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DISSERTATION

Effects of slow oscillatory transcranial direct current stimulation (so-tDCS) on sleep-dependent memory consolidation

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Abstract

I. German

Hintergrund: Es gibt zunehmende Evidenz, dass Schlaf eine aktive Rolle in der Gedächtniskonsolidierung spielt. Insbesondere wird in diesem Zusammenhang die Bedeutung langsamer Oszillationen (< 1 Hz) für die schlafbezogenen Gedächtniskonsolidierungsprozesse diskutiert. In einer wegweisenden Studie, in der eine langsam oszillierende transkranielle Gleichstromstimulation (so-tDCS) appliziert wurde, konnte bei jungen Probanden eine erfolgreiche exogene Manipulation dieser langsamen Oszillationen sowie eine Verbesserung des deklarativen Gedächtnisses beobachtet werden. Spätere Studien, die mit ähnlicher Methodik durchgeführt wurden, zeigten jedoch widersprüchliche Ergebnisse. Die Wirksamkeit dieser neuromodulatorischen Technik wird deshalb in Frage gestellt.

Ziel: In dieser Studie wurde untersucht, ob mittels so-tDCS spezifische neurale Oszillationen während des Schlafes moduliert werden können und das deklarative Gedächtnis gesteigert werden kann. Das Ziel war es, die Ergebnisse der Pionierstudie an gesunden jungen Probanden zu replizieren. Dazu wurde ein leicht modifiziertes Stimulationsprotokoll verwendet, welches zuvor an älteren Probanden angewandt wurde.

Methoden: In einem doppelblinden, placebo-kontrollierten Laborexperiment mit randomisiertem Crossover-Design wurde der Effekt von bifrontal applizierter anodaler so-tDCS (Frequenz 0,75 Hz) während des Schlafstadium 2 (N2) des Non-REM Schlafes auf die Ergebnisse eines Wortpaar-Assoziationstests und einer Finger-Tapping-Aufgabe an 23 gesunden Probanden (Mittelwert ± Standardabweichung: 23.2 ± 1.9 Jahre; 13 Frauen) überprüft. Stimulationseffekte wurden für Schlafstadien, die Schlafspindeldichte und die EEG-Power analysiert. Weiterhin wurde der Einfluss der so-tDCS auf die deklarative und prozedurale Gedächtniskonsolidierung überprüft.

Ergebnisse: Weder auf Verhaltens-, noch auf physiologischer Ebene wurden signifikante Stimulationseffekte beobachtet. Unter beiden Stimulationsbedingungen verbesserte sich die Gedächtnisleistung über Nacht bei der prozeduralen Aufgabe, während sie sich bei der deklarativen Aufgabe verschlechterte. Hatten die Probanden jedoch zusätzliche Lernmöglichkeiten, verringerte dies die Abnahme der deklarativen Gedächtnisleistung. Unabhängig von der Stimulation kam es zu einer Abnahme der schnellen parietalen Spindeldichte von der Baseline (vor Stimulation) zu den

stimulationsfreien Intervallen, während bei der langsamen frontalen Spindeldichte kein signifikanter Unterschied auftrat.

Schlussfolgerungen: Die vorliegende Studie konnte die Ergebnisse der Pionierstudie nicht reproduzieren. Unsere Ergebnisse stimmen jedoch mit einer früheren Studie überein, die das gleiche Stimulationsprotokoll bei älteren Probanden verwendete. Das Ausmaß der nächtlichen Konsolidierung von deklarativen Gedächtnisinhalten war davon abhängig, ob es eine Möglichkeit zur Wiederholung der Lerninhalte gab. Die Standardisierung des Studienprotokolls und eine Berücksichtigung individueller Variabilität sind essentiell für so-tDCS Studien.

II. English

Background: There is growing evidence that sleep plays an active role in memory consolidation. Specially, there are indications that slow oscillations (< 1 Hz) might be involved in sleep-dependent memory consolidation processes. Employing slow oscillatory transcranial direct current stimulation (so-tDCS) during slow-wave sleep, a pioneer study reported a successful exogenous manipulation of slow oscillations accompanied by an enhancement of declarative memory in young participants. However, subsequent studies using similar methodologies yielded contradictory results questioning the effectiveness of this neuromodulatory technique.

Aim: This study attempted to modulate specific neural oscillations during sleep and boost declarative memory using so-tDCS with the aim to replicate the findings of a seminal study in young healthy adults, using a slightly modified stimulation protocol previously implemented in elderly participants.

Methods: The effect of anodal so-tDCS applied bifrontally (frequency 0.75 Hz) during non-rapid eye movement (NREM) stage 2 sleep (N2) was assessed on a word-pair task and a sequential finger tapping task in 23 healthy participants (mean ± Sd: 23.2 ± 1.9 years; 13 women) in a double-blind, placebo controlled, counterbalanced, randomized crossover design. Stimulation effects were analyzed on sleep stages, sleep spindle densities, and EEG power, as well as on declarative and procedural memory performances.

Results: No significant stimulation effects were observed neither on the behavioral performance nor at the physiological level. Under both stimulation conditions, overnight retention raised in the procedural task and declined in the declarative task. However, when participants had additional learning opportunities, the decline in declarative memory performance diminished. Regardless of stimulation, fast parietal spindle densities decreased from baseline (prior to stimulation) to stimulation-free intervals, while slow frontal spindle density showed no significant changes.

Conclusion: The present study failed to replicate the results of the pioneer study in this field. However, our findings are in line with a previous study that used the same stimulation protocol in elderly participants. Overnight retention performances in declarative memory were dependent on re-encoding opportunities. Finally, it should be noted that protocol standardization and variability control are essential in so-tDCS studies.

Abbreviations

µA	Microampere
µV	Microvolt
AASM	American Academy of Sleep Medicine
AC	Alternating current
ADHD	Attention-deficit/hyperactivity disorder
BI	Blinding Index
BMI	Body Mass Index
CI	Confidence interval
DC	Direct current
DST	Digit Span Test
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
ESS	Epworth Sleepiness Scale
EWL-N	Adjective Check List (*Eigenschaftswörterliste*)
FSA	Fast spindle activity
Hz	Hertz
LTM	Long-term memory
LTP	Long-term potentiation
mA	Milliampere
MCI	Mild cognitive impairment
MEQ	Morningness-Eveningness Questionnaire
MWT-B	Vocabulary-Intelligence-Test-B (*Mehrfachwahl-Wortschatz-Intelligenztest-B*)
N1	NREM stage 1 sleep
N2	NREM stage 2 sleep
N3	NREM stage 3 sleep
NMDA	N-methyl-D-aspartate receptor
NREM	Non-rapid eye movement

PANAS	Positive and Negative Affect Schedule
PSG	Polysomnography
PSQI	Pittsburgh Sleep Quality Index
REM	Rapid eye movement
RWT	Regensburg Word Fluency Test
S3-LPS	Subtest 3 of the Performance Testing System (*Leistungsprüfsystem*)
SAS	Self-Rating Anxiety Scale
Sd	Standard deviation
SD	Spindle density (Sleep spindle counts per 30 seconds)
SDS	Self-Rating Depression Scale
SEM	Standard error of the mean
SFI	Stimulation-free intervals
SFTT	Sequential Finger Tapping Task
SO	Slow oscillations (< 1 Hz)
so-tDCS	Slow oscillatory transcranial direct current stimulation
SPW	Sharp waves
SPW-R	Sharp wave-ripple
SSA	Slow spindle activity
SWA	Slow-wave activity
SWS	Slow-wave sleep
tACS	Transcranial alternating current stimulation
tDCS	Transcranial direct current stimulation
TES	Transcranial electrical stimulation
WPT	Word Pair Task

Manteltext

The description of the present study is also presented in Bueno-Lopez et al. (1).

1. Introduction

New information is acquired every day, from learning that our solar system is 4.6 billion years old (a fact) to knowing how to ride a bike (a motor skill). How these memories are remembered (or forgotten) and which neuronal processes are involved in their storage (or retrieval), are important questions in neuroscience (2, 3). Studies on amnesic patients and on animals endorse the development of models and theories about memory and its consolidation processes at the synaptic and system levels (4). In the past decades, the relationship between memory and sleep has become an important topic of research. Moreover, it is generally accepted that sleep is involved actively in the process of memory consolidation, that is, in the integration, transformation and strengthening of newly labile memory traces into a more stable form and a long-term storage (5, 6). Indeed, sleep-dependent memory consolidation refers to the involvement of sleep in post-encoding processes that allow the stabilization, reorganization, enhancement and long-term integration of memory representations (7). Despite the fact, that the exact mechanisms by which memory representations are reactivated during post-encoding sleep are still unclear, it is widely accepted that oscillations during sleep in general - and slow oscillations (SO; < 1 Hz) specifically - are crucial for memory consolidation and correlate with memory enhancement (8).

Motivated by the important role that sleep plays in memory consolidation, different approaches have been used to manipulate oscillations during sleep (usually SO and spindles), not only to better understand the underlying physiological mechanisms that take place during specific sleep stages in relation to memory consolidation processes, but also to translate this knowledge into possible therapeutic strategies. The most common approaches include pharmacological manipulations, sensory stimulation, and transcranial electrical stimulation (TES), but other approaches have also been implemented, such as vestibular stimulation or even hypnosis (for recent reviews, see (9, 10)). Focusing on TES approaches, at the beginning of this century two studies (11, 12) were able to enhance declarative memory by applying direct currents (DC) transcranially during slow-wave rich periods of sleep. Since then, however, a mixture of positive and negative results have been reported using similar stimulation procedures (13). This heterogeneity in the literature challenges the effectiveness of this stimulation technique. Thus, the purpose of this slightly modified replication study was to confirm the results of the first study (12) that

showed an improvement in sleep-dependent declarative memory prior to the application of slow oscillatory transcranial direct current stimulation (so-tDCS) in young subjects.

1.1. Theories and models of memory and sleep

During learning, an initial neural representation of new information is formed (encoding) (14). This novel information is consolidated and organized by the formation of neuronal networks that allow a flexible access (retrieval) to this memory trace (14). Depending on the access capacity, memory has commonly been divided into working memory and long-term memory (LTM). Long-term multiple memory systems have been broadly classified into two main groups depending on whether these memories are expressed during recollection explicitly (in a conscious manner), also known as declarative memories; or by performance implicitly (in an unconscious manner), also known as non-declarative memories (15). Declarative memory is considered to be representational and can be broken down into two types: semantic memory (facts; general knowledge) and episodic memory (events; autonoetic awareness) (16, 17). In contrast, non-declarative memory is dispositional and can be divided into procedural memory (motor skills and habits), priming, perceptual learning, conditioning (emotional responses and skeletal responses), and non-associative learning (Squire, 2004). These memory systems receive inputs from the association areas of the neocortex and send projections to different brain structures depending on the kind of memory involved (16). Regarding declarative memory, the hippocampal region is involved in the encoding and the integration of new information into neural networks in neocortical areas; and the prefrontal cortex is involved in the organization of overlapping memories and the creation of neocortical memory networks (14). By contrast, non-declarative memory depends more on the striatum and the cerebellum (16, 18).

After new information has been processed in neocortical areas, the brain systems supporting memory initiate a gradual process of reorganization and stabilization of LTM representations, a process referred as system consolidation (19, 20). Memory consolidation is considered a dynamic and lasting process that can take from seconds to years and that not only occurs during wakefulness but also during sleep, a brain state which may promote in an active manner system consolidation (19). Different hypotheses and theories have been developed to explain LTM stabilization with special focus on declarative memory. Derived from studies of amnesic patients, the medial temporal lobe memory system hypothesis (standard consolidation theory) (18, 21) proposed that, initially, structures in the medial temporal lobe, specifically the hippocampal formation, store memory traces for a limited time ("fast-learning*"*), and later on this

initial memory is gradually stored in the neocortex ("slow-learning") and becomes independent of the hippocampus. Therefore, the interaction between the hippocampus and the neocortex is what may support LTM consolidation. Alternatively, the multiple trace theory (22, 23) makes a distinction between the consolidation of episodic and semantic memory. This theory proposes that for episodic memory, the hippocampal complex is not only activated during the initial storage but also during retrieval, in contrast to semantic memory. Thus, episodic recollection is considered to be dependent on the hippocampus during retrieval.

According to these two hypotheses, the two-stage model of memory trace formation (24), supports the idea that memory is consolidated in the neocortex during periods of inactivity and sleep by a gradual reactivation of memory representations via hippocampus-neocortical communication (24, 25). This neurophysiological model proposes that: (i) labile memory traces are formed during "exploratory behaviors" (learning) when the neocortex transfers new information to the hippocampus and theta oscillations are predominant; and (ii) long-lasting memory traces are formed during "consummatory behaviors" (retention intervals) when the hippocampus transfers this newly acquired information to the neocortex (24). This transfer of information is driven by sharp wave (SPW) events, which are a specific pattern of synchronous activity characteristic of the hippocampus and recurrent in slow-wave sleep (SWS) (24, 26). In fact, SPW are associated with short-lasting fast oscillatory bursts (140-200 Hz) called "ripples" (SPW-R), which are proposed to be the key mechanism by which labile memories can be stored in the neocortex (26).

Focusing on the consolidation of newly acquired information during sleep, the concept of sleep-dependent memory consolidation gains strength. It should be noted that sleep is not a uniform state of the brain. Sleep is divided in two main states: non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep (27). These two states alternate throughout the entire sleep period in 70 -110 minute-cycles. In the first half of the night, NREM sleep predominates and is characterized by a synchronous electroencephalogram (EEG) activity that goes from lighter [sleep stage 1 (N1); and 2 (N2)] to deeper [sleep stage 3 (N3); also referred as SWS] sleep stages; the latter defined by high-amplitude and low-frequency EEG (27). REM sleep is more prominent in the last third of the night and is defined by low amplitude and high-frequency EEG (desynchronized EEG) (27).

These dichotomies in sleep (NREM vs REM) and memory systems (declarative vs non-declarative) raised the question of what role sleep stages play in memory consolidation processes (28). One approach to this issue comes from the dual-process hypothesis, which supports the theory that NREM and REM

sleep act differently on different memory representations (29). According to this view, REM sleep is essential for the consolidation of non-declarative memory, and NREM sleep - most precisely SWS - plays a significant role in the consolidation of declarative memory (30-32). However, studies have shown that sleep spindles during N2 are also involved in procedural memory consolidation (e.g. (33, 34)) and that theta activity during REM is related to declarative memory consolidation (35). Another approach is based on the sequential hypothesis, which postulates that a coordinated sequential interaction between NREM and REM periods is crucial, regardless of the kind of memory being consolidated during sleep (36, 37). However, those assumptions are mostly based on studies that used sleep deprivation approaches. Hence, methodological issues have been raised in relation to the paradigms used in the different studies and the characteristics of the memory tasks implemented in the literature (38).

While above-mentioned hypotheses focused more on the roles of the different sleep stages, two further hypotheses (closely related to ones already described) have attempted to elucidate how sleep as a global brain state could sustain memory consolidation at the neuronal level. In this respect, the synaptic homeostasis hypothesis (39) places wake and sleep states along a continuum based on their circadian regulation, where synaptic potentiation increases during wakefulness and is downscaled during subsequent sleep, guided by a homeostatic regulation via slow-wave activity (SWA). Therefore, during sleep the synaptic strength presented in wakefulness might be renormalized, when synaptic weight decreases, weak connections are removed, and synaptic activity is gradually restored to baseline levels (39, 40).

This hypothesis proposes that “sleep-dependent renormalization” or “down-selection” might occur in NREM sleep specifically during slow waves “up/on” periods coincident with SPW-R (41). Furthermore, neocortical SO (< 1 Hz) are characterized by two phases: (i) an extended depolarized “up-state” of neuronal excitation followed by (ii) a prolonged hyperpolarized “down-state” of neuronal silence (42). Indeed, the active system consolidation hypothesis (5) claims that newly acquired memory representations during wakefulness are selectively and repeatedly reactivated and redistributed by a dialog between the hippocampus and the neocortex during SWS, and that REM sleep promotes synaptic consolidation that might stabilize these previously transformed memories. Consequently, synaptic consolidation could be considered as a “subroutine” within system consolidation processes (43). This model proposes that “up-states” of SO generated in the neocortex drive the reactivation of memory representations in the hippocampus, where SPW-R are generated synchronously with thalamo-cortical spindles in parallel, allowing LTM storage in preexisting neocortical networks (5, 30, 44, 45).

Additionally, it has been reported that the occurrence of fast spindles (> 12 Hz) might regulate SO and support sleep-dependent memory consolidation (46).

In addition to the previously described theoretical framework, the general notion that EEG oscillations may contribute to various cognitive functions has encouraged the use of different neuromodulation techniques to manipulate specific brain oscillations, exploring changes in associated cognitive outcomes (47). Particularly, the use of stimulation methods such as so-tDCS, aiming to investigate the specific role of sleep in relation to memory attempts to prove a causal relationship between specific sleep oscillations and sleep-dependent memory consolidation processes (for a review see (48)). Hence, exogenous stimulation of SO and sleep spindles might not only allow to better describe and/or support sleep-dependent memory theories in a more precise manner, but also to improve their related memory performance.

1.2. Non-invasive transcranial brain stimulation

As pointed out before, a variety of neurostimulation approaches (e.g. olfactory, acoustic, vestibular, or non-invasive brain stimulation, among others) have been used mostly to enhance sleep oscillations (9, 10), using reactivation paradigms. Focusing on non-invasive transcranial brain stimulation methods, TES can be divided into transcranial direct or alternating current stimulation (tDCS and tACS, respectively)(49). It is important to mention that stimulation strongly depends on the electrical waveform, current intensity (mA or μA), current density (mA/cm^2), duration, size, and montage of the stimulation electrodes (usually located on specific scalp areas) through which the current is applied, and on individual anatomical factors(49). In studies, which aim was the modulation of sleep oscillations, so-tDCS has been typically used. So-tDCS applies a monophasic sinusoidal wave at low frequencies (0.75 Hz) and could be considered a *hybrid* between direct (DC) and alternating current (AD) stimulation because, although its intensities are regularly modulated, its polarity never changes (e.g. between 0 and 260 μA) (49). In animal models it has been shown, firstly, that the externally applied DC electric fields induces the polarization of the neuronal membrane and, secondly, an enhancement of synaptic activity takes place, following by a NMDA modulation and the polarization of non-neural targets (50). So-tDCS mechanisms are based on the characteristic of alternating current (AC) stimulation that can entrain brain endogenous oscillations either due to their modulation or to shifting of their phase when stimulation is phase-locked (51).

1.3. So-tDCS and sleep-dependent memory consolidation

Based on the previously described characteristics of so-tDCS, this technique has been used to modulate endogenous SO characteristics of SWS, with the aim to increase these specific brain rhythms and therefore induce declarative memory consolidation enhancement (44). Based on this premise, Marshall et al. (11, 12), implemented for the first time an anodal so-tDCS bilaterally over the prefrontal cortex during SWS to manipulate declarative memory consolidation in healthy young adults, being able to improve declarative memory performance. Six additional studies have applied similar stimulation paradigms to enhance memory, not only in young (1, 52, 53), but also in elderly (54-57) participants. While these studies in healthy populations yielded heterogeneous results, positive effects of so-tDCS have been observed in schizophrenic patients (58), subjects with mild cognitive impairment (MCI) (59), and children with attention-deficit/hyperactivity disorder (ADHD) (60, 61). Additionally, a meta-analysis of 13 studies (13) found an overall enhancement in declarative memory after applying tDCS during sleep. This study included results from some of these clinical samples, therefore demographic differences and different stimulation protocols used across the studies could have influenced the reported positive effects on declarative memory.

2. Aim/Objective

The aim of the present study was to replicate the positive findings on sleep-dependent declarative memory consolidation in young adults observed by Marshall et al. (12) using a slightly modified stimulation protocol. Furthermore, this study enables to further examine whether the results found in young participants correspond to previous findings of our research group in elderly participants (54).

3. Method

3.1. Participants

In total, twenty-six healthy young adults participated in this study, three of which had to be excluded due to stimulation protocol deviations (stimulation was applied too early) and technical problems during data acquisition. Thus, data from 23 participants (13 women; mean ± Sd: 23.2 ± 1.9 years) were analyzed. All participants were nonsmokers, had no history of sleep disorders and were fluent German speakers (see Table 1 for additional inclusion criteria). Severe untreated medical conditions, cognitive impairment,

regular intake of medication (except oral contraceptives), hormonal disorders, having metal implants, sensitive skin, and excessive consumption of caffeinated (> 5 cups/day) and/or alcoholic beverages (> 3 glasses/day) were considered as exclusion criteria. Eligible participants underwent a detailed physical and neurological examination. Finally, prior to the experimental nights, participants were screened for sleep disorders and had to fulfil the following inclusion criteria by a polysomnography in the laboratory: sleep latency < 30 min; sleep efficiency > 80 %; apnoe-hypopnoe-index < 10/h; periodic-limb-movement-arousal-index < 10/h).

	Men (n = 10)				**Women (n = 13)**				
	Mean	±Sd	Median	IQR	Mean	±Sd	Median	IQR	p
Age (years)	23.3	±1.7	23.0	1.5	23.1	±2.1	23.0	4.0	0,785
BMI (kg/m²)	22.2	±1.8	22.1	3.5	21.1	±2.1	20.7	3.6	0,191
PSQI (≤ 5)	2.9	±1.2	3.0	1.3	3.5	±1.1	4.0	1.0	0,262#
ESS (≤ 10)	5.6	±2.6	5.0	4.5	6.3	±2.4	7.0	4.0	0,502
MEQ (≥ 31 or ≤ 69)	50.2	±7.8	52.0	12.8	49.0	±9.2	51.0	14.5	0,744
SAS (≤ 36)	24.5	±3.0	25.5	5.3	23.7	±2.8	23.0	4.5	0,506
SDS (≤ 40)	25.3	±2.8	24.5	3.5	25.6	±4.0	24.0	4.0	0,834
MWT-B	31.9	±2.8	32.5	4.8	31.0	±2.7	32.0	3.5	0,445#
S3-LPS	34.2	±5.6	34.5	9.3	35.3	±3.8	37.0	6.0	0,576

Table 1. **Characteristics of the participants**. Demographics, questionnaires scores, and general intelligence measures. Inclusion criteria were having good sleep quality, no excessive daytime sleepiness, no extreme chronotype and no depressive symptoms or anxiety. PSQI = Pittsburgh Sleep Quality Index (62); ESS = Epworth Sleepiness Scale (63); MEQ = Morningness-Eveningness Questionnaire (64); SDS = Self-Rating Depression Scale (65); SAS = Self-Rating Anxiety Scale (66). Cut-off value(s) per questionnaire are represented in parentheses. BMI = Body-Mass-Index. Sd = Standard deviation; IQR = Interquartile range. Fluid and crystallized intelligence aspects were controlled by the subtest 3 of the "Performance-Testing-System" (S3-LPS = Subtest 3 of the *Leistungsprüfsystem*; (67)) and a multiple choice "Vocabulary-Intelligence-Test" version "B" (MWT-B = *Mehrfachwahl-Wortschatz-Intelligenztest-B*; (68)), respectively. T-test and Mann-Whitney-U-test#. Participants were within the range of normal reference values for reasoning and verbal fluency indicating normal cognitive functioning (see (1)).

This study was approved by the ethics committee of the Charité - Universitätsmedizin Berlin (EA4/076/15). All participants gave written informed consent and received monetary compensation for their participation in the study.

3.2. Study design

The study consisted of three nights in the sleep laboratory. Participants underwent a screening and adaptation night followed by a first experimental night and, four weeks later, the second and last experimental night took place. The experimental nights were separated by four weeks in order to control the menstrual cycle in women. All female participants were in the luteal phase in order to avoid cofounding effects (69). For all nights, participants arrived at the laboratory around 19:00 h and were instructed to avoid alcohol and caffeine during these days. First, electrodes and sensors were fixed. After that, during the first night, participants had time to familiarize themselves with the room before going to bed. On the second and third nights (experimental nights), participants filled out two control questionnaires, and general retrieval capability and working memory were assessed, followed by the implementation of a declarative and a procedural memory task in a counterbalanced order. Before going to bed at 23:00 h and after awakening at 6:30 h, participants were asked to fill out an evening and a morning protocol (70) respectively for all nights. In the experimental nights verum or sham so-tDCS was applied following a counterbalanced randomized, double-blind, within-subject crossover design. In the morning (at 7:00 h), memory tasks were administered followed by the control tests and questionnaires. Then, participants were asked to estimate which kind of stimulation they received and whether possible symptoms related to stimulation were associated with it (71). Finally, electrodes were removed and participants had breakfast (for the study design see Figure 1 Bueno-Lopez et al. (1)).

3.3. Memory tasks and control tests

Two memory tasks were administered to assess declarative and procedural memory in both experimental nights. Both tasks consisted of two versions, which were balanced between the experimental nights. Differences in task performance between morning and evening indicated overnight retention for both memory tasks. All tasks were presented using E-Prime 2 (Psychology Software Tools, Pittsburgh, USA).

A word-pair task (WPT) (11, 12) was implemented in order to assess declarative memory (see Figure 1). In this task, participants were instructed to learn a paired associate word list of 54 word-pairs composed of semantically related German nouns. The first and last four word-pairs of the list controlled primacy and recency effects, thus the maximal number of correct word-pairs was 46. During learning in the evening, word-pairs were presented followed by an immediate recall, where cue-words were presented

and participants had to answer the corresponding target-words without time limit. No feedback was given after word recall (similar to (54)). If participants were not able to correctly retrieve a minimum of 60% of the word-pairs, the same associated pairs were presented in a different randomized order until participants reached this learning-to-criterion condition. In the morning, during the retrieval testing, cue-words were presented again in a newly randomized order and participants were asked to recall the associated target-words. Task performance was measured considering the number of correct responses after learning-to-criterion in the immediate recall and in the retrieval phase at the next morning.

For the evaluation of procedural memory a sequential finger tapping task (SFTT) (12) (adapted from (72)) was administered (see Figure 1). Participants were instructed to type repeatedly a sequence composed of five digits as accurately and fast as possible within 30-seconds blocks-trials (with their non-dominant hand) using a computer keyboard. Participants completed twelve trials followed by 30 sec rest periods each. The next morning, during the retrieval period, participants completed three 30 sec trials. Performance was calculated using the mean of the three last trials of the learning period and the three trials of the retrieval period for both the number of correctly typed sequences and the error rate (percentage of the errors relative to the total number of completed sequences).

In order to control that no other factors affected memory performance, verbal fluency, working memory, mood state and motivational aspects were assessed before the learning phase and after the retrieval period. The Regensburg Word Fluency Tests (RWT; *Regensburger Wortflüssigkeits-Test*; (73)) assessed verbal fluency (categorical and phonetical retrieval capacity). The Digit Span Test of the Wechsler Adult Intelligence Sale (WAIS-R; (74)) measured working memory. In this test, orally presented series of numbers had to be repeated forwards and backwards. Finally, mood state was assessed by the Positive and Negative Affect Schedule (PANAS; (75); German version by (76)) and participant's current state were assessed by an adjective check-list (*Eigenschaftswörterliste*, EWL-N, (77)).

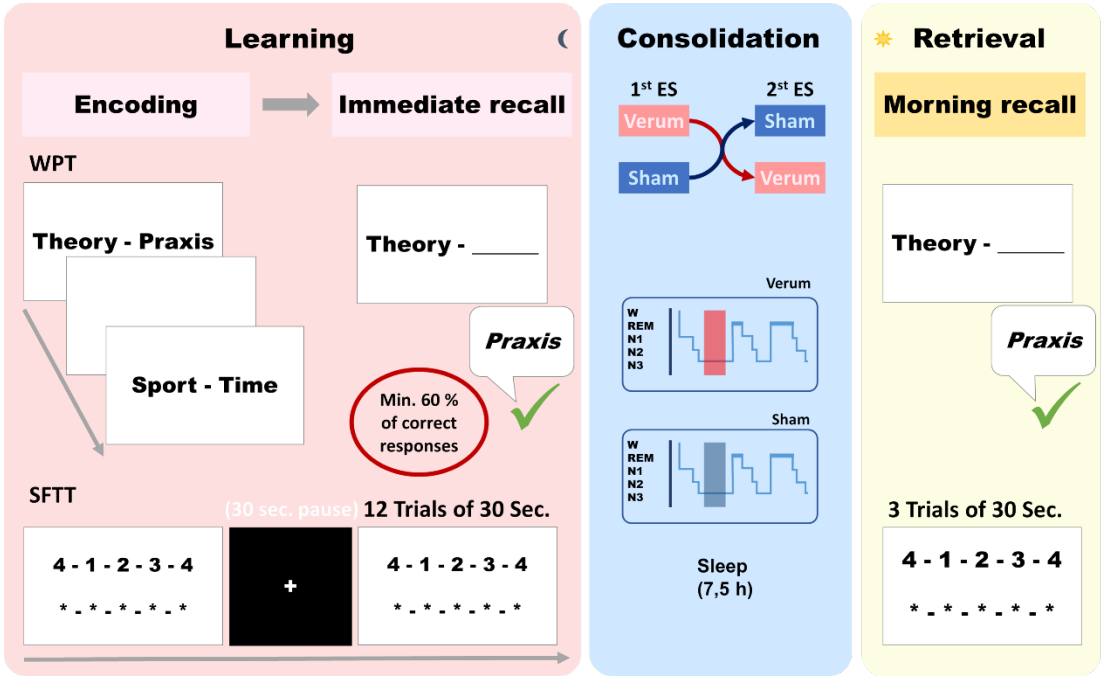

Figure 1. Memory tasks. In both experimental sessions (ES), a word-pair task (WPT) and sequential finger tapping task (SFTT) were administered in the evening (*learning*) and in the next morning (*retrieval*). In the WPT, 54 word-pairs (composed of a cue-word and a target-word) were presented. + = fixation mark. If participants did not correctly retrieve a minimum of 60% of the word-pairs, word-pairs were re-exposed until this learning criterion was reached. In the SFTT participants typed repeatedly a sequence of five digits within 30 sec with 30 sec pause.

3.4. Stimulation blinding and adverse effects

In order to test if participants were able to recognize stimulation conditions, they were asked to guess the stimulation condition that they thought they were exposed to in both experimental nights. Additionally, participants filled out an adverse effects questionnaire (71). This questionnaire assessed the occurrence of possible symptoms (e.g. headache or trouble concentrating, among others) that could be related to so-tDCS (see Suppl. material in Bueno-Lopez et al. (1)).

3.5. So-tDCS

Stimulation was applied by a battery driven Eldith DC-Stimulator Plus (NeuroConn GMbH, Ilmenau, Germany). A tDCS with sinusoidal oscillating waveform between 0 and 260 µA at a frequency of 0.75 Hz with a ramping-period at the beginning and at the end of each stimulation interval (fade-in/fade-out,

8 sec each) was delivered via 10 mm diameter electrodes. Stimulation electrodes were placed bifrontally at F3 and F4 (anodes) and at both mastoids (M1 and M2, cathodes) following the 10-20 system (78). Maximal current density was 0.331 mA/cm^2 for each pair of stimulation electrode. Periodic small current pulses that enable impedance control with no therapeutic effect were applied in the sham stimulation condition (see (54)). In both experimental nights, the stimulation procedure was the same regardless of the stimulation condition. Based on visual online sleep scoring, stimulation was triggered after the first eight consecutive epochs (4 min) of stable N2. Stimulation period (25'8'') was split into five (5'16'') periods of stimulation, each of them followed by a 1-minute stimulation-free intervals (SFI) (see Figure 2 B Bueno-Lopez et al.(1)). The tDCS device was encoded in a sham or verum modus by using 5-digit-codes. Those were previously assigned and randomized by a third investigator, who was neither involved in any data acquisition nor in the stimulation administration processes, nor in the following data analysis. Moreover, the PSG screen was switched off right before triggering the stimulation to assure the double blinding.

3.6. Polysomnography, sleep scoring and sleep micro- and macrostructure

In all three nights, sleep was monitored and EEG data were collected using a Neurofax EEG-9200 device (Nihon Kohden, Tokio, Japan) via gold-coated scalp electrodes attached according to the 10-20 system (78). In the first night, a complete cardiorespiratory PSG was performed following the recommendations of the American Academy of Sleep Medicine (AASM) (79). In the experimental nights, EEG locations (F7, Fz, F8, C3, Cz, C4, T3, T4, P3, Pz, P4, O1, O2, and Fp2 as ground electrode) were referenced to the nose tip (Nz) and recorded (see Figure 2 A Bueno-Lopez et al.(1)) according to previous studies (12, 54). Additionally, electrooculgram (EOG, vertical and horizontal), electromyogram (EMG mental and submental), and electrocardiogram (ECG) signals were recorded. Subsequently, sleep recordings were analyzed according to the AASM guidelines (79) by a sleep scorer expert, not involved in the statistical analysis of the data sets.

Three different periods were assessed in relation to sleep macrostructure and the possible effects of verum stimulation in comparison with sham stimulation. Firstly, the entire night was analyzed based on the percentage of sleep stages referenced to the total sleep period with exclusion of the stimulation period. Secondly, the 60 minutes after the stimulation period were analyzed to assess stimulation after-effects. Finally, the five 1-min SFI were analyzed based on 10-second epochs where the EEG signal was free

from stimulation artefacts. Subsequently, the impact of stimulation on sleep microstructure (sleep spindles and EEG power) was measured as well for the five 1-min SFI under verum and sham stimulation similarly as in Eggert et al. (54). Sleep spindles (11 - 15 Hz) were divided regarding to their topography and frequency into two types: “slow spindles”, that usually have frequency peaks in frontal areas [slow frontal spindles (SFS); 11 - 13 Hz], and “fast spindles”, that usually have frequency peaks in parietal areas [fast parietal spindles (FPS); 13 - 15 Hz] (80). An automatic algorithm was used to detect spindle waves based on their amplitude in terms of their root mean square (threshold 3.5 μV 2 and boundary 31.8 μV 2) and their duration (between 0.5-3.0 seconds) (see (54)). The assessment of the spindle densities from frontal (Fz - Nz) and parietal (Pz - Nz) derivations was referenced to 30-sec epochs for the minute before stimulation (60-sec baseline) and for the five 1-min SFI (5 times, 50-sec) (see (54)). Then, the differences between the baseline and the SFI were calculated and compared. Finally, EEG spectral power for eight frequency bands [SO (0.5 - 1 Hz and 1 - 1.5 Hz), delta (1 - 4 Hz), theta (4 - 8 Hz), alpha (8 - 11 Hz), sigma (11 - 13 Hz and 13 - 15 Hz; slow-spindle and fast-spindle frequencies, respectively), and beta (15 - 25 Hz)] was calculated. Additionally, EEG artifacts were identified and excluded from the final analysis. Artifacts were detected visually and using an amplitude criterion, thus when amplitudes were higher than 150 μV, they were dismissed. After the off-line rejection of the artifacts, the mean (of the 1-min stimulation-free intervals) of the medians (calculated for the five 10s-epochs of each stimulation-free block) was calculated for the eight frequency bands and averaged topographically over three cortical regions: frontal (F7, Fz, F8), central (C3, Cz, C4, T3, T4), and posterior (P3, Pz, P4, O1, O2).

3.7. Statistical analysis

The analysis of the data across the two stimulation conditions was performed with IBM SPSS Statistics 24. Participant’s performance in the declarative and the procedural memory task and in the control tests was analyzed using a repeated measures analysis of variance (rmANOVA) with the within-subject factors STIM (verum vs sham) and TIME (immediate recall at evening vs morning retrieval). Similarly, spectral power of the EEG for all frequency bands was evaluated considering the within-subject factors STIM (verum vs sham) and three scalp regions LOC (frontal vs central vs posterior). Slow- and fast- spindle densities were examined considering the factors STIM (verum vs sham) and TIME (baseline vs SFI). Differences between the stimulation conditions in the sleep macrostructure were analyzed using a t-test for paired observations or the Wilcoxon matched-pairs signed-ranks test depending on the data distribution.

Independently of the stimulation condition, the influence of the number of sessions (1 vs. 2 and 3) needed to reach the 60 % learning criterion for the number of correctly remembered word pairs, as well as the impact of the number of sessions on the overnight retention was tested by t-tests for independent samples. Finally, participant's stimulation blinding was tested using James' Blinding Index (BI), which is sensitive to the degree of disagreement (81). Additionally, the prevalence of adverse effects and their subjective associations were analyzed by McNemar-Test and Stuart-Maxwell Tests respectively. (See also statistical analysis and Suppl. material in Bueno-Lopez et al. (1)).

4. Results

4.1. Memory tasks and control tests

In the declarative memory task, the correctly recalled word pairs did not differ significantly between the two stimulation conditions ($F_{1;22} = 1.52$, $p = 0.231$), whereas they differed significantly with TIME ($F_{1;22} = 27.07$, $p < 0.001$). The interaction between STIM x TIME was not statistically significant ($F_{1;22} = 0.01$, $p = 0.943$) (see Table 2 A in Bueno-Lopez et al (1)). Post-hoc t-tests confirmed significant overnight reductions in the number of correctly recalled word-pairs in both conditions (see Figure 2 A). The non-significant interaction indicates that these overnight reductions do not differ significantly between sham and verum stimulation (see Figure 2 B). The number of sessions to reach the learning-to-criterion in the WPT has a significant effect on the number of recalled words in the evening (one session: n, mean ± SEM: 33, 35.5 ± 1.0; more than one session: n, mean ± SEM: 13, 40.8 ± 1.0, $p = 0.003$). This also has implications for the number of recalled words in the morning (one session: n, mean ± SEM: 33, 34.1 ± 1.1; more than one session: n, mean ± SEM: 13, 40.3 ± 1.0; $p = 0.002$). Overnight retention differed between subjects who needed only one session to reach the learning criterion (mean ± SEM: -1.4 ± 0.3) and subjects who needed more than one learning session (mean ± SEM: -0.5 ± 0.2). The additional chance to learn resulted in a less pronounced overnight forgetting. However, the mean number of sessions that each participant needed to reach the learning criterion was the same under both stimulation conditions 1.3 ± 0.1 (mean ± SEM) for sham and 1.3 ± 1.0 (mean ± SEM) for verum ($p = 1.000$) (see (1)).

For the performance in the procedural memory task again TIME ($F_{1;22} = 42.18$, $p < 0.001$) but not STIM ($F_{1;22} = 0.07$, $p = 0.790$), were statistically significant on correctly typed sequences per 30 second. The interaction between TIME x STIM was not statistically significant ($F_{1;22} = 1.26$, $p = 0.274$), indicating

that the overnight changes were not different between the two stimulation conditions (see Table 2 A in Bueno-Lopez et al (1)). Post-hoc t-tests revealed significant increases in performance from evening to morning sessions, irrespective of the stimulation condition (see Figure 2 C). However, these increases were similar in both stimulation conditions ($p = 0.272$) (see Figure 2 D). Additionally, analyses of the error rate in the performance showed that neither TIME ($F_{1;22} = 2.56$, $p < 0.124$) nor STIM ($F_{1;22} = 0.84$, $p < 0.370$) were statistically significant (see Figure 3 G in Bueno-Lopez et al. (1)). Therefore, differences in typing errors were not statistically significant during the learning phase and the morning recall, and between stimulation conditions. The interaction between TIME x STIM was again not statistically significant ($F_{1;22} = 1.22$, $p = 0.281$), showing that overnight changes were not different between the two stimulation conditions (see Table 2 A in Bueno-Lopez et al. (1)).

There was no evidence that the stimulation affected mood, working memory or verbal word fluency (see Suppl. material and Suppl. Table 2. for the results of rmANOVA in Bueno-Lopez et al (1)).

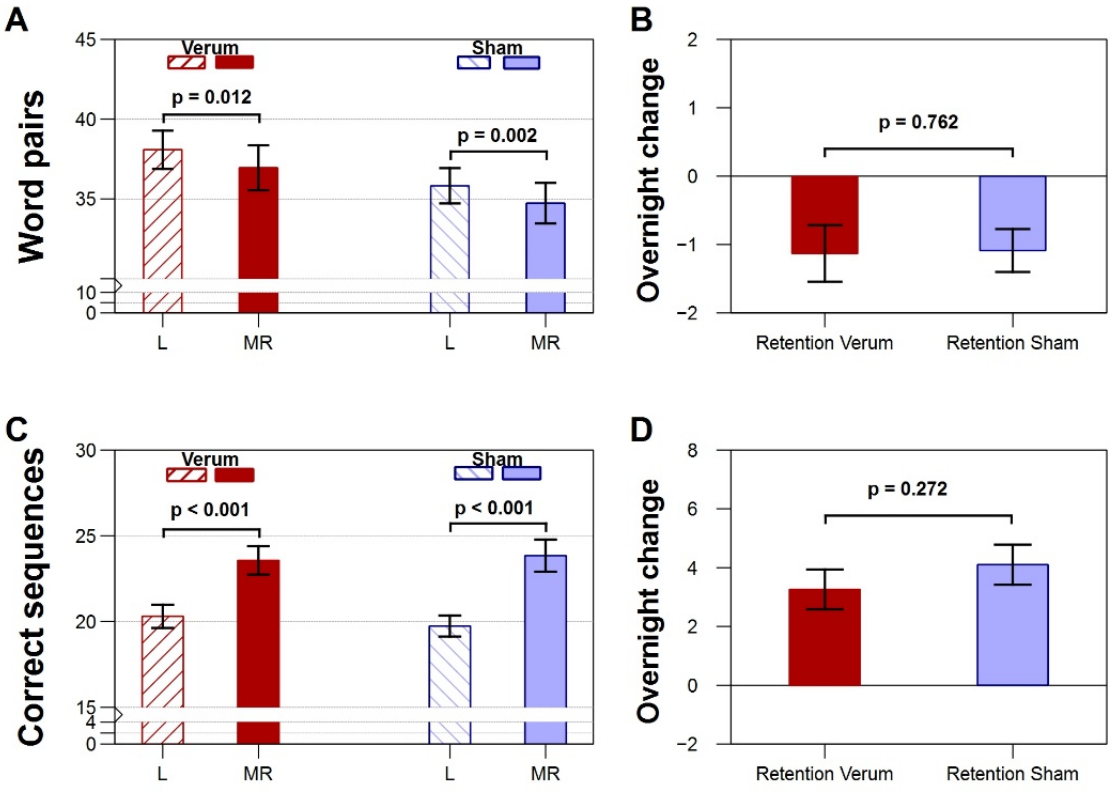

Figure 2. Declarative and procedural memory performances. L = Learning phase; MR = Morning recall. A) Correctly recalled word pairs and B) overnight changes in retention under verum and sham stimulation in the word pair task. C) Correctly typed sequences and D) overnight changes in retention under verum and sham in the sequential finger tapping task. Adapted with permission from Elsevier: Bueno-Lopez et al. (1).

4.2. Sleep macro- and microstructure

Sleep macrostructure for the entire night, the 60 min following exposure, and stimulation-free intervals did not differ significantly between exposure conditions in any parameter (see Table 3 in Bueno-Lopez et al (1)). The effect of so-tDCS stimulation on spindle densities was analyzed separately for fast and slow spindles with the factors STIM (verum vs sham) and TIME (baseline vs SFI). Slow frontal spindle densities did not differ significantly between the two stimulation conditions ($F_{1;22} = 2.79$, $p < 0.108$) nor did they differ significantly with TIME ($F_{1;22} = 2.56$, $p < 0.124$). The interaction between STIM x TIME was not statistically significant ($F_{1;22} = 0.39$, $p = 0.538$). Fast parietal spindle densities did not differ significantly between the two stimulation conditions ($F_{1;22} = 0.18$, $p < 0.676$), however, they do differ significantly with TIME ($F_{1;22} = 19.29$, $p < 0.001$). The interaction between STIM x TIME was not statistically significant ($F_{1;22} = 1.47$, $p = 0.239$). Even though, no stimulation effect was found in the analysis of the spindle density (see Table 2 B in Bueno-Lopez et al (1)), fast parietal spindles densities were significantly lower after both stimulation conditions (see Figure 3). The analysis of the spectral power in the stimulation-free intervals revealed no effects of verum stimulation compared to sham. However, the results indicate that spectral power varies topographically within all the frequency bands (see Table 2 C and Figure 5 in Bueno-Lopez et al (1)).

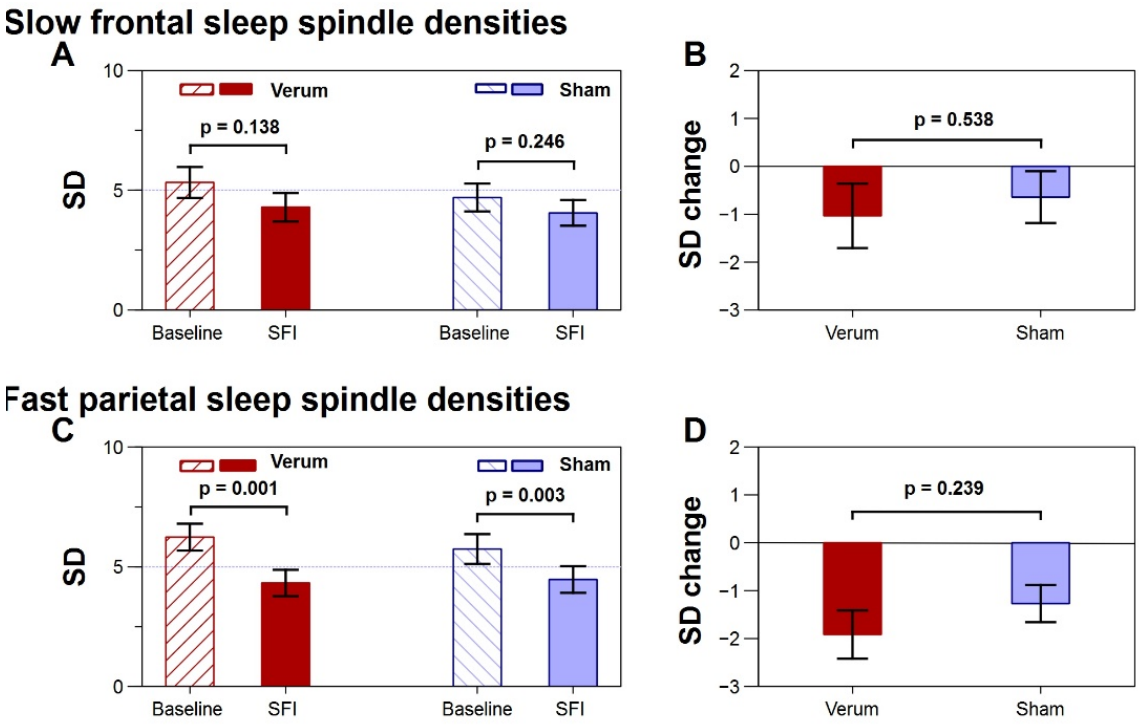

Figure 3. Sleep spindle densities. **A**) Slow frontal sleep spindle density and **B**) change in spindle density from baseline to SFI. **C**) Fast parietal sleep spindle density and **D**) change in spindle density from baseline to SFI. SD = Spindle density; SFI = Stimulation-free intervals. Adapted with permission from Elsevier: Bueno-Lopez et al. (1).

4.3. Stimulation blinding and so-tDCS adverse effects

Stimulation blinding was quantified by James' BI (81). The BI in this study was 0.80 (where 0 means a total lack of blinding and 1 a complete blinding; [95 % CI: 0.69; 0.91]) which indicates a successful blinding (see Suppl. Figure 2S in Bueno-Lopez et al (1)). Self-reported symptoms did not differ significantly between stimulation conditions (see Suppl. material in Bueno-Lopez et al (1)).

5. Discussion

The main purpose of this study was to replicate the results of an earlier study (12), which observed an increase of SO and sleep spindles as well as an enhancement of declarative memory performance after so-tDCS during NREM sleep in young healthy subjects. Our results indicate that anodal so-tDCS at a frequency of 0.75 Hz when applied during slow-wave rich periods of sleep had no effect (a) on the

overnight changes in both procedural and declarative memory tasks; (b) on sleep macrostructure; (c) on slow frontal and fast parietal sleep spindle densities; and (d) on SO in the stimulation free intervals. Furthermore, declarative memory retention decreased and procedural memory increased after sleep, and a decrement of FPS densities was observed after baseline in both experimental nights (see discussion in (1)).

Despite the encouraging results of Marshall et al. (11, 12), participants in the present study did not improve their performance in the WPT after stimulation. This result is consistent with previous studies that followed Marshall's experimental paradigm (52-56) but, in contrast to others (11, 12, 57). The discrepancies between the results of the present study and Marshall's study might be explained firstly by critical factors related to the inherent mechanistic of so-tDCS, and secondly by the different characteristics of the experimental tasks used to measure possible behavioral effects (see discussion in (1)). In the subsequent paragraphs, these two aspects will be discussed separately in order to disentangle these contradictory data in the literature and explain the present results.

A growing number of studies (1, 52-61, 82-85) has been adopted and adapted Marshall's protocol (12) with different stimulation aims and outcomes. Even though so-tDCS has been widely used, its methodology has some limitations that might explain the heterogeneous results in the literature.
A first critical aspect of neuromodulation techniques like so-tDCS is the timing of its application. Thut et al. (86) indicated that in order to exogenously entrain a specific neuronal oscillation rhythm (e.g. SO) it is required that: the neural populations of the stimulated region (e.g. prefrontal areas of the cortex) actually exhibit the target oscillations endogenously (e.g. SWA); the continuous weak electrical currents are periodic; the internal neural networks and the external stimulation are phase aligned (synchronized); and the induced stimulation interacts directly with the endogenous oscillations (86). Theoretically, entraining SO might affect the neural activity of the target regions by the increment of the signal intensity due to the phase alignment and by the progressive synchronization of the neuronal activity to the entraining rhythmic stimulation (87). Conversely, for low stimulation intensities, such as in so-tDCS protocols, it is crucial that the stimulation frequency and the ongoing endogenous frequencies coincide with each other (87). In other words, if the start of the stimulation process does not accurately match the ongoing slow oscillations in the areas of interest (e.g. frontal cortex), the process of entrainment may not occur properly. Indeed, Ozen et al. (88) proved the importance of "phase-aligned" stimulation protocols in a rodent study. In that study, neocortical and hippocampal neurons were recorded during slow frequency sinusoidal transcranial stimulation showing the entrainment of SO during sleep by the

combination of the induction of extracellular fields that affect neurons excitability and the effects mediated by ongoing activity of neuronal networks (88). In line with these results, another rodent study (89) altered hippocampal-cortical communication via slow sinusoidal stimulation due to the entrainment of SO on frontal regions of the neocortex and the hippocampus, which successfully increased hippocampal ripples and spindles. Thus, these findings in animal models emphasize that phase alignment is crucial for an accurate exogenous stimulation of SO.

Considering this, there are a priori three issues which might compromise the necessary time precision required to assure SO entrainment during SWS periods and that could explain our results. Firstly, following Marshall's methodology, stimulation was manually applied based on online visual scoring of sleep stages, which implies possible delays in the beginning of the stimulation. Secondly, the stimulation in our study included fade-in/fade-out periods at the beginning and at the end of each stimulation interval, which might have prevented SO entrainment (54). Finally, once the protocol was started, the stimulation was repeated without further inspection of the EEG signal in order not to compromise double blinding. This approach was based on the assumption that SWS would not be disturbed within this period of time, which might not always be that stable. Some studies (55, 56, 82) addressed this problem by monitoring the EEG signal during the stimulation period, ensuring that all the stimulation blocks (not only the first one) took place during stable periods of N2, at the expense of compromising blindness. Moreover, new methodologies based on closed-loop systems, which can detect SO instantly and automatically, allow to ensure that the beginning of the stimulation is aligned to endogenous SO. A recent study (90) used a closed-loop algorithm that was able to adapt and adjust tACS to the ongoing SO activity in frequency and phase. Another study (91) initiated the stimulation procedure 5 sec after SO were detected, and then applied so-tDCS in a "short-duration" repetitive manner (cycles of 4 sec stimulation, plus 4 sec rest). Both studies reported an enhancement of SO after stimulation showing the effectiveness of these two specific closed-loop approaches. Finally, a more specific stimulation method developed by Lustenberger et al. (92) was able to improve motor memory consolidation and to modulate FSA by applying a feedback-controlled tACS in the spindle frequency range (12 Hz) during NREM sleep.

Further to the above considerations, Horvath et al. (93) reviewed critical aspects reported in the tDCS literature that could also explain the absence of so-tDCS effects in the present study. These issues are associated with (a) inter-individual variability and intra-individual reliability problems; (b) blinding problems; (c) an absence of sham stimulation controls; (d) possible interference of cognitive activity in other tasks while ongoing stimulation; (e) and the importance of variations in the electric current

parameters that affect current density and flow (93). Furthermore, the efficacy of tDCS has been questioned by studies that reported an absence of cognitive modulation after one single session of tDCS (94, 95). Indeed, based on the growing amount of inconsistent results in the TES literature, Krause et al. (96) identified possible confounding variables related to the existent variability between individual differences in behavioral outcomes, cortical excitability and responsiveness to these stimulation methods. For instance, the following factors might affect TES outcomes: (a) pre-existing neurotransmitter levels like glutamate or gamma-aminobutyric acid; (b) head anatomy (size and tissue thicknesses); (c) cortical morphologies of gyri and sulci; (d) age; (e) hormonal levels (progesterone, estradiol, and estrogen levels during the menstrual cycle); (f) circadian rhythms; (g) variations in study designs (dosage, number of sessions, and applied current) (96). In order to reduce tDCS reliability problems, the use of individual modeling approaches of current distribution has been proposed not only to avoid oversimplification of TES mechanisms, but also to better describe the stimulation impact on neural networks (97).

While so-tDCS is widely used, its effectiveness has also been questioned. In a recent study, Lafon et al. (98) assessed the physiological effects of the interaction between induced electrical fields and ongoing brain activity using Marshall's (12) stimulation protocol. They measured intracranial EEG signals from implanted electrodes in patients with epilepsy after the application of tACS at 0.75 Hz in NREM sleep and concluded that the weak-induced electric fields were not able to entrain SO (0.5 - 1.5 Hz), hence to modulate cortical oscillations (98). While a study (99) suggested that the measured electric field at the applied potential is at least one order of magnitude lower than the required electric field to produce any reliable SO entertainment, accumulative effects that lead to resonance processes were not dismissed. Thus, so-tDCS protocols at weak intensities (i.e. 260 μA) may not be strong enough to enhance endogenous SO, which could elucidate why we did not find an increment of SO after stimulation.

Based on the growing number of studies that aim to modulate SO and spindles, given the role that they play in sleep-dependent memory consolidation (5, 48), so-tDCS could be considered as a potential therapeutic tool that can target these specific brain oscillations. Hypothetically, so-tDCS might be implemented to improve memory, for example in patients with insomnia, major depression, schizophrenia, or post-traumatic stress disorder, all of them pathologies associated with sleep disorders and memory impairment (100). For instance, to date, so-tDCS positive effects were reported in schizophrenic patients (58), children with ADHD (60, 61), and in patients with MCI (59). However, bearing in mind the above-mentioned critical aspects of so-tDCS, the implications related to its application and its translation into a therapeutic tool should be reconsidered. The heterogeneous effects

observed in healthy participants after so-tDCS (see Table 1 in Bueno-Lopez et al (1)) point out the need to develop more appropriate stimulation methods and better stimulation protocols capable of modulating SO accurately. In this respect, new stimulation techniques have been implemented over recent years with the purpose to manipulate SO and sleep spindles. For example, studies that used closed-loop acoustic stimulation applied in phase to SO up-states were able to modulate SO and to report memory enhancement in healthy participants (101-103). However, studies in MCI patients (104) and in healthy young participants (105), which used closed-loop acoustic stimulation as well, failed to improve memory performance, although SO were increased after stimulation. In line with this, another recent study (106) that applied an auditory rhythmic stimulation during NREM sleep was able to enhance sleep spindles (memory consolidation was not tested). Further studies in this direction may lead to more effective non-invasive neuromodulation methods that can be implemented during sleep. This is essential for making progress in the understanding of sleep associated memory consolidation processes and in the development of reliable therapeutic tools.

In the present study, the absence of a declarative memory enhancement might not only be due to the stimulation protocol, but also due to the memory task *per se*. One critical aspect of the WPT used in this study was that no feedback was given during the immediate recall in the evening. Contrary to what was expected, our results revealed that overnight retention of word-pairs decreased in both experimental nights, irrespective of the stimulation condition. This finding is in line with previous studies that did not use feedback paradigms (54-56, 83, 105). However, it contradicts to studies that did provide feedback during recall. The latter reported increases of memory retention after sleep (e.g.(11, 12, 53, 57)). These observations could suggest a weak memory encoding due to the absence of feedback during immediate recall and therefore explain this overnight retention decline. A second critical aspect of this task was the use of a learning-to-criterion condition, that is, the opportunity to relearn the word pairs, if less than a 60% of the target words were recalled. This criterion allowed measuring the impact of a re-exposure of word pairs on memory retention. Thus, participants who had additional learning opportunities recalled more words during the immediate recall at the evening and showed a better overnight retention regardless of the experimental condition. In other words, these participants forgot less word pairs than those who only had one learning session.

Both factors, feedback and re-exposure of word pairs, represent two different forms of re-encoding opportunities that might have influenced memory retention. The level of encoding in word learning tasks has been previously discussed in relation to how the strength of memory encoding (weak vs strong) might influence consolidation, pointing out that weakly encoded words could benefit more from sleep (107).

In addition, Pastötter and Bäuml (108) found that only unrecalled words could benefit from re-encoding process such as feedback. Furthermore, if word pairs are reinforced during re-encoding opportunities, they can be more retrievable and this could prevent forgetting (109). Cameron et al. (110) suggested that the activity of hippocampal neurons might be associated with the encoding phase of word pairs and that this activity may predict a better performance during recall. Thus, during recall, memory traces might be re-activated and feedback should reinforce these word pairs associations.

In conclusion, our findings indicated that so-tDCS applied during NREM sleep has no beneficial effects on sleep-dependent memory consolidation in young healthy adults. Moreover, so-tDCS did not affect macro- and microstructure of sleep. In contrast to the results of Marshall et al. (11, 12), the present study could not exogenously enhance SO and/or boost declarative memory (see Table 1 in Bueno-Lopez et al. (1)). Finally, factors such as so-tDCS stimulation protocols, individual variability and memory task specifications, may limit the comparability between studies. These issues contribute to the actual replication problems of so-tDCS beneficial effects on declarative memory (see conclusions in Bueno-Lopez et al (1)).

6. References

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Eidesstattliche Versicherung

„Ich, Ana Bueno-Lopez, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: Effects of slow oscillatory transcranial direct current stimulation (so-tDCS) on sleep-dependent memory consolidation selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; http://www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst."

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Ausführliche Anteilserklärung an der erfolgten Publikation

[Die Anteile an der ausgewählten Publikation sind so deutlich und detailliert zu erklären, dass es der Promotionskommission und den wissenschaftlichen Gutachtern ohne Probleme möglich ist zu erkennen, was Sie selbst dazu beigetragen haben. Wünschenswert wäre ein konkreter Bezug zur Publikation selbst wie z. B.: „aus meiner statistischen Auswertung sind die Tabellen 1, 4, 47 und 60 entstanden."

Sie teilen sich die Erstautorenschaft mit jemand anderem: Dies ist sichtbar anzugeben. In diesem Fall ist die Erklärung von beiden Autoren abzugeben. Nur Ihre eigene Erklärung ist mit in Ihre Dissertation einzubinden. Auf diesem Exemplar brauchen nur Sie selbst zu unterschreiben. Darüber hinaus legen Sie bitte noch eine weitere von Ihnen und Ihren Betreuer/innen unterschriebene Erklärung sowie die Erklärung des/der anderen Erstautors/in mit dessen Unterschrift für die Akte vor.]

Publikation 1:
Bueno-Lopez, A., Eggert, T., Dorn, H. and Danker-Hopfe, H. 2019. Slow oscillatory transcranial direct current stimulation (so-tDCS) during slow wave sleep has no effects on declarative memory in healthy young subjects. Brain Stimulation; S1935-861X(19)30065-8. doi: 10.1016/j.brs.2019.02.012.

Beitrag im Einzelnen:
Hiermit bestätige ich, dass die Erhebung der in dieser Studie berücksichtigten Daten größtenteils von mir durchgeführt wurde (22 von 26 Probanden). Die Datenerhebung umfasste ein mehrstufiges Screeningverfahren, die Durchführung von Polysomnographien sowie die Betreuung der Testabläufe am Abend und am Morgen. Die medizinische Voruntersuchung, an der die Probanden im Rahmen der Rekrutierung teilnehmen mussten, erfolgte im Beisein einer unserer beiden Studienärztinnen (Dr. Marie-Luise Hansen und Dr. Anita Peter). Für das Scoring der polysomnographischen Aufzeichnungen war Frau Esther Marasanov verantwortlich. Die Powerspektralwerte sind von Dr. Hans Dorn berechnet worden. Bis auf den Blinding-Index, der von Dr. Torsten Eggert ermittelt wurde, sind alle statistischen Analysen von mir durchgeführt worden. Die Erstellung des Manuskripts sowie dessen Überarbeitung gemäß den Anmerkungen der Co-Autoren und der Gutachter lag komplett in meinem Verantwortungbereich.

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Unterschrift, Datum und Stempel des betreuenden Hochschullehrers/der betreuenden Hochschullehrerin

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Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI
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Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NATURE REVIEWS NEUROSCIENCE	40,834	32.635	0.069940
2	NATURE NEUROSCIENCE	59,426	19.912	0.153710
3	ACTA NEUROPATHOLOGICA	18,783	15.872	0.041490
4	TRENDS IN COGNITIVE SCIENCES	25,391	15.557	0.040790
5	BEHAVIORAL AND BRAIN SCIENCES	8,900	15.071	0.010130
6	Annual Review of Neuroscience	13,320	14.675	0.016110
7	NEURON	89,410	14.318	0.216730
8	PROGRESS IN NEUROBIOLOGY	13,065	14.163	0.015550
9	BIOLOGICAL PSYCHIATRY	42,494	11.982	0.056910
10	MOLECULAR PSYCHIATRY	18,460	11.640	0.047200
11	JOURNAL OF PINEAL RESEARCH	9,079	11.613	0.008600
12	TRENDS IN NEUROSCIENCES	20,061	11.439	0.026860
13	BRAIN	52,061	10.840	0.075170
14	SLEEP MEDICINE REVIEWS	6,080	10.602	0.010720
15	ANNALS OF NEUROLOGY	37,251	10.244	0.053390
16	Translational Stroke Research	2,202	8.266	0.005260
17	NEUROSCIENCE AND BIOBEHAVIORAL REVIEWS	24,279	8.037	0.048460
18	NEUROSCIENTIST	4,738	7.461	0.008730
19	NEURAL NETWORKS	10,086	7.197	0.015290
20	FRONTIERS IN NEUROENDOCRINOLOGY	3,924	6.875	0.006040
21	NEUROPSYCHOPHARMACOLOGY	24,537	6.544	0.042870
22	CURRENT OPINION IN NEUROBIOLOGY	14,190	6.541	0.034670
23	Molecular Neurodegeneration	3,489	6.426	0.009850
24	CEREBRAL CORTEX	29,570	6.308	0.058970
25	BRAIN BEHAVIOR AND IMMUNITY	12,583	6.306	0.026850
26	BRAIN PATHOLOGY	4,952	6.187	0.007750
27	**Brain Stimulation**	**4,263**	**6.120**	**0.014510**
28	NEUROPATHOLOGY AND APPLIED NEUROBIOLOGY	3,654	6.059	0.006350
29	JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM	19,450	6.045	0.028280
30	JOURNAL OF NEUROSCIENCE	176,157	5.970	0.265950
31	Molecular Autism	1,679	5.872	0.006320
31	Translational Neurodegeneration	589	5.872	0.002280
33	GLIA	13,417	5.846	0.020530
34	Neurotherapeutics	3,973	5.719	0.008980
35	PAIN	36,132	5.559	0.038000
36	NEUROIMAGE	92,719	5.426	0.152610
37	Acta Neuropathologica Communications	2,326	5.414	0.011550
38	Multiple Sclerosis Journal	10,675	5.280	0.021890

Publication

Bueno-Lopez A, Eggert T, Dorn H, Danker-Hopfe H. Slow oscillatory transcranial direct current stimulation (so-tDCS) during slow wave sleep has no effects on declarative memory in healthy young subjects. Brain Stimul. 2019 Jul-Aug;12(4):948-958.

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Curriculum vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlich.

List of publications

Bueno-Lopez, A., Eggert, T., Dorn, H. and Danker-Hopfe, H. 2019. Slow oscillatory transcranial direct current stimulation (so-tDCS) during slow wave sleep has no effects on declarative memory in healthy young subjects. Brain Stimulation; S1935-861X(19)30065-8. doi: 10.1016/j.brs.2019.02.012.

Schmid, G., Hirtl, R., Bueno-Lopez, A., Dorn, H., Eggert, T. and Danker-Hopfe, H. 2020. Design and Dosimetric Analysis of an Exposure Facility for Investigating Possible Effects of 2.45 GHz Wi-Fi Signals on Human Sleep. Bioelectromagnetics; doi: 10.1002/bem.22256

Danker-Hopfe, H., Bueno-Lopez, A., Dorn, H., Schmid, G., Hirtl, R. and Eggert, T. 2020. Spending the night next to a router – Results from the first human experimental study investigating the impact of Wi-Fi exposure on sleep. Submitted.

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