, a study using simultaneous EEG and fMRI to examine brain activation time-locked to spindles found that the neural activations in the thalamus, anterior cingulate cortex and putamen were correlated with reasoning ability but not with short-term memory or verbal abilities (Fang *et al.*, 2019). The same group later reported that spindle-related functional connectivity of the cortical-striatal and TC circuitries was associated with reasoning abilities, meaning participants with higher reasoning ability possessed a higher number of sleep spindles (Chatburn, 2013). These findings suggest that memory capacity may be less relevant to the inter-individual difference in spindles during baseline sleep (i.e. no learning was administered prior to sleep).

Although one may argue that the lack of sufficiently matched in physical and psychometric variables of the five learning tasks in CTRL condition may confound the difference in slow spindle activity between groups, the higher attention and difficulty level were reported in the learning session suggests that plasticity changes were actually induced by the learning tasks.

In sum, the result supported the hypothesis 1a, such that learning can indeed lead to network modification, however, the inter-individual difference in MQ could be linked to both the learning performance as well as task-induced changes in spindle activity.

4.2 Efficacy of so-tDCS on EEG is dependent upon task-induced spindles

Hypothesis 1b: So-tDCS efficacy is dependent upon such task-induced network modifications

To account for hypothesis 1b, the increased slow spindles after learning may further lead to the facilitation of ongoing brain oscillations by so-tDCS. During the 150-min post-stimulation period, fast spindle parameters at centro-parietal regions were significantly

increased in STIM as compared to SHAM for all participants. This result partially supports the postulation of hypothesis 1b that efficacy of so-tDCS is dependent upon the underlying network modification. The result, however, also proposes that the efficacy of so-tDCS is subjected to inter-individual difference in the network modification. Specifically, only high-MQ participants showed significant improvement in FPA (details will be discussed in **section 4.3**) and only this group of individuals showed task-induced fast spindle power during the acute period.

Furthermore, fast spindle parameters during the 150-min post-stimulation period failed to reach significance when examined the groups separately, suggesting such an increase appears not to be of functional relevance for consolidation of the investigated memories. Indeed, mounting evidence supports the notion that the beginning of SWS is more relevant for the declarative memory consolidation compared to the later time period (Plihal & Born, 1997; 1999; Lahl *et al.*, 2008; Diekelmann *et al.*, 2009). The enhancement of fast spindle power during the acute period in SHAM compared to CTRL may imply a greater task-specific level of NMDA-dependent LTP or spike time dependent plasticity (Piantoni *et al.*, 2016; Ulrich, 2016) that reflects learning process and thus, leads to the facilitation of FPA memory consolidation by so-tDCS.

Despite the failure of the fast spindles induced during the 150-min post-stimulation period to affect any of the memory functions tested across all participants, it is the first time that such long-lasting so-tDCS after-effects are reported. As constant DC stimulation is known for its long-lasting after-effects (Nitsche *et al.*, 2008; Monte-Silva *et al.*, 2013), the anodal component of so-tDCS could contribute in some way to the spindle enhancement. Another plausible mechanism for such a prolonged after-effect lies in the corresponding stimulation frequency relative to the intrinsic oscillatory brain state. Since exogenous stimulation applied in the frequency that matches the natural frequency will induce resonance (Buzsaki, 2006), such resonance could sustain the ongoing slow oscillating network for a long period of time (Reato *et al.*, 2013; Veniero *et al.*, 2015).

4.3 Efficacy of so-tDCS is susceptible to inter-individual difference

Hypothesis 1c: so-tDCS efficacy is dependent upon an inter-individual network component

On the behavioural level, there was no significant improvement in any of the memory tasks in STIM as compared to the SHAM condition across all participants. However, when the participants were split according to their MQ score, high-MQ individuals showed an enhanced memory performance in STIM compared to SHAM in the FPA task. Such enhancement was not observed in the low-MQ group. Furthermore, fast spindle power during the acute period showed an increase in STIM compared to SHAM in the high-MQ but not low-MQ group, although the effect is only marginal. These results support the hypothesis 1c that the efficacy of so-tDCS is dependent on an inter-individual network component.

While the inter-individual difference in MQ explains the distinct efficacy of so-tDCS, it is intriguing that only the FPA task, but not the WPA task was enhanced. Previous studies using so-tDCS during sleep to modulate SWS and declarative memory have mainly reported, although not limited to, enhancement of WPA (Barham *et al.*, 2016). One plausible explanation for the failure of so-tDCS to enhance consolidation on all other three hippocampus-dependent tasks may be owing to an interaction between all the tasks employed. Available evidence shows that the declarative (hippocampus-dependent) and non-declarative (procedural) memory systems operate in a competitive manner during acquisition, whereby an impairment of one system could lead to the facilitation of another (Dagher *et al.*, 2001; Poldrack & Packard, 2003; Gagne & Cohen, 2016). Thus, off-line consolidation processes of these memory tasks could likewise directly interfere with one another and to a certain extent impair the retention (Brown & Robertson, 2007). The neuronal networks of individuals with high MQ after learning may thus be sturdier in preserving the acquired information despite such competitive state (Deary *et al.*, 2010).

Aside from the effect of so-tDCS on behaviour, efficacy to modulate brain rhythms needs to be discussed. Surprisingly, so-tDCS failed to enhance SO activity in any way

despite its beneficial effect on the FPA task. Given the frequently reported increase of SOs accompanying improved memory consolidation with so-tDCS (Marshall *et al.*, 2006; Prehn-Kristensen *et al.*, 2014; Munz *et al.*, 2015; Westerberg *et al.*, 2015; Ladenbauer *et al.*, 2016; Paßmann *et al.*, 2016), the tight relationship between the two is undoubtedly expected. Nonetheless, a study used optogenetics to induce SO in mice also failed to affect memory, leading to the conclusion that the effect on SO incidence alone was insufficient to alter memory consolidation (Latchoumane *et al.*, 2017). Along this line, when SO events were examined closely, Heib *et al.* (2013) found that specific SO properties like the duration of up-state and down-state contribute to the consolidation process. Moreover, in light of recent studies on the relevance of coupling of spindles to a SO phase for memory consolidation, the interplay between these rhythms indeed appears to be a closer correlate of consolidation than the power of SOs per se (Cox *et al.*, 2012b; Ngo *et al.*, 2013a; Weigenand *et al.*, 2016; Ngo *et al.*, 2019). Furthermore, it has recently been reported that so-tDCS has the capacity to increase coupling strength of fast spindle to SO, and that increased coupling strength was associated with enhanced memory consolidation (Ladenbauer *et al.*, 2017). Thus further underscoring a crucial role of SO-fast spindle coupling for memory consolidation, and raising the question of whether previous reports on so-tDCS efficacy were linked to facilitating this coupling; a concept of ongoing investigations.

The finding that only the high-MQ group with the increased FPA memory performance showed a significant increase of fast spindle power after learning and tended to reveal an increase in fast spindle power over frontal and decrease over the parietal region after so-tDCS may lead to the following speculation: The increase in fast spindle power may reflect an efficient coupling between fast spindle and SOs. Considering this study is the first to employ individual SO frequency for stimulation, the matching frequencies between the exogenous stimulation and the intrinsic ongoing brain rhythm may consequentially resonate and thereby construct an efficient temporal coordination between SO and spindle. This speculation can be supported by mounting

evidence that demonstrates matching exogenous stimulation to the ongoing brain rhythm is favorable for neural entrainment and/or plasticity (Hutcheon & Yarom, 2000; Kanai *et al.*, 2008; Fröhlich & McCormick, 2010; Ozen *et al.*, 2010; Vosskuhl *et al.*, 2018). Albeit to note, the coupling was not measured here and further studies are needed in this regard.

Taken together, better learning performance in high-MQ individuals could translate into a post-learning neuronal circuitry. In this state, the network would be highly susceptible to the sub-threshold inputs from anodal so-tDCS and eventually leads to better memory consolidation.

4.4 So-tDCS during wakefulness suppresses the ongoing dominant alpha network

Hypothesis 2a: so-tDCS can induce EEG frontal SO and theta activity

In contrast to the hypothesis 2a, so-tDCS applied over the DLPFC during quiet wakefulness did not induce EEG SO or theta activity in Study II. However, so-tDCS suppressed the ongoing alpha power. One plausible explanation for the effect on alpha activity is that so-tDCS is a frequency-dependent technique (Marshall & Born, 2011). Alpha oscillations prevail during quiet wakefulness with eyes closed (Pfurtscheller, 1997), largely due to the blockage of incoming visual input (Barry *et al.*, 2007). The large discrepancy between SO (0.75 Hz) frequency and the endogenous oscillating frequency (alpha peak ~ 10 Hz) presumably perturbed the ongoing synchronized alpha rhythm. A comparable observation has been previously reported by Marshall *et al.* (2011), where the authors utilized theta-tDCS during SWS during which endogenous SOs are dominant. Such stimulation disrupted the SWS as well as decreased SO and slow spindle power. On the other hand, when theta-tDCS was applied during REM sleep where theta activity is prominent, an increment in corresponding gamma activity was seen (Marshall *et al.*, 2011).

The above effects of so-tDCS are however not absolute. Another study utilized anodal theta-tDCS and so-tDCS during eyes-closed resting state, as in Study II, and reported an increase of alpha power after so-tDCS as well as an increase in delta power during theta-tDCS, although no task was introduced prior to the stimulation (D'Atri *et al.*, 2016). Based on results reported by Alagapan *et al.* (2016), the authors proposed that task-related input could tune the subsequent intrinsic network into a state that is more susceptible to perturbations. Thus, the stimulation effect depended on behavioural state, which is similar to the conclusion in Hypothesis 1 above. Another study using alpha-tACS during eyes closed (i.e. high alpha power) and eyes open (i.e. low alpha power) observed that stimulation could enhance EEG alpha power under the condition involving a state of low but not high endogenous alpha power, suggesting that the brain may be more susceptible to oscillatory electric stimulation when the corresponding endogenous neural oscillation is weakly pronounced (Neuling *et al.*, 2013). However, whether the response of tACS is similar to anodal so-tDCs would need to be systematically studied. Assuming the brain's responses to so-tDCs are similar, it could be speculated that the suppression in alpha activity was due to the prior procedural task, which may have strengthened post-task EEG alpha power (Henz & Schöllhorn, 2016). It could more likely be, that the applied so-tDCs was sufficient in hampering the ongoing neural network generating alpha activity, but was not strong enough to override the activity of endogenous brain state. Thus, SO was not induced. This is supported by the supplementary experiment that used IAF-tDCS where alpha power in this cohort was no longer suppressed. Moreover, the applied current intensity over the DLPFC was rather weak compared to other tDCS studies (Bikson *et al.*, 2016). A higher dose with a larger electrode size may be more appropriate to induce the desired oscillations when introducing discrepant frequencies between endogenous and exogenous rhythms (Ho *et al.*, 2016).

4.5 SO-tDCS during wakefulness disrupts motor consolidation

Hypothesis 2b: so-tDCS has no effect on the procedural behavioural task performance

The behavioural task was used to induce a comparable brain state between sessions and individuals, thus no difference at retest was expected as stated in hypothesis 2b. The finding that retention performance was impaired in STIM leads to a rejection of the hypothesis. Increased accuracy on the FSTT after wakefulness in SHAM suggests that during the relatively short experimental session, an undergoing consolidation process took place. It could be postulated that the neural processes after learning a motor skill continue to evolve with the passage of time (Doyon *et al.*, 2003; Krakauer & Shadmehr, 2006; Henz & Schöllhorn, 2016), and this process is susceptible to disruption (Brashers-Krug *et al.*, 1996). Consequentially, the impaired motor performance at retest was due to the interference of the SO component with the putative ongoing frontoparietal network - a network found to be increased after motor learning (Albert *et al.*, 2009b), evidenced by indifference between STIM and SHAM in FSTT performance after IAF-tDCS (supplementary experiment).

However, it is worth mentioning that the lack of a significant correlation between the suppressed alpha and impaired behaviour suggests these two factors are not directly associated. In addition, the sample size in supplementary experiment is rather small and results are to be interpreted with caution.

In sum, the procedural task prior to stimulation presumably resulted in a network that was susceptible to perturbation. Hence, so-tDCS applied during this period may have disrupted the ongoing functional network and ultimately lead to decrement in FSTT performance.

4.6 ACLS acutely augments SO and fast spindle activity during NREM sleep

Hypothesis 3a: Auditory stimulation delivered in-phase with the SO depolarisation up-state (ACLS) will induce SOs, slow and fast spindle activity in NREM sleep

In agreement with hypothesis 3a, ACLS successfully enhanced the amplitude of SO and fast spindle RMS across several topographies during NREM sleep. EEG power in other frequency bands (i.e. SWA, delta, theta, fast and slow spindles) during NREM sleep was nevertheless not affected by the stimulation. The augmentation of SO amplitude closely resembled results of previous studies, analysing effects during nocturnal sleep as well as an afternoon nap (Ngo *et al.*, 2013a; Ngo *et al.*, 2015; Ong *et al.*, 2016; Leminen *et al.*, 2017; Papalambros *et al.*, 2017; Ong *et al.*, 2018; Patanaik *et al.*, 2018; Henin *et al.*, 2019), illustrating ACLS is an efficient method to target SOs and the fast spindles that nested within the depolarised up-state. Slow spindle activity was however not affected in NREM sleep.

Although it is well-known that SO in many regions interacts with sensory-evoked responses (Gao *et al.*, 2009), the underlying mechanism of ACLS on enhancing the SOs are still unclear. One hypothetical mechanism proposed by Bellesi *et al.* (2014) is that the auditory stimulus interacts with the non-lemniscal pathway, a pathway that is secondary to sensory modalities in which it involves the thalamic networks that project to cortical layer I; this interaction may thus lead to a large and efficient synchronization of large cortical population which is reflected as a slow wave in the EEG. While comparing different stimulation modalities, Riedner *et al.* (2011) observed that stereotypic KCs occurred in response to all sensory modalities, suggesting more generally that a stereotypic KC may be the result of the involvement of non-lemniscal pathway (referred to nonspecific pathway), and that auditory stimulation evoked the greatest stereotypic KC slow wave as compared to the other modalities. This phenomenon resonates with other studies using auditory stimulation (Fernandez & Luthi, 2019) as well as the results demonstrated in Study III that ACLS during NREM sleep evoked P1, N550, and P900.

Therefore, it is rational to assume, although the non-lemniscal pathway may not be the only route, through which ACLS enhances SO.

Fast spindle activity was enhanced during the peak of the up-state but decreased thereafter. The increment suggests a strengthening of the temporal coupling between these two oscillations (Mölle *et al.*, 2011; Klinzing *et al.*, 2016). The subsequent spindle reduction is in line with the reduced amplitude of the subsequent SO hyperpolarisation. The diminished subsequent spindle power upon the second click in NREM may imply the refractoriness of spindle generation (Antony *et al.*, 2018). Indeed, a similar study employed more than two clicks during ACLS found no further temporal enhancement of SO as well as spindles, further affirmed the refractoriness of spindle generation (Ngo *et al.*, 2015; Ong *et al.*, 2016; Papalambros *et al.*, 2017; Ong *et al.*, 2018).

Slow spindle activity, on the other hand, was not enhanced during NREM sleep as reported previously for nocturnal sleep (Ngo *et al.*, 2013a). This could in part be explained by the timing of stimulation. As fast spindle are nested preferentially at the down-to-up state of a SO — the phase where ACLS targeted, it is credible that ACLS had a larger effect on fast spindle than on slow spindle. Another aspect is the shallow SWA depth in an afternoon nap compared to nocturnal sleep. As slow spindles occur more frequently in SWS than in N2 (Mölle *et al.*, 2011), ACLS might have only a weak effect on slow spindles due to the limited amount of occurrence. In short, ACLS efficiently augmented SO amplitude and possibly strengthened the temporal coupling of SO-fast spindle, evidenced by increased in fast spindle activity during the up-state of SO.

4.7 Differential electrophysiological effect of ACLS during N2 and N3 sleep

Hypothesis 3b: ACLS will have a unique effect on SO, slow and fast spindles in N2 compared to N3

When examined the effect of ACLS separately in N3 and N2, there was no significant upsurge of SO amplitude responding to the ACLS in N2, although a slight increase in hyperpolarisation down-state was visible. On the other hand, SO was significantly

enhanced across midline sites in N3. Moreover, fast spindle and slow spindle power in N2 showed a rather distinct effect. Whereas the fast spindle was enhanced only at ca. 1 s in N3, two significant peaks were found at ca. 1 s and 2 s in N2. Slow spindles, in general, were not significantly affected in N3 but had a global increase in power in N2. These observations are consistent with the hypothesis 3b that ACLS does have a unique effect in N2 as compared to N3.

Many earlier studies that employed auditory stimulation during different sleep stages have already shown disparate AEPs response, suggesting different brain-states profoundly modified its response to external stimuli (Nielsen-Bohlman *et al.*, 1991; Plihal *et al.*, 1996; Cote *et al.*, 2000). From the micro-state however, the ACLS was applied specifically at the optimal time point (i.e. SO down-to-up state) when the brain is most receptive to external stimuli (Massimini *et al.*, 2005; Schabus *et al.*, 2012) and the optimal timing to increase likelihood of spindle occurrence and enhanced SO amplitude (Navarrete *et al.*, 2019). The enhancement of SO in N3 is undoubtedly consistent with many other studies using both ACLS or auditory stimulation (Bellesi *et al.*, 2014; Zhang & Gruber, 2019). The lack of significant enhancement of SO in N2 could be attributed to a number of factors. First, the small number of stimulus amount applied during this sleep stage may yield a large variability (evidenced by a large standard error) that could consequentially hamper the ACLS effect. Second, the SOs detected in N2 were profoundly single event KCs rather than a train of SOs, due to the prominent emergence of KC in this sleep stage (Colrain, 2005), thus, a following SO train was absent in N2.

In terms of the differential spindle responses found in N2 and N3, the distinct emergence of fast and slow spindles between sleep stages have been previously reported (Mölle *et al.*, 2011; Ayoub *et al.*, 2013; Cox *et al.*, 2017; Dehnavi *et al.*, 2019) (see **section 1.3.1.2**). Likewise, a differential function of the two types of sleep spindles has also been observed (Barakat *et al.*, 2011). Given the stimulation phase of ACLS, it is likely that ACLS optimizes the synchronization of SO and fast spindle in N3. On the other hand, the global

enhancement of slow spindle power in N2 is comparable to the observation that auditory stimulation applied during N2 revealed enhanced numbers of the spindle (Sato *et al.*, 2007). It is well-established that spindles modulate the transmission of auditory inputs during sleep (Schabus *et al.*, 2012) and that the more sleep spindles are associated with better tolerance for noise during sleep (Dang-Vu *et al.*, 2010), thus, the increase in slow spindle activity in N2 sleep might be due to the sensory gating mechanism. Furthermore, differences in neuromodulators/neurochemical status, which change within NREM sleep of different depths (Feld & Born, 2020) presumably contribute to the differential responses in N2 and N3.

4.8 ACLS during sleep improves only post-sleep FPA encoding performance

Hypothesis 3c: If ACLS induces SO and spindles, encoding capacity will be enhanced

Aside from the prominent enhancement of SO and spindle activities, post-sleep learning on the FPA task was marginally improved. This result is consistent with the hypothesis 3c but contains potentially open questions. The enhanced SO and better FPA encoding in ACLS parsimoniously supports the hypothesis 3c. These results are concomitant with previous findings that showed experimentally enhanced SO activity was associated with better subsequent encoding (Antonenko *et al.*, 2013; Ong *et al.*, 2018). Nevertheless, besides of the augmented SO, fast spindle in N3 and slow spindle in N2 were also enhanced, therefore, it is difficult to resolve if the enhanced spindles also in some way contributed to the subsequent encoding. Although there are only a handful of studies that explored the function of spindles in encoding, Mander *et al.* (2011) showed that prior episodic learning followed by sleep had better subsequent learning performance compared to when wakefulness substituted sleep. The authors further revealed the left prefrontal fast spindles are positively correlated with the post-sleep episodic learning ability. A following study reported an association between prefrontal fast spindles and next day hippocampal functioning, and such association determined the episodic learning ability (Mander *et al.*, 2013). More importantly, the authors also indicated that the

reduction of prefrontal fast spindles in older individuals led to poorer sleep-dependent restoration of next day learning ability, underscoring the importance of fast spindles in new memory formation (Mander *et al.*, 2013). Based on these findings, the enhanced SO and fast spindles, which both serve the key function in sleep and memory processes, are presumably involved in the synaptic downscaling process and are relevant for the subsequent encoding ability.

Interestingly, the post-sleep encoding performance was only improved in FPA but not in the other declarative tasks. This observation raises the question of why the other tasks, especially WPA and VLMT, which are both SWS relevant, were not improved. Due to the small sample included, it is arbitrary to provide any conclusive statement. Nonetheless, one can postulate that it is the novelty component in the FPA task that leads to better encoding (Poppenk *et al.*, 2010; Poppenk *et al.*, 2013). Moreover, one study employed so-tDCS during an afternoon nap enhanced SWA together with better WPA and VLMT encoding performance, proposing the depth of SWA in a single sleep cycle is relevant to impact these tasks (Antonenko *et al.*, 2013). Thus, the mere enhancement of SO and spindles as an event may not be robust enough to facilitate the whole hippocampus-dependent memory neural network system. Furthermore, sleep-dependent encoding has been reported to be task-specific (i.e. declarative vs. procedural) (Mander *et al.*, 2011; Antonenko *et al.*, 2013).

The electrophysiology during learning revealed no significant changes after the stimulation. Albeit the activation seems higher in SHAM upon the presentation of figural pairs, the limited amount of trials and the small sample size in Study III restricts generalization of the result and should be taken with caution.

Taken together, ACLS in-phase with the SO depolarisation up-state could enhance SO and fast spindles activity but not slow spindle in NREM sleep. The ACLS effect differed greatly between N2 and N3 on SO, slow and fast spindles, conceivably due to distinct dominant neuronal oscillations upon the delivery of ACLS that were modified. It is likely

that the enhanced SO and fast spindles that represent hippocampus-dependent memory mechanism during N3 accounts for the enhancement of FPA encoding.

5. General discussion

Study I and Study II not only showed that so-tDCS is an efficient tool to modulate functional networks and their relevant behavioural performance but also indicated that the efficacy of so-tDCS is brain state dependent. Study III further confirmed the importance of brain state upon the application of stimulation, by indicating differential modulations in N2 and N3. These observations coincide with other experimental studies (Ben-Simon *et al.*, 2008; Kanai *et al.*, 2008; Zaehle *et al.*, 2010; Marshall & Binder, 2013; Alagapan *et al.*, 2016). In fact, it should come as no surprise that different human brain states constitute distinct underlying neuronal network properties in order to function appropriately according to the surrounding environment (Cote *et al.*, 2000; Cantero *et al.*, 2002; Buzsaki, 2006). Subsequently, when applying exogenous stimuli aiming to alter the temporal dynamics underlying networks or neuronal properties, these stimuli tend to resonate with the dominant endogenous ongoing oscillations (Zrenner *et al.*, 2016). This is supported by the results of Study I, where learning profoundly enhanced subsequent TC spindle activity at the essential time period in individuals who learned better. The enhanced spindle activity may resonate with the exogenously applied currents and thus facilitate the active memory consolidation system, ultimately leading to better memory performance. On the other hand, exogenous stimuli deviating from the brain rhythms upon stimulation interfered with the ongoing neural oscillations. In the case of Study II, SO frequency perturbed the dominant alpha rhythms and led to impaired behaviour.

Interestingly, only the FPA task was altered in both Study I and Study III but not the WPA task as reported by previous studies. It may well be the different form of retrieval (i.e. free recall and recognition) that contribute to the different results. Indeed, a previous study depicted that although free recall and recognition memory shared underlying encoding mechanism; the activation in DLPFC and posterior parietal cortices

involved in free recall suggested a different order of internal mnemonic representation for embedding the encoded item (Staresina & Davachi, 2006). Tasks similar to FPA would thereby involve encoding into a rich associative network that would facilitate later recall. One could also speculate that the greater recollection component together with the novelty property of the FPA task has a greater dependency on the hippocampus (Poppenk *et al.*, 2013). This is supported by the results that fast spindle activity, which co-occurs with hippocampal sharp-wave ripples (Niknazar *et al.*, 2015; Cox *et al.*, 2019) and is associated with hippocampal activation and hippocampal-cortical functional connectivity (Schabus *et al.*, 2007; Andrade *et al.*, 2011), was enhanced on both so-tDCS and ACLS.

The enhancement of hyperpolarisation down-state and depolarisation up-state of SO in Study III indicated SO may contribute to the synaptic downscaling process, perhaps through modulating the sodium-dependent potassium current. This process may strongly rely on activity of the sodium-dependent potassium current which was shown to lead to a longer and more pronounced hyperpolarisation down-state (Tononi & Cirelli, 2003; Tononi & Cirelli, 2006). Furthermore, considering the involvement of cortical SOs in synchronizing TC spindles and hippocampal sharp-wave ripples, thereby transferring memory traces from temporary storage in hippocampus into neocortex for long term storage (Rasch & Born, 2013), it is conceivable to infer that SOs attribute to some extent to the synaptic downscaling and SOs enhanced by ACLS further facilitate the process. However, since concurrent augmented SO together with enhanced fast spindle were found, it is at this stage not possible to rule out the role of spindle in subsequent learning.

A few limitations of this dissertation should be addressed. First, the lack of sufficiently matched non-learning paradigm in the control session of Study I dampened the effect of task-induced network modification, as learning may have occurred while watching the documentary. Second, the small sample size in Study III limits any

generalisation of the results. Third, the large difference in the number of detected SO in N2 and N3 sleep made it difficult to perform a direct comparison between sleep stages.

In summary, learning is profoundly influenced by the type of tasks and inter-individual differences. These factors could lead to a difference in post-learning macro-state as well as micro-state activity/levels, and thereby influence the efficiency of stimulation. As different brain rhythms occur at different brain states, stimulation applied during a particular brain state would interact with the ongoing oscillations, thus potentially influence network activity and subsequent behavioural performances in a modified way. It is to note hereby, that visible modification in brain electric activity is not mandatory for a behavioural change and vice versa (see **Figure 20**).

6. Conclusion

NIBS has enabled researchers to obtain a broader perspective of neuroelectrophysiological processes during sleep and wake. It has brought us a step towards gaining a deeper understanding of the interaction between (human) brain rhythms. However, there are many potential variables confounding the effects of stimulation that result in different outcomes. The findings of all three studies only provide a glimpse into a “secret garden”. They reveal important aspects to consider in future research with NIBS and investigations on brain rhythms, learning and memory. Below is a schematic summary of this dissertation.

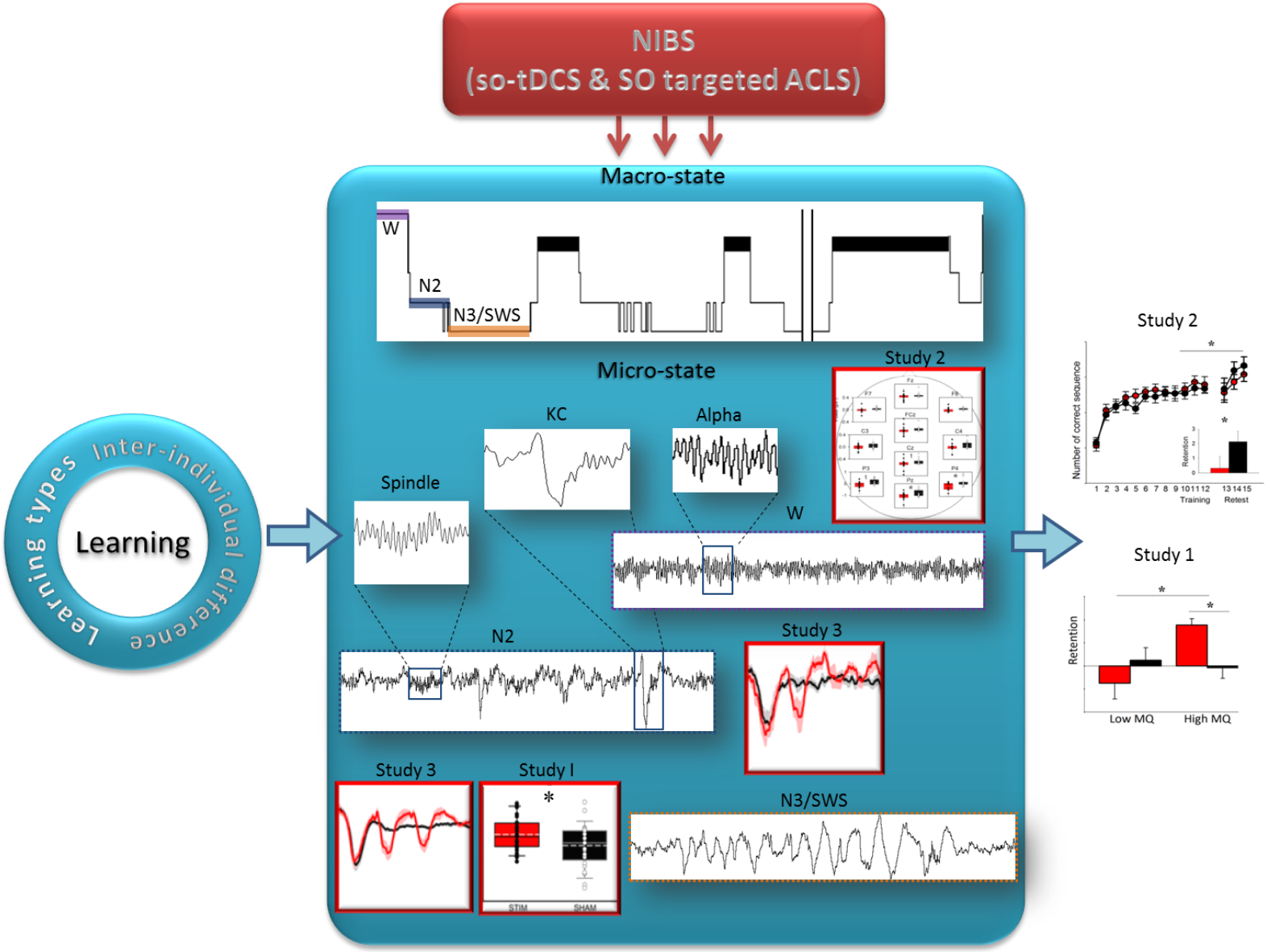

Figure 20. Schematic summary of this dissertation. Macro-state comprises of different vigilance states such as wake (in purple), N2 (in blue), and N3/SWS (in orange), respectively. Depending on the macro-state when NIBS was delivered, it could profoundly modulate the ongoing micro-state, such as sleep spindle, K-complex (KC) or wakeful alpha oscillations. Such modulations could influence the behavioural performance subsequently (e.g. enhanced or impaired).

7. Bibliography

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8. Appendices

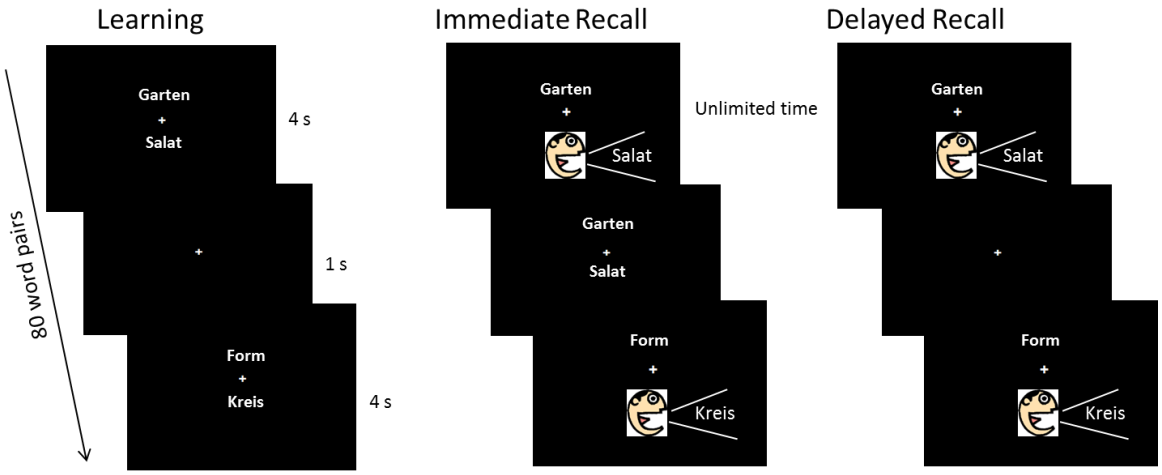
8.1 Abbreviation

AASM	American Academic of Sleep Medicine
ACLS	Auditory Closed-Loop Stimulation
AEPs	Auditory Evoked Potentials
COND	Condition
CTRL	Control
DC	Direct Current
DLPFC	Dorsal lateral prefrontal cortex
EEG	Electroencephalography
EMG	electromyogram
EOG	electrooculograms
ERD	Event-related Desynchronization
ERP	Event-Related Potential
ERS	Event-related Synchronization
FFT	Fast Fourier Transformation
fMRI	Functional Magnetic Resonance Imaging
FPA	Figural-paired Associate
FSTT	Finger Sequence Tapping Task
IAF	Individual Alpha Frequency
ICA	Independent Component Analysis
IIR	Infinite Impulse Response
IL	Interference List
IQ	Intelligent Quotient
KCs	K-Complexes
LTD	Long term depression
LTP	Long term potentiation
MQ	Memory Quotient
MT	Mirror Tracing
N1	Sleep stage 1
N2	Sleep stage 2
N3	Sleep stage 3
NIBS	Non-Invasive Brain Stimulation

NREM	Non-Rapid Eye Movement
PANAS	Positive and Negative Affect Scale
PVT	Psychomotor Vigilance Test
REM	Rapid Eye Movement
rmANOVA	Repeated-Measured Analysis of Variance
RMS	Root-Mean-Square
SEM	Standard Error Mean
SO	Slow Oscillation
So-tDCS	Slow oscillatory-transcranial Direct Current Stimulation
SSS	Standford Sleepiness Scale
STIM	Stimulation
SWA	Slow Wave Activity
SWS	Slow Wave Sleep
tACS	transcranial Alternating Current Stimulation
TC	Thalamocortical
tDCS	transcranial Direct Current Stimulation
TFR	Time Frequency Representation
TIB	Time in Bed
TMT	Total Movement Time
TOPO	Topographic
TST	Total Sleep Time
TWT	Total Wake Time
VLMT	Verbal Learning Memory Test
WPA	Word-paired Associate

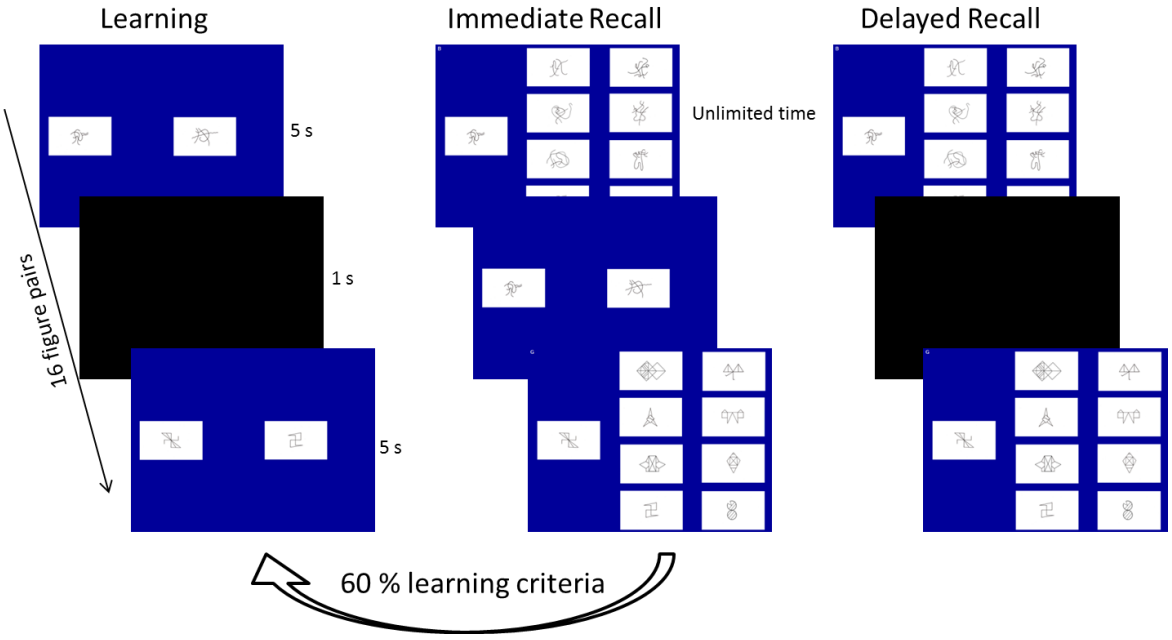
Supplementary note/figures

Supplementary Figure 1



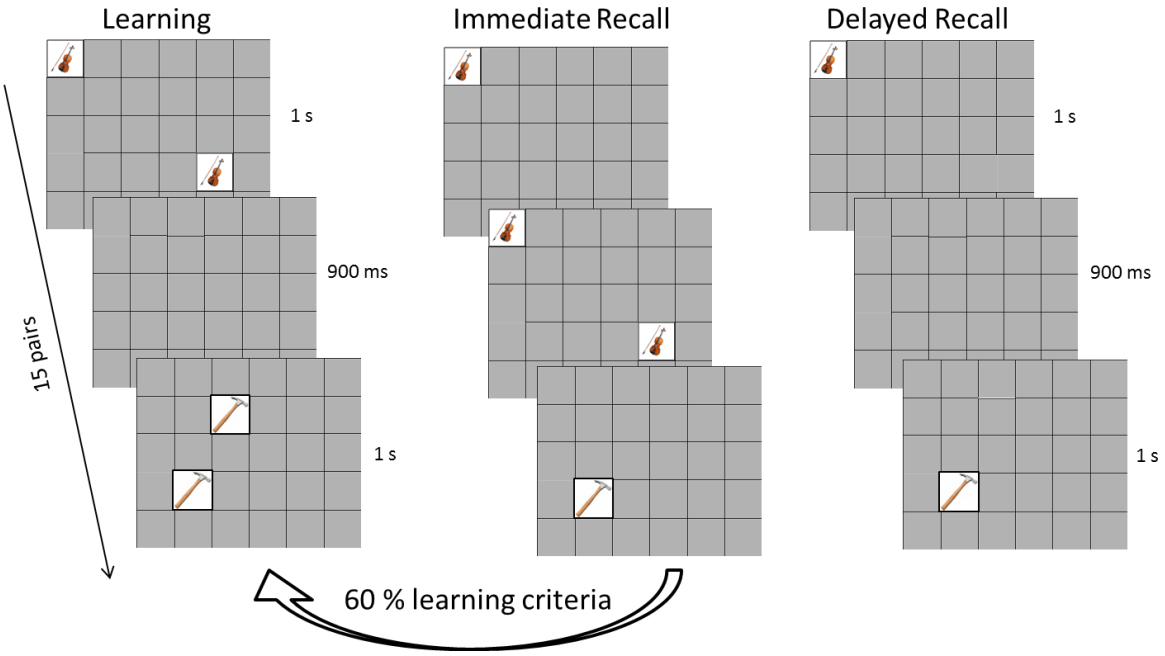
Supplementary Figure 1. WPA task procedure employed in Study I. Feedback was given during immediate recall but not delayed recall. See **section 2.1.4.1.**

Supplementary Figure 2



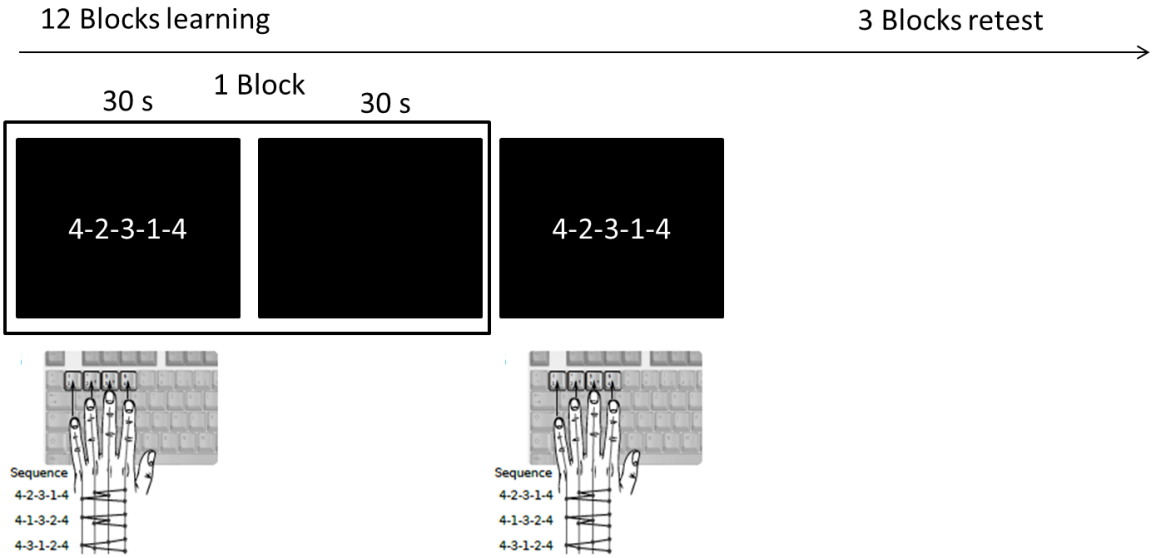
Supplementary Figure 2. FPA task procedure employed in Study I. Feedback was given during immediate recall but not delayed recall. A 60 % of learning criterion was required during the immediate recall. The maximal learning blocks amongst the participants were 6 times. See **section 2.1.4.2**.

Supplementary Figure 3



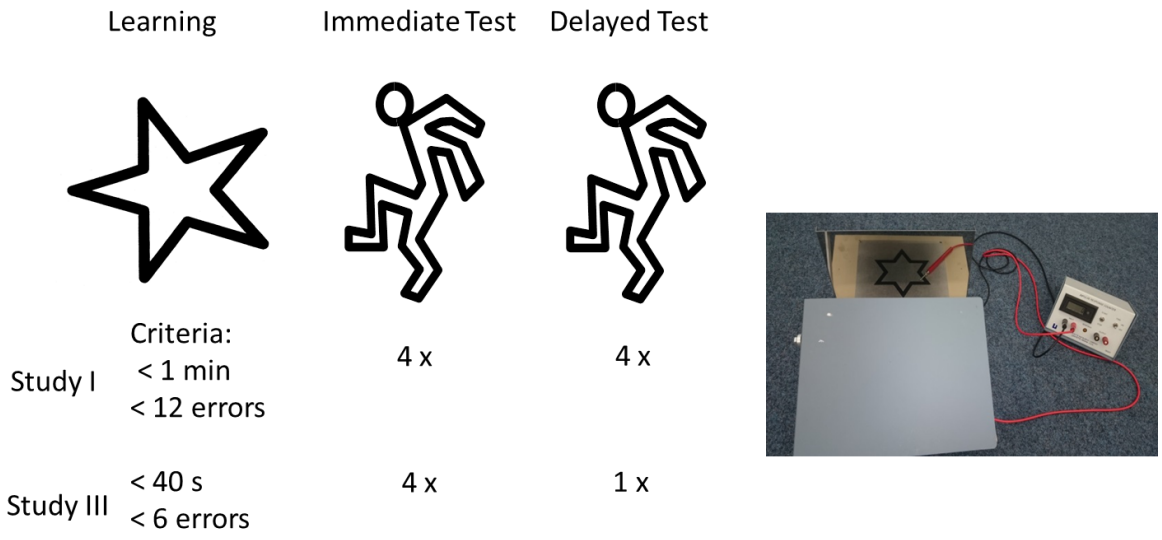
Supplementary Figure 3. 2D-object location task procedure employed in Study I. Feedback was given during immediate recall but not delayed recall. See **section 2.1.4.3**.

Supplementary Figure 4



Supplementary Figure 4. FSTT procedure employed in Study I and Study II. The learning consists of 12 learning blocks and the subsequent recall consisted 3 blocks of retest. See **section 2.1.5.1**.

Supplementary Figure 5

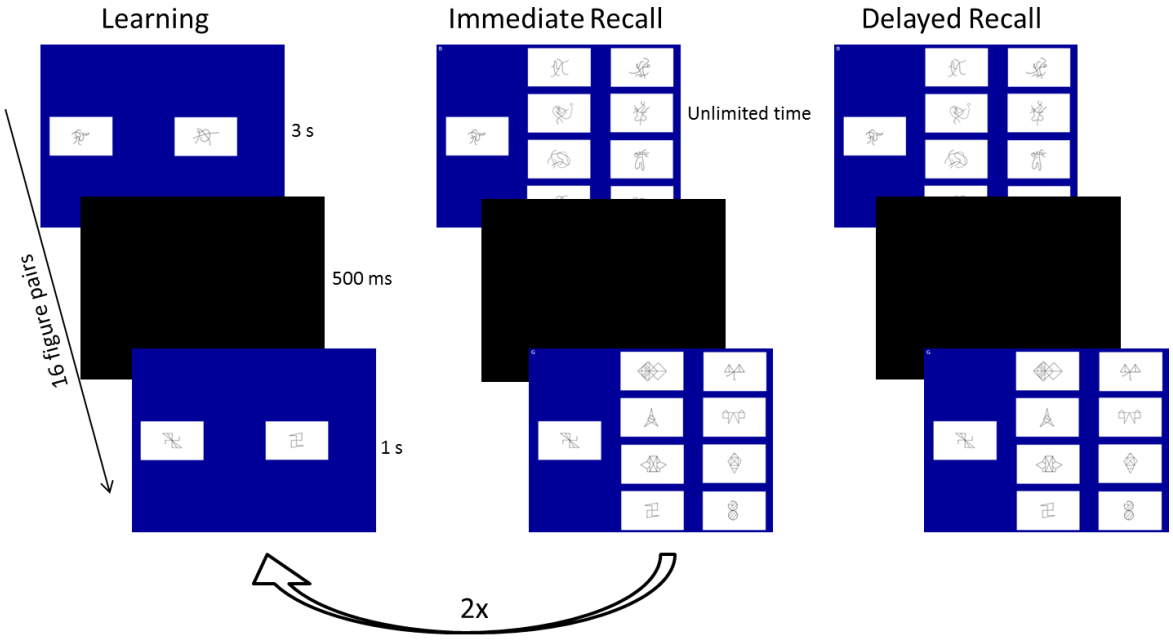


Supplementary Figure 5. MT procedure employed in Study I and Study III. To focus on the learning component, stricter criteria were used in Study III. See **section 2.1.5.2**.

Supplementary Figure 6

Supplementary Figure 6. The WPA task encoding procedure. Feedback was neither given during immediate nor delayed recall. See **section 2.3.3.1**.

Supplementary Figure 7

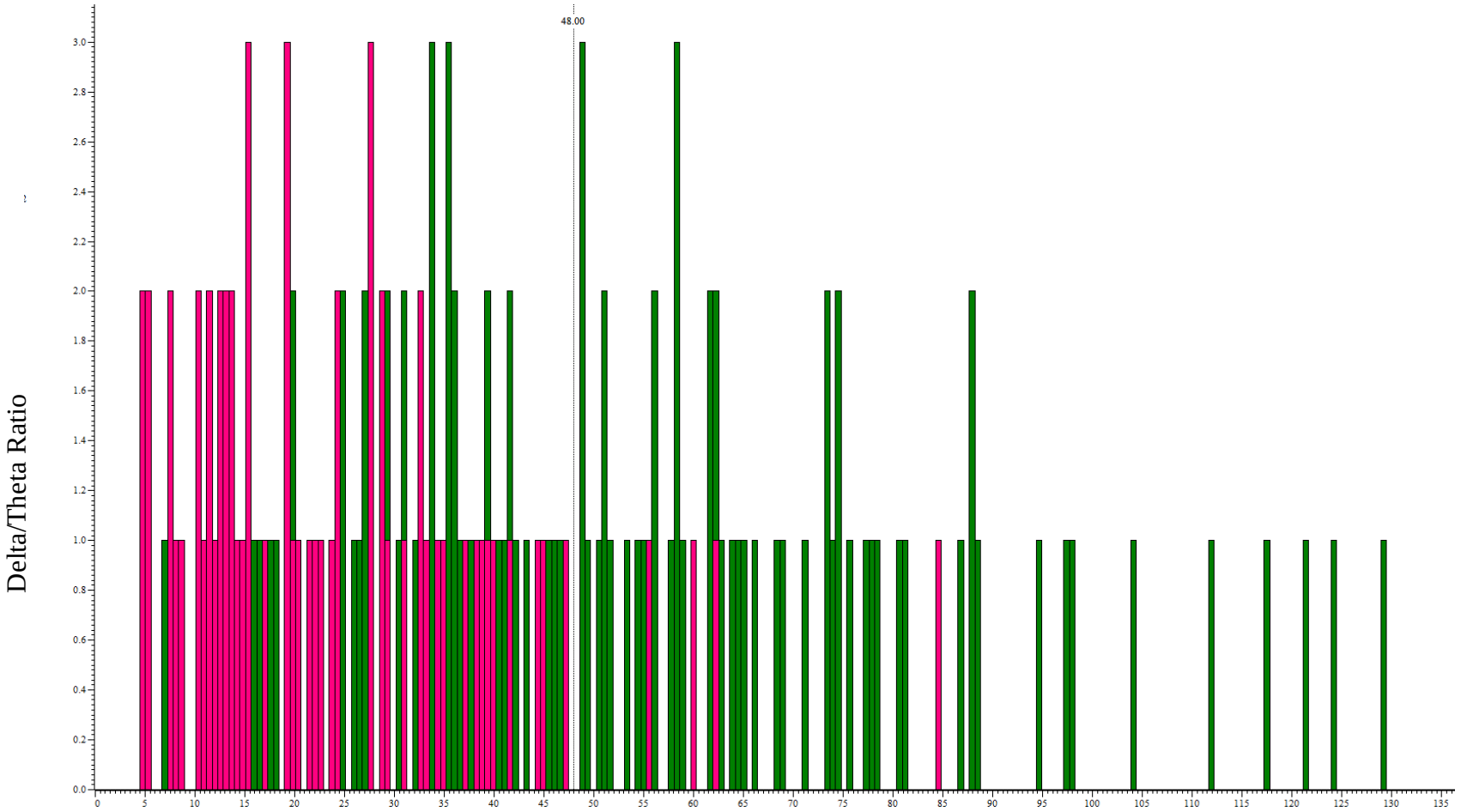


Supplementary Figure 7. FPA task encoding procedure. To note, the presentation time and inter-stimulus interval is shorter in Study III to promote learning and feedback was not given during immediate recall. The learning blocks were fixed to two blocks. See **section 2.3.3.2**.

Supplementary note – Study III

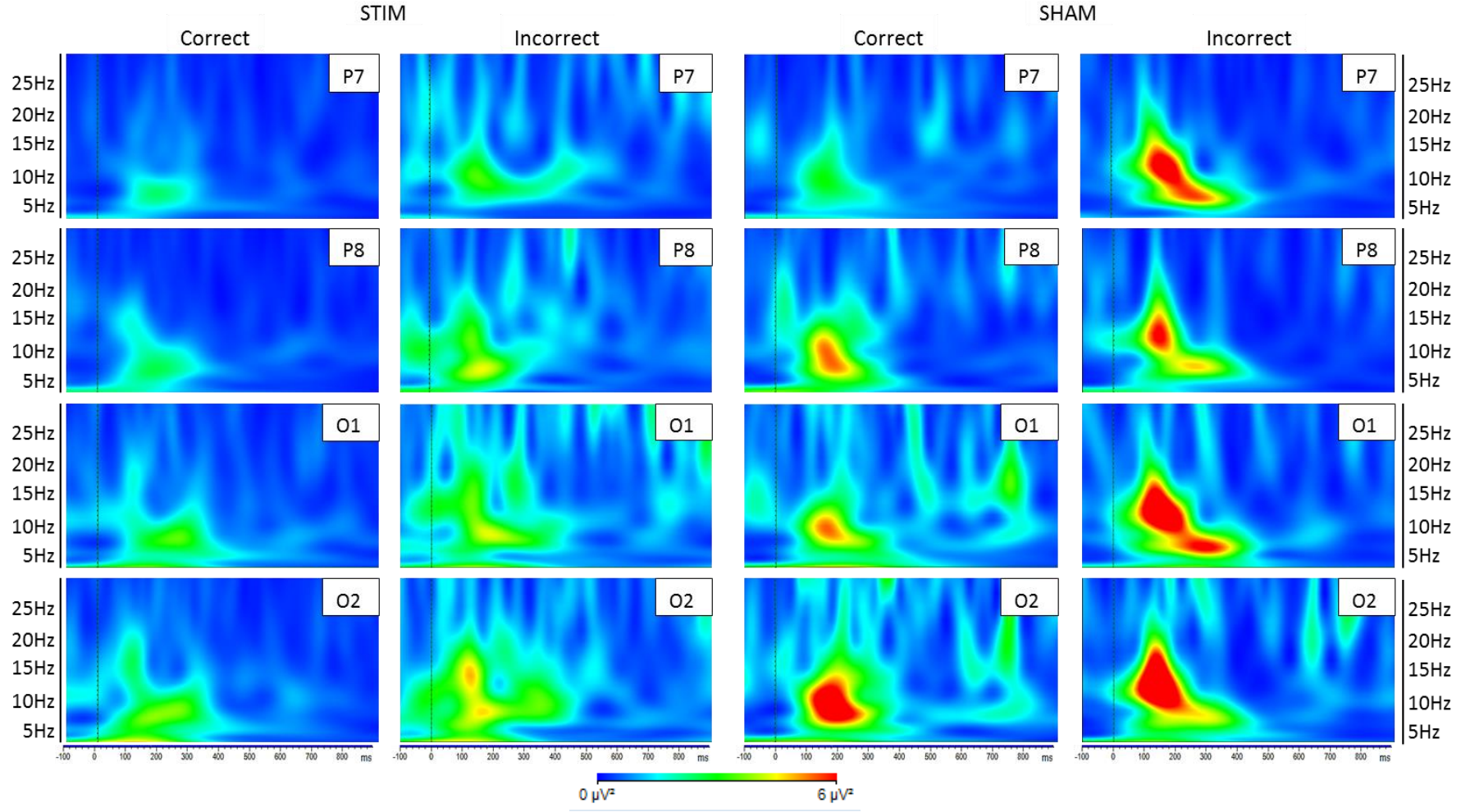
As wavelets analysis comprises of time-frequency trade-off, a higher resolution decrease the frequency resolution and vice versa. The morlet parameter *c* can modulate such trade-off by determines the number of cycles of the wavelets. The lower the morlet parameter *c*, the larger the spectral width is in the lower frequency (Herrmann *et al.*, 2005). The morlet parameter *c* was defined as 4 due to our interest in lower frequency (theta and alpha) (Herrmann *et al.*, 2005). Given that the wavelet length entails the formular *WL(f)=c/f*. Hence, *WL(f)=4/9 Hz* leads to the end result of 444 ms. Safety margin at borders and stimulation is calculated as *WL/2*, thus 222 ms. The maximum baseline interval was calculated as *stimulation interval-WL/2* and minimum baseline interval was calculated as *0-WL/2,* in which it yielded -278 ms and minimum -222 ms. This will result in only 56 ms of reference interval. Thus the baseline normalization was omitted.

Supplementary Figure 8



Supplementary Figure 8. Example of Delta/Theta ratio plot obtained from an adaptation. The vertical line was placed at the approximate interchange between NREM (green bars) and REM (red bars), where number of NREM increase along with decrease in REM. In this case delta/theta ratio was defined as 48 for the following main sessions. The x-axis represents the number of delta/theta ration, and y-axis is the number of occurrence of the delta/theta ratio.

Supplementary Figure 9



Supplementary Figure 9. Wavelet for correct and incorrect pairs in FPA task separately in STIM and SHAM. Due to technical issues, the triggered of two participants in SHAM and one in STIM were not sent, hence excluded for analysis. The low sample size might partially contribute to the insignificant difference due to larger variance.

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In memory of my father, I dedicate this dissertation to him, whom he dedicated his whole life caring and loving me.