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Slow oscillatory transcranial direct current stimulation (so-tDCS) during slow wave sleep has no effects on declarative memory in healthy young subjects

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ABSTRACT

Background: The manipulation of specific brain oscillations by applying transcranial electrical stimulation techniques in order to enhance memory processes during sleep has become an intriguing field of research. A seminal study found a positive effect of slow-oscillatory transcranial direct current stimulation (so-tDCS) on sleep-dependent consolidation of declarative memories. Since then several studies have tried to replicate this result with inconsistent findings.
Objective/Hypothesis: This study aimed to reexamine effects of so-tDCS on declarative memory observed in young participants based on a previously described stimulation protocol used in elderly subjects.
Methods: 23 healthy participants (mean ± SD: 23.2 ± 1.9 years; 13 women) completed a word-pair test and a sequential finger tapping test before and after sleep. Participants received anodal so-tDCS bifrontaly at a frequency of 0.75 Hz or sham stimulation during NREM sleep N2, following a double-blind, placebo controlled, counterbalanced, randomized crossover design. Data were analyzed with respect to possible effects of stimulation on memory performances, sleep staging, spindle densities and EEG power in eight frequency bands.
Results: Stimulation had no significant effect on sleep dependent memory consolidation or on sleep macro- and microstructure. Independent of stimulation, procedural memory performances increased and declarative memory performances decreased overnight. This decline was less pronounced when participants had more than one learning opportunity. Fast parietal but not slow frontal spindle densities diminished from baseline to stimulation-free intervals under both stimulation conditions.
Conclusion: The present study could not reproduce the results of the seminal study in young subjects, but it is consistent with results observed in elderly subjects using the same protocol. Irrespective of stimulation, re-encoding opportunities in the word-pair test had an impact on memory strength and retrieval performance.

Introduction

There is a growing body of literature that recognizes the importance of sleep as a mediator of memory consolidation processes (e.g., reviews [1,2]). When new information is encoded and initially stored during waking, new memory traces are formed [3]. During sleep, these newly labile encoded memory representations are reactivated, reorganized and reinforced, a process known as sleep-dependent memory consolidation [3–6]. According to the two-stage model of hippocampal memory storage [7], declarative memory consolidation is mediated at the cortical level by slow oscillations (< 1 Hz) that present global neuronal excitation ("up-states" of depolarization) and neuronal rest ("down-states" of hyperpolarization) during slow wave sleep (SWS) [8,9]. During the "up-states", associated thalamo-cortical spindle activity and hippocampal sharp wave-ripples allow the reactivation, stabilization and integration of this labile memory representations into the neocortex [1,6,8,10–12].

Based on these theories, a variety of methods has been implemented to modulate sleep physiology and memory consolidation [13]. At this respect, brain activity modulation by transcranial

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electrical stimulation with direct (DC) and alternating (AC) currents has been extensively investigated (e.g. Refs. [14–17]). The combination of AC with DC offset at low frequencies is defined as slow oscillatory transcranial direct current stimulation (so-tDCS) [17,18]. When electrical currents are externally applied into the brain, the generated weak electric fields can induce neuronal membrane polarization (facilitating or inhibiting neuronal firings) and modulate the amplitude of endogenous brain oscillations via entrainment [19,20]. Transcranial stimulation with AC can modulate amplitude, frequency and phase/phase coherence of brain oscillations at almost no perceptible current strengths [19,21]. Animal studies have shown that AC stimulation at low intensities can modulate neuronal spiking timing and ongoing slow oscillations when the stimulation was phase-aligned with endogenous brain activity [18,19]. Active neural networks seems to have more sensitivity to electric fields, thus ongoing stimulation may amplified the effects of polarization [18,20]. Taking into account these mechanisms, slow wave activity during sleep might be amplified by externally applying so-tDCS [8,22].

Over the last years, eight studies [23–30], which applied anodal-tDCS bilaterally over the prefrontal cortex in order to enhance endogenous slow-oscillations during SWS to modulate sleep-dependent memory consolidation in healthy participants (see Table 1 and Suppl. Table 1), reported inconsistent effects (see Table 1). However, in non-healthy population so-tDCS revealed positive results [31–33].

In a previous study of our research group, so-tDCS did not modulate declarative memory in elderly participants [25] using similar stimulation parameters as the seminal study in young subjects [24]. The deviating results were attributed to differences in the stimulation parameters (see Suppl. Table 1) and to differences in the ongoing cortex activity during stimulation due to sleep fragmentation in the elderly [25].

Given that, the aim of the present study was a conceptual replication of the original results in young adults [24] with the focus on reexamining effects of so-tDCS on declarative memory in this specific age group by applying the same stimulation parameters used in our study on elderly [25]. Furthermore, since it is known that the outcome parameters vary with the menstrual cycle [34] this factor was controlled for. This new effort to confirm previously published results contributes to a better understanding of factors and mechanisms involved in so-tDCS effects and in the importance of the study design to measure memory modulation.

Methods and materials

Participants

Twenty-six healthy young adults aged 20–27 years (13 women; mean age ± SD: 23.3 ± 1.9 years) participated in this study (for sample size calculation see Suppl. material). Major exclusion criteria were a disturbed sleep (Pittsburgh Sleep Quality Index (PSQI) score > 5; [35]), excessive daytime sleepiness (Epworth Sleepiness Scale (ESS) score > 10; [36]), extreme chronotype (Morningness-Eveningness Questionnaire (MEQ) score ≤ 31 or ≥ 69; [37]), and increased depression and/or anxiety score (Self-Rating Anxiety Scale (SAS) score ≥ 36; [38]; Self-Rating Depression Scale (SDS) score ≥ 40; [39]), intake of central nervous system effective medication, sensitive skin, severe untreated medical condition, cognitive impairment, any metal implants; metabolic or hormonal disorder (oral contraceptives were allowed for women); daily excessive consumption of caffeine and/or alcohol. Participants had to be non-smoker, and native German speakers. Participants underwent a physical, mental, and neurological medical examination. Eligible participants were polysomnographically screened for possible sleep disorders. Subjects with an apnoe-hypopnoe-index > 10/h and/or a periodic limb movement arousal index > 10/h were excluded. All participants were Caucasian and university students.

The ethics committee of the Charité - Universitätsmedizin Berlin (Germany) approved this study (EA4/076/15). All the participants

Table 1
Effects of transcranial direct current stimulation. In all studies, the aim of the stimulation was memory enhancement in healthy participants.

Study	Sleep	Participants (n)	Stimulation effects						
			Behavioral measures		Sleep macrostructure			Sleep microstructure	
			Declarative memory task[1)]	Procedural memory task[2)]	Entire sleep time[3)]	Time after stimulation[3)]	Stimulation-free intervals[3)]	EEG power[4)]	Sleep spindles[5)]
Marshall et al. (2004)	Nap	Young (18)	WPT[ab] ↑	MTT n.s.	(1.5 h) n.s.	(15 min) S2 S3 ↑	N/A	SO[6,8] δ[6] ↑ θ[9] α[6,8,9] β[8,9] ↓	SC ↓
Marshall et al. (2006)	Night time	Young (13)	WPT[ab] ↑ FPA n.s.	SFTT n.s. MTT n.s.	(7.5 h) n.s.	(60 min) n.s.	SWS ↑	SO[6] ↑ SSA[6] ↑	SSD[6] ↑
Sahlem et al. (2015)	Night time	Young (12)	WPT[ab] n.s.	SFTT n.s.	(8.0 h) n.s.	(60 min) S3 ↑	N/A	n.s.	N/A
Koo et al. (2018)	Night time	Young (25)	WPT[a] n.s. FPA ↑[d] 2D-L n.s.	SFTT n.s. MTT n.s.	(7.5 h) n.s.	(150 min) n.s.	n.s.	FSA[10] ↑	FSD[10] ↑
Eggert et al. (2013)	Night time	Elderly (23)	WPT[b] n.s.	SFTT n.s.	(7.5 h) n.s.	(60 min) n.s.	S3 ↓ W ↑	δ[6,11] ↓	n.s.
Westerberg et al. (2015)	Nap	Elderly (19)	WPT[a] ↑ FRT n.s.	OP n.s.	(1.5 h) n.s.	(N/S min) n.s.	n.s.	SO[6] ↑	FSD[8] ↓
Passmann et al. (2016)	Night time	Elderly (21)	WPT[bc] n.s. VST ↓	SFTT n.s.	(8.5 h) S4 ↓	(60 min) n.s.	n.s.	SO[6,7] ↑ FSA[8,10] ↑ SSA[6,7] ↑	N/A
Ladenbauer et al. (2016)	Nap	Elderly (18)	WPT[bc] n.s. VST ↑	SFTT n.s.	(1.5 h) n.s.	(N/S min) n.s	n.s.	SO[6] ↑ FSA[6,10] ↑	FSD[6,10] ↑

[1)] **Declarative memory tasks**: WPT = word pair test; FPA = figural paired-associate test; FRT = fact recognition test; VST = visuo-spatial memory task; 2D-L = 2D-object location. Declarative memory test followed by feedback [a], with learning criteria [b] or first a learning period with feedback and the second period without [c]. Results for n = 13 subgroup with high *memory quotinent* [d]. [2)] **Procedural memory tasks**: SFTT = sequential finger tapping task; OP = object priming; MTT = mirror tracing test.[3)] **Sleep macrostructure**: NREM sleep stage 2 (S2), stage 3 (S3) and stage 4 (S4); SWS = slow wave sleep (S3 + S4); W = wake. [4)] **EEG-Power** (within the stimulation-free intervals): SO = slow oscillations; SSA = slow spindle activity; FSA = fast spindle activity. [5)] **Spindle**: SC = spindle counts; FSD = fast sleep spindles density; SSD = slow sleep spindle density. [6)] Frontal. [7)] Prefrontal. [8)] Central. [9)] Parietal. [10)] Centro-parietal. [11)] Temporal. (For exact definition of the regions, see Suppl. Fig. 1). ↑ = significant increase after verum stimulation compared to sham. ↓ = significant decrease after verum stimulation compared to sham; n.s. = non-significant; N/A = not available; N/S = not specified.

signed a written informed consent and received a monetary compensation.

Study design and procedure

Participants spent three nights in the laboratory, where the first night was an adaptation and screening night with no further intervention (see Fig. 1). A time-lapse of four weeks between the sessions aimed to control the menstrual cycle in female participants. All women were in the luteal phase in order to avoid possible confounding effects [34]. Stimulation conditions were applied in a counterbalanced randomized, double-blind, within-subject cross-over design.

Sleep recording

Sleep data was collected with a Neurofax EEG-9200 device (Nihon Kohden, Tokio, Japan). In the adaptation night, a full cardiorespiratory polysomnography (PSG) recording was performed according to the AASM guidelines [40]. For the two experimental nights, electroencephalogram (EEG) was recorded with 13 gold-coated scalp electrodes placed according to the 10–20 EEG system [41] referenced to the nose tip (Nz) and grounded to Fp2 as in Marshall et al. [24] and Eggert et al. [25] (see Fig. 2 A). Additionally, an electrooculgram (EOG), chin electromyogram (EMG mental and submental), and electrocardiogram (ECG) were recorded.

So-tDCS

The stimulation protocol followed the same procedure as described in Marshall et al. [24] and the stimulation parameters were identical to those implemented by Eggert et al. [25]. A tDCS with sinusoidal oscillating waveform between 0 and 260 μA at a frequency of 0.75 Hz was delivered by a battery driven Eldith DC-Stimulator Plus (NeuroConn GMbH, Ilmenau, Germany). Anodal electrodes (10 mm diameter) were placed bilaterally at prefrontal positions (F3-F4), and cathodal reference electrodes were fixed at the ipsilateral mastoids (M1-M2) according to the 10–20 system of electrode placement [41] (see Fig. 2 A). Electrode resistance was limited at < 2 kΩ for the stimulation electrodes. The maximal current density reached per anodal electrode was 0.331 mA/cm^2. In order to avoid painful current steps, a ramping period for 8 s was implemented at the beginning and at the end of stimulation. In the sham stimulation, periodic small input currents with no therapeutic significance were applied [25].

The stimulation procedure was the same for both experimental conditions: Stimulation was triggered after the first eight consecutive epochs of stable NREM sleep 2 based on visual online sleep scoring. The total stimulation period (25′8″) was divided into five intervals of stimulation (5′16″), each followed by a 1-min break without stimulation. Double-blinding was assured by switching off the PSG screen during the stimulation. See Fig. 2 B-C for so-tDCS protocols and parameters.

Memory tasks

A declarative (semantic) and a procedural memory task were administered during a first learning phase before bedtime and in a recall phase after a night of sleep. Both tasks were presented on a computer screen (diagonal: 48 cm) using E-Prime 2 (Psychology Software Tools, Pittsburg, USA).

Semantic memory was assessed by a word-pair association task (WPT) [24]. This task included two different lists of 54 pairs of semantically related German nouns. Word-pair lists were randomly presented in both experimental sessions. Primacy and recency effects were controlled by eight dummy word-pairs: four of them presented at the beginning and the other four at the end of the list. Each word-pair consisted of a cue word and a target word. During evening and morning recall, participants had no time limit to verbalize the target word and once the participant answered, no feedback was provided. If after the learning trial the participant was

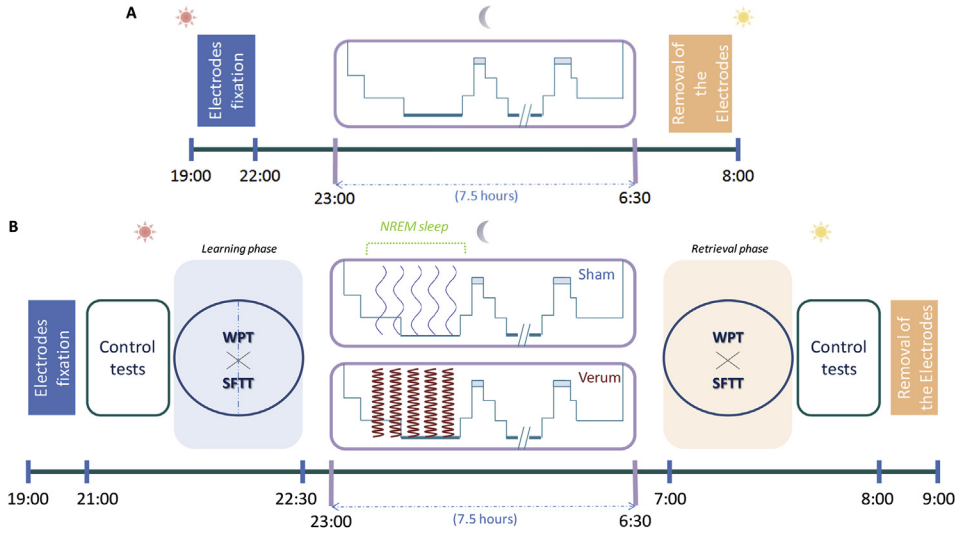

Fig. 1. Timelines of the experimental procedures. Arrival time was scheduled at 19:00 h for all three nights. **A)** Adaptation and screening night: Electrodes were placed for a full cardiorespiratory PSG. After fixation of all PSG sensors, participants filled out an evening sleep protocol and went to bed at 23:00 h. Intake of caffeine and alcohol was prohibited on the days of the experimental sessions. **B)** Experimental sessions: Thirteen electrodes were placed to record the EEG. In addition EOG, chin EMG, ECG and stimulation electrodes were placed. Around 21:00 h, participants completed different psychometric control tests followed by a word pair task (WPT) and a sequential finger tapping task (SFTT). The order of both memory tasks was counterbalanced across and within participants. After the learning phase, participants filled out an evening sleep protocol and went to bed with lights out at 23:00 h. During the sleep period, verum or sham stimulation was applied after the first four minutes of consolidated NREM sleep stage 2 (N2). Thirty minutes after awakening retrieval of both memory tasks was assessed followed by the administration of the psychometric control tests. In addition, participants filled out a morning sleep protocol and they were asked to guess the stimulation condition, they had been exposed to during the night. Participants were permitted to have breakfast and drink caffeinated beverages only after the removal of the electrodes.

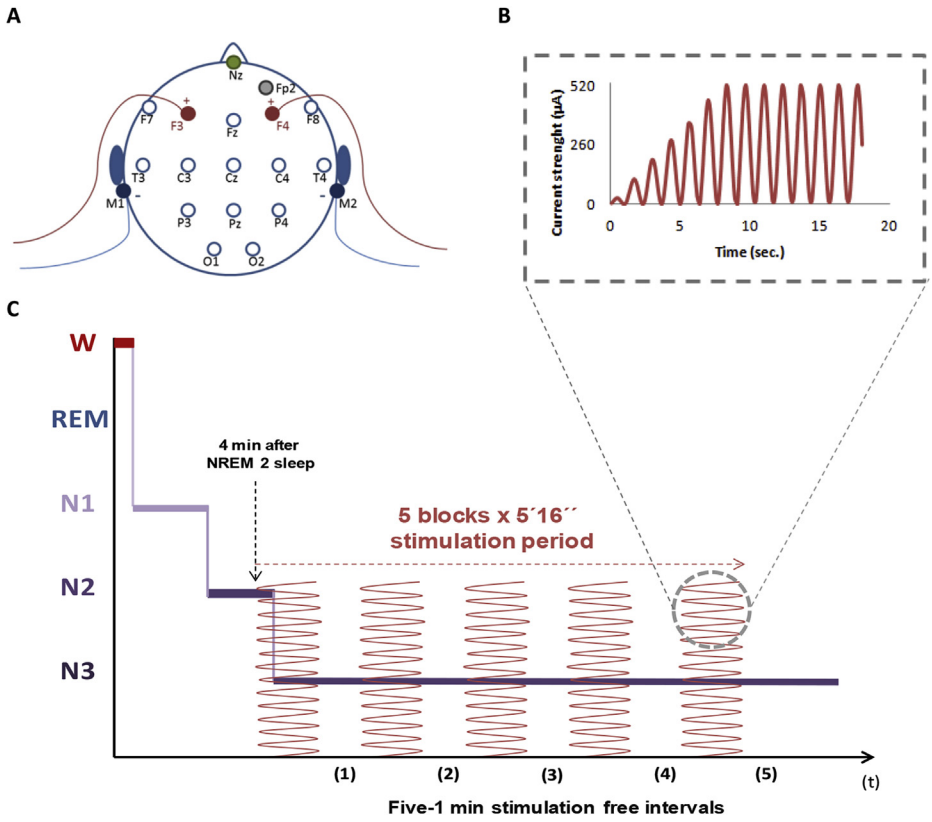

Fig. 2. Stimulation protocol and EEG electrode localizations. A) 13 EEG electrodes (open circles) were placed according to the 10/20 system, referenced to Nz and grounded to Fp2. The anode stimulations electrodes were placed bilaterally at F3 and F4 and the reference stimulation electrodes (cathodes) at the ipsilateral mastoid positions (M1 and M2). **B) and C)** A sinusoidal waveform tDCS oscillating at a frequency of 0.75 Hz between 0 and 260 μA for each stimulation electrode (total: 520 μA) was delivered via 10 mm diameter electrodes resulting in a maximum current density of 0.331 mA/cm^2. A ramping period for 8 s was present at the beginning (*fade-in*) and at the end (*fade-out*) of stimulation in order to avoid painful current steps. The stimulation was delivered five times for 5 min and 16 s each. Every stimulation period was followed a by 1-min stimulation-free interval.

not able to reach a predefined learning criterion of 60 % of correct word-pairs, the same list of word-pairs was presented again but in a different randomized order of words. This was repeated until the learning criterion was achieved. Overnight change was calculated as the differences in performance between the retrieval phase in the morning and the last recall in the evening.

A sequential finger tapping task (SFTT) [24] was performed to evaluate procedural memory. After a short training, participants were asked to tap five-digit-sequences (e.g. 4-2-3-1-4) on a computer keyboard, as quickly and accurately as possible, with their non-dominant hand. The learning phase consisted of twelve 30 s block-trials. At the next morning, participants completed three additional trials during the retrieval phase. After each trial, the number of correct sequences and the number of tapped sequences were shown. For analyses, the correct number of typed sequences per 30 s and the error rates (number of errors expressed as percentage of the total number of completed sequences) were assessed. The mean of the three last trials of the learning phase and the mean of the three trials of the retrieval phase were considered to measure the performance of this task. Overnight change was calculated as the differences between morning and evening performances.

Control tests, stimulation blinding and so-tDCS adverse effects

To ensure normal cognitive functioning two tests were performed prior to the first night in the lab. Control tests were implemented prior to the learning phase and after the recall period in the morning in order to assess mood state, motivation, verbal ability of retrieval, working memory and sleep quality. In addition, after the experimental nights, participants were asked about the stimulation condition and whether or not they experience any symptoms related to the stimulation (see Suppl. material).

Macrostructure of sleep

Sleep recordings were scored according to the standards of the AASM [40]. The impact of stimulation on sleep macrostructure was measured for: (1) the entire night, where the percentage of sleep stages was referenced to the total sleep period time (SPT) excluding the stimulation period; (2) scoring of the 60 min following the end of the stimulation period; and (3) the 1-min stimulation-free intervals (scored based on 10 sepochs).

Microstructure of sleep

Effects of so-tDCS on the sleep microstructure were assessed for the five stimulation-free intervals by analyzing the spectral power and the sleep spindle densities (for details see Ref. [25]). EEG spectral power was calculated for five 50 s periods (each divided in 10 s mini-epochs) for eight frequency bands: Slow oscillations (SO_1: 0.5–1 Hz; SO_2: 1–1.5 Hz), delta (1–4 Hz), theta (4–8 Hz), alpha (8–11 Hz), slow spindles (11–13 Hz), fast spindles (13–15 Hz) and beta (15–25 Hz). EEG artifacts were identified automatically by an amplitude criterion (amplitudes > 150 μV) and by visual inspection. Artifacts were excluded from final analysis. The mean (over the five stimulation-free intervals) of the medians (calculated for the five 10 s epochs of each stimulation-free interval) was computed for

every frequency band and averaged over frontal (F7, Fz, F8), central (C3, Cz, C4, T3, T4) and posterior (P3, Pz, P4, O1, O2) electrode locations.

Spindle activity was assessed for slow frontal (11–13 Hz) and fast parietal (13–15 Hz) sleep spindles (see Ref. [25]). Spindle densities (referenced to 30 s epochs) were determined from frontal (Fz-Nz) and parietal (Pz-Nz) derivations at baseline (60 s prior to stimulation) and for the stimulation-free intervals (5 × 50 s). Differences were computed between densities at baseline and stimulation-free intervals.

Statistical analyses

Statistical analyses were performed with IBM SPSS Statistics 24. A repeated measures analysis of variance (rmANOVA) with the within-subject factors STIM (verum vs sham) and TIME (learning phase at evening vs retrieval phase at morning) was applied to the performances in both memory tasks and in the control tests. For spindle density the within-subject factors STIM (verum vs sham) and TIME (baseline vs stimulation-free interval) were considered. For EEG power the factors STIM (verum vs sham) and electrode locations LOC (frontal vs central vs posterior) were considered for each frequency band. Partial eta-square (η^2_p; small: $\eta^2_p = 0.01$; medium: $\eta^2_p = 0.06$ and large: $\eta^2_p = 0.14$) was used as measure of effect size [42].

Depending on the results of the Shapiro-Wilk-test, differences in sleep macrostructure between the stimulation conditions were analyzed either by a *t*-test for paired observations or the Wilcoxon matched-pairs signed-ranks test. For the sake of consistency the size of effects resulting from these pairwise comparisons were expressed as Cohens'd (small: $d = 0.2$, medium: $d = 0.5$ and large: $d = 0.8$) [43].

The impact of the number of sessions (1 vs. > 1) on the number of correctly remembered word pairs obtained at evening recall and morning retrieval was examined independent from stimulation, i.e. both analyses comprised all 46 observations. As the number of sessions needed to reach the learning criterion did not only vary between but also in a non-unidirectional manner within most of the 23 subjects, this aspect could not be considered as an additional between-subject factor in the rmANOVAs but had to be analyzed separately by t-tests for independent samples.

The efficacy of the stimulation blinding was evaluated by James' Blinding Index (BI) [44]. This Index is sensitive to disagreement taking into account the number of “don't know” responses. Differences in the prevalence of adverse effects between sham and active stimulation was tested by McNemar-Test. Subjective associations between symptoms and stimulation were tested by Stuart-Maxwell Tests (see Suppl. material).

Results

Three participants were excluded from data analyses. According to the stimulation protocol, the stimulation was applied too early for one participant. In the second case, the stimulator did not work properly and no current was applied. A third participant was excluded due to technical problems during data acquisition. Therefore, the final sample included in the statistical analysis consisted of 10 men and 13 women.

Stimulation blinding and so-tDCS adverse effects

The blinding index in this study was 0.80 indicating a successful blinding (Suppl. Fig 2S). James' BI [44] varies within a range from 0 to 1, where 0 represents total lack of blinding and 1 a complete blinding while 0.5 indicates a completely random blinding (i.e. 50 % correct and 50 % incorrect guesses; [45]). Furthermore, reported symptoms after stimulation did not differ significantly between stimulation conditions (see Suppl. material).

Memory tasks

The correctly recalled number of word pairs did not differ significantly between the two stimulation conditions, whereas they differed significantly with TIME, while the interaction between STIM x TIME was not statistically significant (see Table 2 A). Post-hoc t-tests yielded a significant overnight reduction in the number of correctly recalled word-pairs in both conditions (Fig. 3 A). Additionally, evening performances showed no statistically significant differences between both experimental conditions ($p = 0.130$) (Fig. 3 A). The non-significant interaction indicates that these overnight reductions do not differ significantly between sham and verum stimulation (Fig. 3 B). The distributions of individual overnight changes for both conditions are displayed as waterfall plots in Fig. 3 C1-C2.

For the correctly typed sequences per 30 s in the procedural memory task, the effect of TIME was statistically significant, whereas STIM and the interaction between both was not (see Table 2 A). Post-hoc tests revealed overnight improvements (Fig. 3 D–F). Analysis of the performance error rate showed that none of the factors TIME and STIM nor their interaction were statistically significant (see Table 2 A and Fig. 3 G–J).

Sleep macrostructure

The macrostructure of sleep for the entire night, the 60 min following stimulation, and stimulation-free intervals did not differ significantly between stimulation conditions (see Table 3).

Sleep microstructure

The effect of so-tDCS stimulation on spindle densities was analyzed separately for fast and slow sleep spindles. No stimulation effect was found in the analysis of the spindle density (see Table 2). However, the fast parietal spindle densities were significantly lower after both stimulation conditions (see Table 2B, Fig. 4).

The analysis of the spectral power in the stimulation-free intervals revealed no so-tDCS effects in any of the frequency bands. Post hoc analyses showed no statistically significant variations between verum and sham stimulation (see Fig. 5). However, the results indicate that irrespective of the stimulation condition spectral power varies topographically (see Table 2 C). Pairwise post-hoc tests revealed statistically significant results (frontal < central < posterior; all $p \leq 0.003$) for all frequency bands, except for the slow spindle frequency band (11–13 Hz) for which the power was not different for the posterior and central location ($p = 0.156$). There was no significant interaction between stimulation and scalp electrode localizations.

Discussion

The present study showed that while the performances in both memory tasks differed significantly between the morning and evening assessments so-tDCS had no effect on these overnight changes. The macro- and microstructure of sleep (slow frontal and fast parietal sleep spindle densities as well as spectral power during the five stimulation-free intervals) were also not affected by so-tDCS.

Comparing the present results with other relevant studies in this field of research (Table 1) it emerges that procedural memory was consistently not affected by stimulation. With regard to the

Table 2
Results of rm ANOVA. **A)** Memory tasks. **B)** Sleep spindle densities (counts per 30 s). **C)** Spectral power.

A	STIM			TIME			TIME x STIM		
	$F_{(1;22)}$	p	η^2_p	$F_{(1;22)}$	p	η^2_p	$F_{(1;22)}$	p	η^2_p
WPT									
Correct WP	1.52	0.231	0.064	27.07	**<0.001**	0.552	0.01	0.943	<0.001
SFTT									
Correct SQ	0.07	0.790	0.003	42.18	**<0.001**	0.657	1.26	0.274	0.054
Error Rate	0.84	0.370	0.037	2.56	0.124	0.104	1.22	0.281	0.053
B	**STIM**			**TIME**			**TIME x STIM**		
	$F_{(1;22)}$	p	η^2_p	$F_{(1;22)}$	p	η^2_p	$F_{(1;22)}$	p	η^2_p
SFS	2.79	0.108	0.113	2.56	0.124	0.104	0.39	0.538	0.017
FPS	0.18	0.676	0.008	19.29	**<0.001**	0.467	1.47	0.239	0.062
C	**STIM**			**LOC**			**STIM x LOC**		
	$F_{(1;22)}$	p	η^2_p	$F_{(2;44)}$	p	η^2_p	$F_{(1;44)}$	p	η^2_p
SO_1	0.45	0.509	0.020	48.24	**<0.001**	0.687	0.49	0.618	0.022
SO_2	1.75	0.200	0.073	34.42	**<0.001**	0.610	2.91	0.065	0.117
Delta	2.29	0.145	0.094	47.31	**<0.001**	0.683	0.64	0.531	0.028
Theta	3.33	0.082	0.131	83.69	**<0.001**	0.792	0.96	0.390	0.042
Alpha	1.08	0.311	0.047	61.49	**<0.001**	0.736	0.15	0.865	0.007
SSf	0.06	0.805	0.003	8.84	**0.001**	0.287	0.21	0.809	0.010
FSf	0.07	0.799	0.003	52.56	**<0.001**	0.705	0.32	0.725	0.015
Beta	0.24	0.627	0.011	106.94	**<0.001**	0.829	0.18	0.839	0.008

rm ANOVA with the factors STIM (verum vs sham), TIME (memory tasks (**A**): evening vs. morning; sleep spindles (**B**): baseline vs. stimulation-free intervals), and LOC (Spectral power (**C**): frontal, F7, Fz, F8; central C3, C4, Cz, T3, T4; and posterior, P3, P4, Pz, O1, O2 electrode localizations); η^2_p = partial η^2; Correct WP = correctly recalled word pairs in the word pair task (WPT); Correct SQ = correctly typed sequences in the sequential finger taping task (SFTT); SFS = slow frontal spindles; FPS = fast parietal spindles; SO_1 = slow oscillations (0.5–1.0 Hz); SO_2 = slow oscillations (1.0–1.5 Hz); Delta = 1.0–4.0 Hz; Theta = 4.0–8.0 Hz; Alpha = 8.0–11.0 Hz; SSf = slow spindle frequencies (11.0–13.0 Hz); FSf = fast spindle frequencies (13.0–15.0 Hz); Beta = 15.0–25.0 Hz.

macrostructure of sleep our stimulation related results are consistent with the vast majority of studies (Table 1). One possible explanation for deviating results could be the use of different standards to score sleep. Moser et al. [46] reported that the time spent in NREM sleep stage 1, 2 and SWS (NREM 3 and NREM 4) varies between the scoring standard (AASM [40] vs Rechtschaffen and Kales [47]). Results concerning stimulation effects on declarative memory as assessed by the WPT and on the microstructure of sleep (EEG-power and spindle densities) are more heterogeneous (Table 1). Two factors may explain the heterogeneity of the spindle results: (i) spindle definitions and the overlap of spindle frequency ranges (Suppl. Fig. 1), and (ii) the use of different algorithms to detect and measure spindle activity.

It is well documented that sleep is crucial for the consolidation of memories. It is however less clear which specific facilitatory role the different sleep stages play in this process and whether its beneficial impact manifests a real memory enhancement or merely a memory stabilization. For procedural memories, motor skill performance gains after a night of sleep are a frequently observed finding (e.g. Refs. [48,49]) supporting the theory of a sleep-based enhancement of procedural memory formation [50]. The procedural memory results of the present study are in line with this theory as the finger-tapping performance improved significantly over night, independent of the stimulation condition. However, results of a recent random effects quantitative meta-analysis [51] question this sleep-related enhancing effect on motor sequence learning, arguing instead in favor of a sleep-based stabilization of procedural memories. The authors showed that the offline performance improvements reported in the selected literature vanish when confounding factors unrelated to sleep consolidation (e.g. data averaging, reactive inhibition) are controlled for. That motor skill performances following sleep remained better than those following wakefulness underlines the sleep-stabilization effect. As the strong moderating effects of these confounding variables were also not considered in the present study, it suffers from the same weakness.

With regard to the declarative memory domain, an at least stabilizing effect of sleep is well accepted. The results of the present study, however, reflect a general overnight retention decline of the verbal recall of word pairs, independent of the stimulation condition. Although similar findings have also been reported in young [52] and in elderly participants [25,28,29], this was not expected. A plausible explanation for this performance decline could be the lack of feedback during initial recall in the WPT. Studies that used feedback paradigms mainly observed an overnight retention increase after sleep [23,24,26,27,30,53–60]. When no feedback is applied, initial incorrect responses are only rarely answered correctly during a final retrieval test [61]. It has been shown that when word pairs are reinforced due to a re-encoding opportunity, these items become more retrievable and prevent forgetting [62]. However, if feedback is provided during the encoding phase, it may be difficult to disentangle the effects of feedback and sleep on memory consolidation.

The “learning-to-criterion” constitutes another re-encoding opportunity. If the learning criterion (e.g. 60%) is not reached, the word-pairs are presented again and thus subjects have a further re-encoding opportunity. Additional analyses showed that this re-exposure facilitated the retention of these memories. The number of remembered word pairs in the evening depends on the number of sessions needed to reach the learning criterion (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). The number of correctly recalled words in the morning was also higher for those participants who had additional learning sessions (one session: n, mean ± SEM: 33, 34.1 ± 1.1; more than one session: n, mean ± SEM: 13, 40.3 ± 0.9; p = 0.002). This additional chance to learn resulted obviously in a less pronounced forgetting and was more predictive than stimulation effects. Importantly, 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). Thus, this factor was not relevant for the evaluation of stimulation effects.

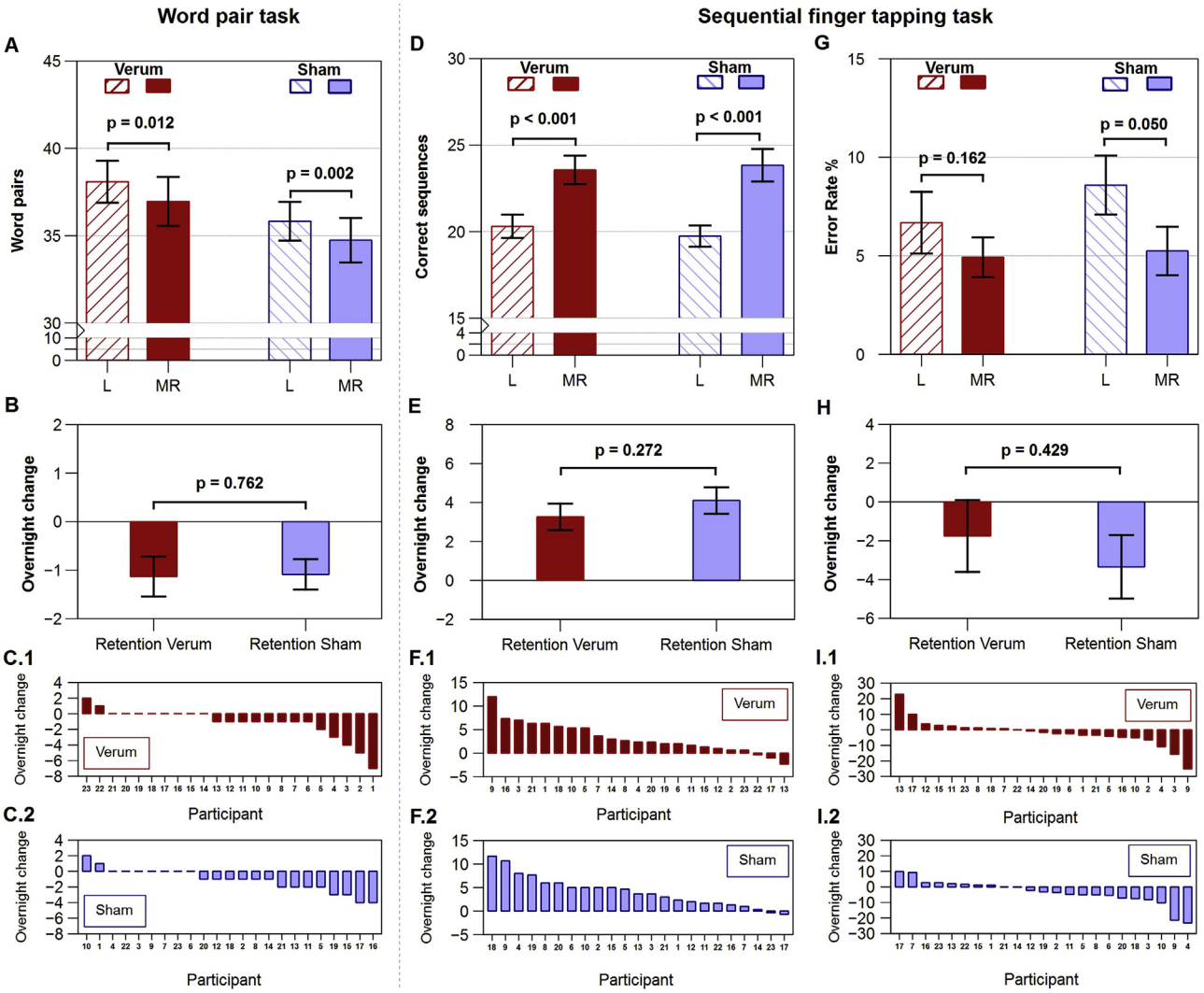

Fig. 3. Memory performances in the declarative and the procedural task. A) Correctly remembered word pairs in the word pair task (WPT) given as mean ± SEM. Performances in the evening recall after learning (L): 38.1 ± 1.2 and 35.8 ± 1.1 under verum and sham stimulation, respectively. Performances in the morning recall (MR): 37.0 ± 1.4 and 34.7 ± 1.3 under verum and sham stimulation, respectively. **B)** Overnight changes (mean ± SEM) [verum: −1.3 ± 0.4; sham: −1.1 ± 0.3] and **C)** Waterfall plot of individual overnight changes in the WPT under **C.1)** verum and **C.2)** sham stimulation. **D)** Number of correctly typed sequences in the finger tapping task (SFTT, mean ± SEM). Performances in the L: 20.3 ± 0.7 and 19.7 ± 0.6 under verum and sham stimulation, respectively. Performances in the MR: 23.6 ± 0.8 and 23.8 ± 0.9 under verum and sham stimulation, respectively. **E)** Overnight changes (mean ± SEM) [verum: 3.3 ± 0.7; sham: 4.1 ± 0.7] and **F)** Waterfall plot of individual overnight changes in the SFTT under **F.1)** verum and **F.2)** sham stimulation. **G)** Error rate (%) in the SFTT. Performances in the L: 6.7 ± 1.6 and 8.6 ± 1.5 under verum and sham stimulation, respectively. Performances in the MR: 4.9 ± 1.0 and 5.2 ± 1.3 under verum and sham stimulation, respectively. **H)** Overnight changes (mean ± SEM) [verum: −1.8 ± 1.8; sham: −3.3 ± 1.6] and **I)** Waterfall plot of individual changes in the SFTT under **I.1)** verum and **I.2)** sham stimulation.

With regard to sleep microstructure, fast parietal spindle counts decreased from baseline to stimulation-free intervals independently of the stimulation condition. This finding may be explained by the natural transition from NREM sleep stage N2 to N3 in the time course after stimulation (see Table 3 and Suppl. Fig. 3S). The absence of an increment in fast and slow spindle densities, which are related to declarative memory consolidation [63], may explain the missing overnight improvement in WPT results. Despite this, increases in spindle density are not necessarily associated with declarative memory improvements after so-tDCS (see Table 1). Lustenberger et al. [60] suggested that spindle densities could reflect the strength of the encoding in a word-pair test, showing learning peculiarities. Thus, it might be speculated that the decrement in fast parietal spindles in both stimulation conditions could reflect a lack of encoding strength due to an absence of feedback in the WPT.

There are several possible explanations why we did not observe stimulation related effects at the physiological and behavioral level. 1) The lack of a more accurate phase-adjusted stimulation protocol in this study may have impaired entrainment of active network oscillations. In single-blind studies, in which so-tDCS was applied in a “phase-locked” manner (i.e. application of so-tDCS when slow oscillations correspond to the endogeneous brain activity), an enhancement in slow oscillations [28,29], and spindle activity [28,29] has been observed. This approach, however, contradicts double-blind studies. 2) The use of ramping pulses at the beginning and the end of each stimulation-block in the present study might have precluded entrainment in phase with ongoing brain activity [25]. 3) In order to entrain neural oscillations it was reported that even imperceptible current strengths are sufficient to interact with the active network and modulate their excitability [21]. Recent studies, however, contradicts this earlier hypothesis. Lafon et al.

Table 3
Sleep stage scoring results.

Entire Night	Verum	Sham	p	|Effect size|
	mean ± SEM	mean ± SEM		
TST [min]	387.04±7.31	382.37±5.11	0.218	0.155
SPT [min]	414.46±2.55	409.52±2.42	0.084	0.413
Wake [% SPT]	6.77±1.45	6.71±1.01	0.429	0.009
REM [% SPT]	22.44±1.34	23.24±0.89	0.693	0.016
N1 [% SPT]	10.29±1.51	8.37±5.56	0.563	0.350
N2 [% SPT]	46.54±1.41	46.56±1.32	0.484	0.002
N3 [% SPT]	14.01±1.44	15.18±1.33	0.356	0.176
60-min following so-tDCS [%]	**mean ± SEM**	**mean ± SEM**		
Wake	5.65±1.40	6.30±1.72	0.837	0.086
REM	15.54±2.62	15.68±2.40	0.967	0.012
N1	8.69±1.68	6.88±0.93	0.314	0.276
N2	39.31±2.84	34.38±3.51	0.276	0.322
N3	30.79±3.33	36.73±5.04	0.276	0.290
Stimulation-free intervals [s]	**mean ± SEM**	**mean ± SEM**		
Undefined	12.17±2.08	8.70±1.70	0.167	0.382
Wake	10.00±5.72	8.69±3.73	0.677	0.056
REM	0.00±0.00	4.34±4.34	0.317	0.295
N1	5.65±4.82	9.56±4.51	0.228	0.175
N2	107.39±14.21	121.30±15.15	0.372	0.197
N3	164.78±16.01	144.34±17.01	0.212	0.258

SEM = standard error of the mean; TST = total sleep time; SPT = sleep period time minus stimulation duration.

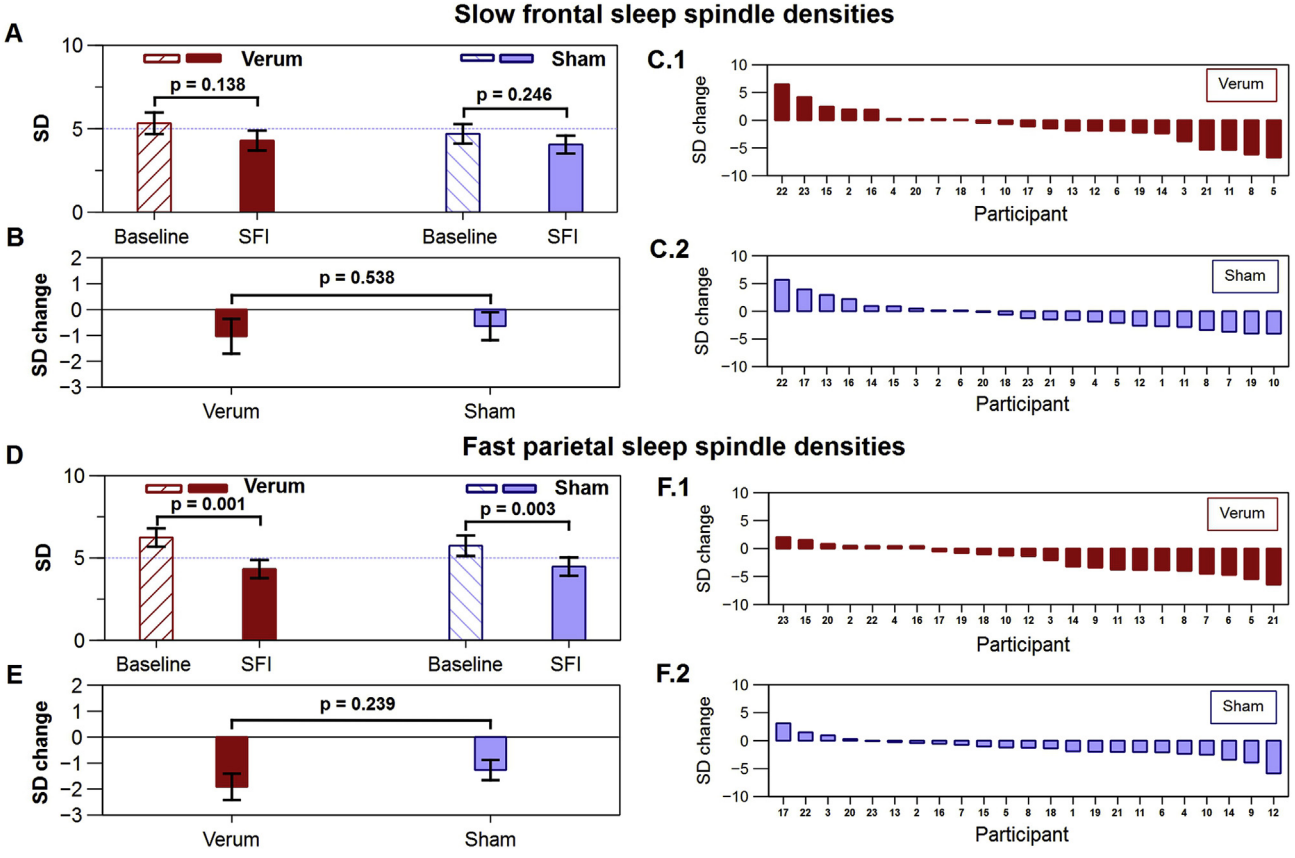

Fig. 4. Sleep spindle density (SD) results (sleep spindle counts per 30 s). A) Slow frontal sleep spindle densities given as mean ± SEM during baseline (verum: 5.3 ± 0.6; sham: 4.7 ± 0.6) and stimulation-free intervals (SFI; verum: 4.3 ± 0.6; sham: 4.1 ± 0.5), **B)** changes in sleep spindle density from baseline to SFI, **C)** waterfall plot of individual changes under **C.1)** verum and **C.2)** sham stimulation. **D)** Fast parietal spindle densities during baseline (verum: 6.2 ± 0.6; sham: 5.7 ± 0.6) and SFI (verum: 4.3 ± 0.6; sham: 4.5 ± 0.6), **E)** changes in sleep spindle density from baseline to SFI, **F)** waterfall plot of individual changes under **F.1)** verum and **F.2)** sham stimulation. Baseline refers to the 60 s interval before stimulation.

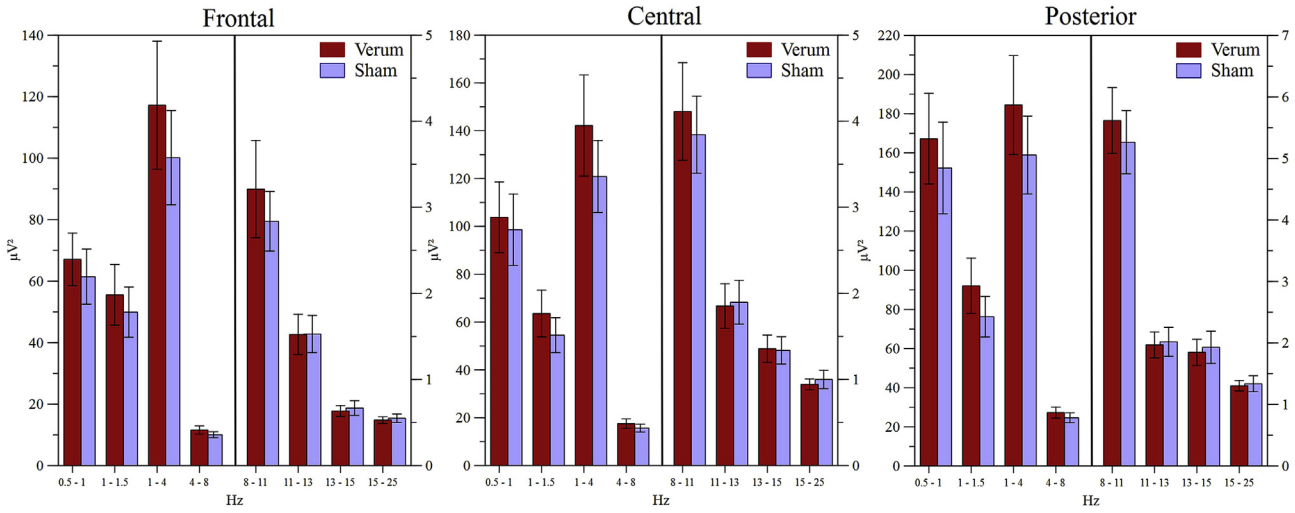

Fig. 5. Spectral power calculated for the stimulation-free intervals. EEG spectral power at frontal (Fz, F7, F8), central (Cz, C3, C4, T3; T4) and posterior (Pz, P3, P4, O1, O2) regions after verum und sham stimulation.

[64] measured the entrainment of slow oscillations intracranialy by using different stimulation parameters. They could show that by applying the same stimulation parameters as used in the pioneer study [24] it was not possible to observe significant entrainment of slow oscillations. Furthermore, Vöröslakos et al. [65] reported that approximately only one fourth of the current reaches the neocortex and that a minimal intensity of 4.5–6 mA might be needed to modulate EEG-power [65]. Thus, the current intensity applied in the present study may have been underdosed and had not the required magnitude to restart slow oscillations.

Overall, additional issues might also compromise the reproducibility of so-tDCS effects. Horvath et al. [66] discussed critical factors for tDCS such as the implementation of sham stimulation and subjects blinding, electric current influences, inter-subject variability, and intra-subject reliability. Li et al. [67] added among others morphological differences as well as genetic, and age-related variability, that might contribute to inter-individual tDCS effects. Esmaeilpour et al. [68] discussed the application of individualized tDCS doses as one approach to reduce inter-individual variability of effects. This implies the assumption that an effect can be observed in every subject at an individually varying level of stimulation intensity. The choice of the individual intensity has to take into account safety limits. In order to standardize cortical targeting, modelling technics that can estimate the induced electric fields by taking in to account the anatomical variability should be used in stimulation studies [69].

Given the multitude of factors that might affect so-tDCS results, it is recommended to standardize and control as many of them as possible (see also Buch et al. [70]).

Conclusion

The results of the present study have shown that so-tDCS, applied during NREM sleep, has neither an impact on memory consolidation nor on macro- and microstructure of sleep. Thus, the specific beneficial effects of so-tDCS on sleep dependent declarative memory consolidation as assessed by the WPT in young healthy adults observed in some studies [23,24] could not be confirmed. This is in line with previous studies that used so-tDCS to manipulate memory consolidation processes during sleep (see Table 1). Given that the stimulation design used in these studies is very similar, it is unlikely that this factor contributes most to the heterogeneous results. Since re-encoding opportunities (by feedback or number of learning possibilities) seems to be an important factor with regard to overnight changes in memory consolidation, differences in the implementation of this task between studies might further limit comparability of results. Variation of factors between and within subjects might have an impact on so-tDCS responses and thus contribute to difficulties in reproducibility. The standardization of protocols and the control of variability in tDCS studies are necessary to assure accurate replications of stimulation effects.

Conflicts of interest

None conflict of interest declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2019.02.012.

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