Behavioral/Systems/Cognitive

Transcranial Direct Current Stimulation during Sleep Improves Declarative Memory

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In humans, weak transcranial direct current stimulation (tDCS) modulates excitability in the motor, visual, and prefrontal cortex. Periods rich in slow-wave sleep (SWS) not only facilitate the consolidation of declarative memories, but in humans, SWS is also accompanied by a pronounced endogenous transcortical DC potential shift of negative polarity over frontocortical areas. To experimentally induce widespread extracellular negative DC potentials, we applied anodal tDCS (0.26 mA/cm^2) repeatedly (over 30 min) bilaterally at frontocortical electrode sites during a retention period rich in SWS. Retention of declarative memories (word pairs) and also nondeclarative memories (mirror tracing skills) learned previously was tested after this period and compared with retention performance after placebo stimulation as well as after retention intervals of wakefulness. Compared with placebo stimulation, anodal tDCS during SWS-rich sleep distinctly increased the retention of word pairs ($p < 0.005$). When applied during the wake retention interval, tDCS did not affect declarative memory. Procedural memory was also not affected by tDCS. Mood was improved both after tDCS during sleep and during wake intervals. tDCS increased sleep depth toward the end of the stimulation period, whereas the average power in the faster frequency bands (θ, α, and β) was reduced. Acutely, anodal tDCS increased slow oscillatory activity <3 Hz. We conclude that effects of tDCS involve enhanced generation of slow oscillatory EEG activity considered to facilitate processes of neuronal plasticity. Shifts in extracellular ionic concentration in frontocortical tissue (expressed as negative DC potentials during SWS) may facilitate sleep-dependent consolidation of declarative memories.

Key words: **declarative memory; direct current (DC) potential; electroencephalography (EEG); nonrapid eye movement (NonREM) sleep; slow oscillation; transcranial direct current stimulation (tDCS)**

Introduction

Application of DC electric fields to the scalp has been shown to modify acutely neuronal membrane potentials and spike firing (Creutzfeldt et al., 1962; Purpura and McMurtry, 1965; Gartside, 1968; Liebetanz et al., 2002) and also to produce long-lasting changes in bioelectric activity of underlying brain tissue (Bindman et al., 1962, for reviews, see Lolas, 1977; Lutzenberger and Elbert, 1987; Nitsche et al., 2003a; Priori, 2003). The effects of cortical polarization depend particularly on polarity, orientation of current flow relative to the axonal-dendritic axis of pyramidal neurons, and on the strength of the electric field (Jefferys, 1995; Nitsche et al., 2003a). Assuming current flow direction is parallel to the axonal-dendritic axis of the dominant neuron type, an anodal electrode on the scalp (i.e., an electrode attracting negative charges and repelling positive charges) causes a depolarization of the membrane potential via an extracellular negative sink in underlying neural tissue (Creutzfeldt et al., 1962; Bindman et al., 1964). *In vitro* experiments in cortical slice preparations revealed maximal membrane polarization at the tips of basal and apical dendrites, and that even very weak electric fields modify the excitability of neurons with a linear relationship between applied field and induced polarization (Bikson et al., 2004).

Sleep facilitates the consolidation of memories, presumably through a "covert" reactivation of the newly encoded materials (Maquet, 2001; Pennartz et al., 2002; McNaughton et al., 2003). Periods rich in slow-wave sleep (SWS) have been found consistently to enhance declarative memories in particular (Plihal and Born, 1997, 1999), although nondeclarative types of memory can benefit also from this period of sleep (Gais et al., 2000; Walker, 2004). Regarding declarative memory, SWS may provide a state during which newly acquired representations, temporarily stored in the hippocampus, are transferred to the neocortex for integration into long-term memories (Buzsaki, 1989; Hasselmo, 1999; McNaughton et al., 2003). This replay of information in the hippocampus and its hippocampo–neocortical transfer is presumably linked to a sharp wave–ripple pattern in the hippocampus (Kudrimoti et al., 1999; Nadasdy et al., 1999), which at the neocortical level occurs in temporal correlation to sleep spindles, δ waves, and their grouping by the slow oscillation (Siapas and Wilson, 1998; Sirota et al., 2003). In humans, SWS is characterized electrophysiologically by maximal spectral power in the δ and slow oscillatory frequency ranges and increased, but submaximal, spindle power (Achermann and Borbely, 1997; Mar-

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shall et al., 2003). In addition, within the nonrapid eye movement (NonREM)–rapid eye movement (REM) sleep cycle, an endogenous DC potential shift, best visible over frontal cortical areas, reveals a pronounced negative-going DC potential shift peaking typically at about the onset of SWS, and subsequently a high level of DC potential is maintained that then decreases very gradually in the remaining period of SWS. Notably, the DC potential shift during the passage into SWS was correlated in time with coefficients as high as 0.9 to slow oscillatory activity, suggesting the mechanisms generating these changes are associated (Marshall et al., 2003). An enhanced excitability characterizing the depolarizing up phase of slow oscillatory activity compared with the down phase and presumably also to the EEG activity during quiet waking may prove that this type of generated rhythm is particularly susceptible to slow changes in exogenous or endogenous DC potential shifts (Steriade et al., 2001; Bikson et al., 2004). The working hypothesis for the present experiments is that during early SWS-rich sleep, transcranial direct current stimulation (tDCS) affects declarative memory consolidation. We applied anodal tDCS during a period of sleep characterized by SWS-rich early sleep and slow oscillatory activity as well as an enhanced negative level of the endogenous DC potential to induce, or rather potentiate, a widespread negative DC potential with a focus over frontocortical areas. We aimed to test whether this anodal tDCS applied repeatedly enhances declarative memory consolidation. The effects of tDCS induced depolarization on slow oscillation activity as a possible mediator of DC potential effects, as well as on other sleep-related EEG rhythms, were of interest. In addition, associated changes in sleep stages and sleep-specific hormonal activity were monitored.

Materials and Methods

Subjects, experimental design, and procedure

Thirty men with a mean age of 23.8 years (range, 19–28 years) who were nonsmokers and free of medication participated in these studies after giving informed written consent. Subjects with, or with a history of any of the following, were excluded: epilepsy, paroxysms, cognitive impairments, mental, hormonal, metabolic, or circulatory disorders, or sleep disturbances. The experimental protocol was approved by the ethics committee of the University of Lübeck.

Two experiments were conducted to assess the effect of anodal tDCS on memory, one during sleep (Sleep experiment) and the other during wakefulness (Wake experiment). In both experiments, subjects were tested in two conditions, a stimulation condition and a placebo condition, according to a double-blind cross-over design. The two sessions of a subject were separated by an interval of at least 1 week. Time course of the Sleep experiment is schematized in Figure 1. Subjects ($n = 18$) arrived at the laboratory at 7:00 P.M. After preparation for tDCS, EEG recording, and blood sampling, subjects were tested on learning tasks for both declarative memory [paired associate learning (PAL)] and procedural memory [mirror tracing (MT)] between 9:30 P.M. and 10:30 P.M. The order of the tasks was randomized across subjects. In the Sleep experiment, subjects subsequently went to bed, and EEG and polysomnographic recordings were started. tDCS began after the subject entered SWS (i.e., after on-line scoring confirmed the presence of 30 sec of sleep stage 3 or 4). After the end of the first NonREM–REM sleep cycle, subjects were awakened. At this time, they were usually in light NonREM sleep (stages 1 or 2) after the first REM sleep period. About 20 min after awakening, recall on the memory tasks was tested. Because sleep is characterized by prominent neuroendocrine regulation, blood was sampled for determination of hormone concentration (norepinephrine, cortisol, growth hormone) before and after learning and recall testing, as well as after lights off and every 30 min during the sleep interval. Before learning and after recall testing, psychometric tests [d2, Positive and Negative Affect Schedule (PANAS), Eigenschaftswoerterliste (EWL)] were given also to assess capabilities to concentrate and feelings of tiredness and mood.

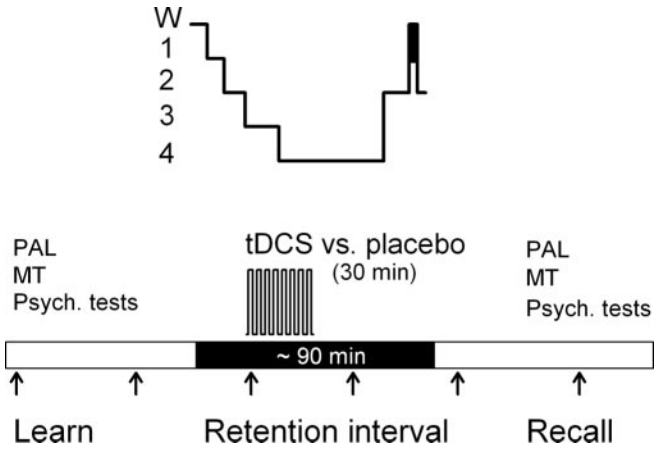

Figure 1. Procedure of the Sleep experiment. Time points of learning and recall of the memory tasks (PAL, MT), psychometric tests (d2, EWL, PANAS), tDCS, blood sampling (arrows), period of lights off (horizontal black bar), and sleep, represented by the schematized hypnogram, are indicated. W, Wake; 1–4, sleep stages 1–4; vertical black bar, REM sleep.

The procedure of the Wake experiment ($n = 12$) was the same as in the Sleep experiment, except that the period of sleep was replaced by a period of wakefulness. During this wake period, subjects, seated in a reclining chair, were shown a video presentation ("Koyaanisqatsi" or "Powaqqatsi," films with only instrumental accompaniment). tDCS was given 10 min after the beginning of the presentation. Mood was also tested directly after tDCS. No EEG was recorded in the Wake experiments.

Transcranial DC stimulation

For transcranial DC stimulation, electrodes (8 mm diameter) were applied bilaterally at frontolateral locations [F3 and F4 of the international 10:20 system (Jasper, 1958)] and at the mastoids. Anodal tDCS (i.e., positive polarity at both frontal sites) was applied intermittently (15 sec on, 15 sec off; current density, 0.26 mA/cm^2) over a period of 30 min by a battery-driven constant-current stimulator. In the placebo control session, the electrodes were applied as in the stimulation sessions, but the stimulator remained off. Stimulation was not felt by the subjects.

Memory tasks and psychometric tests

Paired associate word lists. To assess declarative learning, a PAL task with category–instance word pairs similar to one used previously (Plihal and Born, 1997) was used. A different word list was used for each of the subject's two experimental sessions. Each list consisted of 46 pairs of German nouns adapted from a normative study. In addition to the 46 word pairs, four dummy pairs of words at the beginning and end of each list served to buffer primacy and recency effects, respectively. Response words represented instances for the categories of the respective stimulus words (e.g., train–track, bird–wing). To prevent serial learning, the sequence of word-pair presentations within the lists was randomized between repeated trials. In the learning condition, the list was displayed on a color monitor with a presentation rate of 0.20 sec and an interstimulus interval of 100 msec. During learning, presentation of the list was followed by a task of cued recall. Here, only the 46 stimulus words of the word list appeared on the screen, in a different order than the foregoing presentation. The subject had unlimited time to recall the appropriate response word and write it down. The number of correct responses was calculated immediately. If a minimum of 60% correct responses was not obtained, word pairs were presented again in a newly randomized order, and cued recall was repeated. In the recall condition, after the retention interval of sleep or wakefulness, the 46 word pairs were again displayed in a newly randomized order. The subject was required to recall the appropriate response words and write them down.

Mirror tracing. A task of procedural learning, with improved memory performance shown to depend on sleep during the second half of the night but not on sleep after the first half alone, was conducted as a control memory condition (Plihal and Born, 1997). To assess procedural learning, subjects traced figures as fast and as accurately as possible while these

figures and their hand movements were visible to them only through a mirror. Two different sets with seven different figures were used. One set consisted of a line-drawn five-pointed star, for practice, and six line-drawn human figures made up of 26–28 segments joined by 25–27 angles. In the second set, the straight segments were curved. The apparatus was as described in detail by Plihal and Born (1997). Performance was assessed by a light sensor of a tracing stylus that indicated whenever the stylus left the line to be traced. Subjects traced the figures with a stylus starting and ending at the same point. An error consisted of moving the stylus off the line of the figure. Subjects first practiced with the star until a maximum of only six errors was made and continued with the line figures. In the recall condition, subjects traced the same figures, starting with the star to warm up. Performance measures were mean draw time and mean error count, collapsed across the six line-drawn figures.

d2-test, PANAS, EWL. On the d2-test of attention (Brickenkamp and Zillmer, 2002), subjects are required to cross out specifically marked target letters in several sequels of signs. Total count of signs processed within 45 sec, and errors were calculated as an estimate of the capability to concentrate. The PANAS describes, by a five-point self rating, the subject's current mood on two dimensions: positive (enthusiastic, active, and attentive) and negative (irritability, nervousness, and fear) affect (Watson et al., 1998). The EWL (Janke and Debus, 1978) is an adjective checklist describing the subject's mood on 15 dimensions (e.g., activated, tired, high spirits, irritable, excited, fearful).

Polysomnographic and EEG recordings and analyses

EEG (Fz, Cz, Pz, Oz, C3, C4, P3, P4, F7, F8, T3, T4, T5, T6) and vertical and horizontal electro-oculograms were recorded continuously by a DC/AC amplifier (Toennies DC/AC amplifier; amplification, 200 μV/V; 1–35 Hz; Jaeger GmbH and Co. KG, Würzburg, Germany). Analog DC EEG signals were digitized at 200 Hz per channel (CED 1401; Cambridge Electronics, Cambridge, UK).

Three types of comparisons were performed between the conditions of tDCS and placebo control. First, sleep structure was compared between the sessions based on standard polysomnographic criteria (Rechtschaffen and Kales, 1968). For the total sleep epoch as well as for a 45 min interval beginning with the onset of tDCS (i.e., the first appearance of SWS), every 30 sec epoch was scored as NonREM sleep stage 1, 2, 3, 4, or REM sleep. SWS was determined as the sum of sleep stages 3 and 4. For the placebo condition, sleep stages were determined for corresponding intervals. The time in minutes for each sleep stage, the total sleep time, and the percentage of sleep time in each stage with reference to total sleep time were determined. Mean time spent in the different stages beginning with the onset of stimulation and ending 15 min after termination of the stimulation interval was calculated and compared with respective intervals of the control session. In a second analysis, average EEG power was compared for the 30 min interval of DC stimulation and the corresponding interval during the placebo condition for the following bands: θ (4–8 Hz), α 1 (8–10 Hz), α 2 (10–12 Hz), spindle frequency (12–15 Hz), β 1 (15–20 Hz), and β 2 (20–25 Hz). This analysis was run separately for periods of SWS and stage 2 sleep. A third analysis concentrated on the immediate effects of DC polarization. For this purpose, average power spectra for all the above frequency bands were compared during the 60 15 sec intervals of acute cortical polarization with that obtained for the 60 intermittent 15 sec breaks in which the DC stimulation was discontinued. The time course of short-term effects across the 15 sec epochs was also assessed. As a result of on–off artifacts in the EEG induced by the stimulation, 13 sec rather than 15 sec intervals were analyzed. Power spectra and corresponding bands were calculated using three overlapping or for time course analyses moving windows of 5 sec intervals (2048 data points), resulting in a resolution of 0.098 Hz per bin. Only artifact-free intervals were used. A five-point moving average was applied to the individual data before averaging.

Hormones

For blood sampling, a catheter was connected to a long, thin tube enabling blood collection from an adjacent room without disturbing the subject. Standard HPLC with electrochemical detection was used for plasma norepinephrine detection [sensitivity, 35.64 pmol/l; interassay coefficients of variation (CV), <6.1%]. Assays used for determination of cortisol and growth hormone were an ES300 (sensitivity, 1.0 μg/dl; intraassay CV, <6%; interassay CV, <4%; Boeringer Mannheim, Mannheim, Germany) and a RIA (sensitivity, 0.9 μg/l; intraassay CV, <5%; interassay CV, <9%; Diagnostic Products Corporation, Bad Nauheim, Germany), respectively.

Statistical analyses

Statistical analyses relied in general on ANOVA with Stimulation (tDCS, placebo) as repeated-measures factor and mental state (Sleep, Wake) as group factor. When appropriate, a Greenhouse–Geisser correction for degrees of freedom was used. A p value <0.05 was considered significant. Paired t tests were used for comparisons of time courses.

Results

Memory tasks and psychometric tests

On the PAL task, learning before sleep (Sleep experiment) and wakefulness (Wake experiment) was comparable for all conditions. The number of trials required until the criteria of 60% correct responses was obtained at immediate recall in the Sleep experiment was 1.37 ± 0.09 and 1.33 ± 0.09 for tDCS and placebo conditions, respectively. In the Wake experiment, 1.50 ± 0.15 and 1.42 ± 0.15 trials were needed to reach the learning criteria ($p > 0.6$, for respective differences between stimulation conditions). The number of words recalled at the criterion trial during learning (shown in Table 1) also did not differ among conditions ($p > 0.2$, for all comparisons). For assessing effects of tDCS on subsequent memory formation, recall performance after the retention trials was compared with the individual performance at learning before (Fig. 2, Table 1). In the Sleep experiments, recall generally improved across the sleep retention interval, and this improvement was distinctly greater when tDCS was applied than placebo stimulation ($F_{(1,17)} = 10.44$; $p < 0.005$). In the Wake experiments, recall performance on average did not improve but slightly decreased across the wake retention interval ($F_{(1,28)} = 4.81$; $p < 0.05$, for the difference between Sleep and Wake experiments). Moreover, there was no effect of tDCS on retention performance in the Wake experiment ($F_{(1,11)} = 0.04$; $p > 0.8$) (Table 1).

Table 1 also summarizes results of draw time and error count on the mirror tracing task. Performance at learning before the retention intervals was comparable between tDCS and placebo conditions of both experiments ($p > 0.5$), although subjects of the Wake experiment made more errors than subjects of the Sleep experiments ($F_{(1,28)} = 7.48$; $p \leq 0.01$). Compared with placebo, tDCS did not affect memory for mirror tracing, as assessed by the improvements in speed and accuracy at recall, neither in the Sleep experiments ($p > 0.5$ and $p > 0.8$ for speed and accuracy, respectively) nor in the Wake experiments ($p > 0.3$ and $p > 0.5$, respectively). Subjects of the Wake experiments showed a greater reduction in error count across the retention interval than those of the Sleep experiments ($F_{(1,28)} = 7.51$; $p \leq 0.01$), which might be a result of the generally higher error rate at learning in these subjects.

The d2 attention test did not indicate differences in concentration between tDCS and placebo conditions neither at learning before sleep (total count of processed signs, 511 ± 11 vs 513 ± 14; error count, 18 ± 4 vs 18 ± 4) nor at recall after sleep (total count of processed signs, 531 ± 9 vs 516 ± 12; error count, 15 ± 3 vs 16 ± 4; $p > 0.2$). There were also no differences in d2 performance at learning and recall testing in the Wake experiments ($p > 0.3$).

The PANAS questionnaire indicated that positive affect decreased generally across the retention interval. However, during

Table 1. Mean ± SEM number of correctly recalled word pairs on the PAL task and speed and accuracy of performance on the MT task before (Learning) and after (Recall) the retention interval

	Learning (mean ± SEM)	Recall (mean ± SEM)	Retention (mean ± SEM)	Significance (*p*)
PAL				
Sleep				
tDCS	33.0 ± 1.4	35.7 ± 1.4	2.7 ± 0.6	$p < 0.005$
Placebo	33.6 ± 1.5	34.5 ± 1.5	0.9 ± 0.8	
Wake				
tDCS	36.2 ± 1.5	36.3 ± 1.2	0.0 ± 1.0	$p > 0.8$
Placebo	34.0 ± 1.4	33.7 ± 1.4	−0.3 ± 0.5	
MT draw time (in milliseconds)				
Sleep				
tDCS	72.63 ± 9.57	55.77 ± 6.06	−16.86 ± 4.58	$p > 0.5$
Placebo	72.00 ± 7.88	58.68 ± 5.02	−13.32 ± 3.67	
Wake				
tDCS	60.00 ± 6.72	52.50 ± 6.75	−7.51 ± 5.80	$p > 0.3$
Placebo	63.33 ± 14.21	46.81 ± 9.60	−16.52 ± 5.76	
MT error count				
Sleep				
tDCS	7.1 ± 1.4	5.4 ± 1.3	−1.8 ± 0.6	$p > 0.8$
Placebo	6.6 ± 1.6	4.5 ± 1.4	−1.9 ± 0.7	
Wake				
tDCS	14.6 ± 3.5	8.7 ± 1.5	−5.9 ± 1.7	$p > 0.5$
Placebo	12.7 ± 3.1	8.8 ± 1.2	−3.9 ± 2.3	

Retention is defined by the difference in retrieval performance before and after the retention interval. The retention interval was filled with Sleep or wakefulness (Wake), during which either tDCS or placebo stimulation was applied. The right column indicates significant differences in retention, compared with the respective placebo condition. Corresponding performances during learning and recall did not differ significantly (see Results for details).

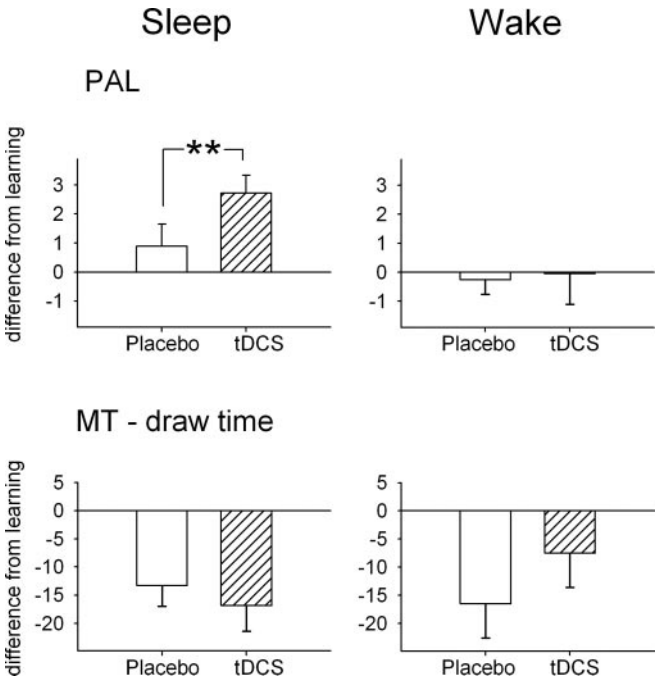

Figure 2. Memory performance on the PAL and MT tasks across retention periods of sleep (left) and wakefulness (right) during which either tDCS (hatched bar) or placebo stimulation (white bar) was applied. Recall after the retention interval is expressed as difference from performance at learning in number of words (for PAL) and in milliseconds for draw time (for MT). $^{**}p < 0.01$, for differences between the effects of tDCS and placebo stimulation. Error bars represent SEM.

tDCS, this decrease was smaller than in the placebo conditions (−0.31 ± 0.10 vs −0.60 ± 0.11; $p < 0.05$) regardless of sleep or wakefulness in the retention interval. Likewise, the EWL revealed that after tDCS, subjects reported decreased feelings of "depression" (−0.50 ± 0.26), whereas in the placebo condition, such feelings increased across the retention intervals of sleep and wakefulness (+0.37 ± 0.32; $p < 0.05$).

Table 2. Sleep during the tDCS and placebo stimulation conditions of the Sleep experiment

	tDCS (mean ± SEM)	Placebo (mean ± SEM)
Awake %	6.0 ± 2.5	5.7 ± 1.3
S1 %	9.3 ± 1.7	9.6 ± 2.5
S2 %	46.9 ± 4.4	48.9 ± 3.6
S3 %	18.4 ± 2.1	16.6 ± 1.6
S4 %	16.6 ± 4.1	17.5 ± 4.2
SWS %	35.0 ± 4.2	34.1 ± 4.4
REM %	2.3 ± 0.8	1.2 ± 0.6
Total time (in minutes)	96.1 ± 4.9	88.6 ± 3.7
Latency to		
S2 (in minutes)	3.6 ± 1.1	4.9 ± 5.4
SWS (in minutes)	25.4 ± 6.3	22.9 ± 3.7

Percentage (%) of time spent in different sleep stages and latency to stage 2 sleep, SWS, and REM sleep (with reference to sleep onset) is shown. Total time defines the time from sleep onset until awakening. There were no significant differences between the conditions.

S1–S4, Sleep stages 1–4.

Polysomnographic EEG recordings and sleep-associated neuronal activity

Table 2 summarizes the time spent asleep and in the different sleep stages in the Sleep experiments for the tDCS and placebo conditions. There were no significant differences between the two conditions, also when this analysis was restricted to a 45 min interval beginning with the first appearance of SWS (i.e., with anodal stimulation in the tDCS condition). However, when the time course for the mean sleep stage was determined (with sleep stage 1–4 given the values 1–4, respectively, and REM sleep the value 0) (Marshall et al., 1998), subjects toward the end of the tDCS stimulation and during the subsequent 15 min showed deeper sleep than during the corresponding interval of the placebo condition, with this difference transiently reaching statistical significance (Fig. 3). Power spectra determined separately for periods of SWS and stage 2 sleep during the 30 min interval of stimulation indicated that tDCS, compared with placebo, reduced power in the lower β frequency range (15–20 Hz) during

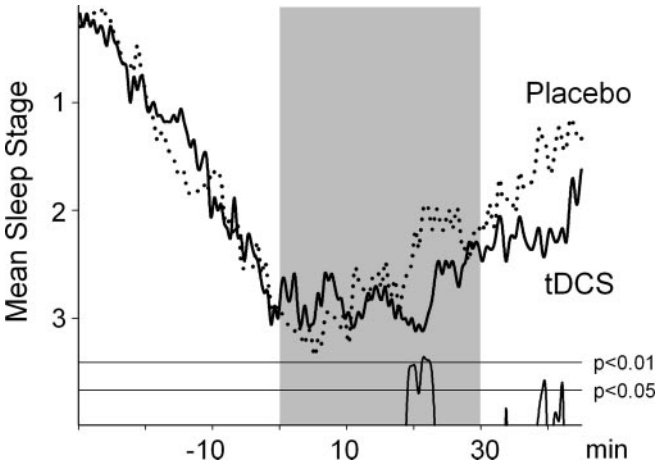

Figure 3. Time course of mean sleep stage for tDCS (solid line) and placebo (dotted line) conditions of the Sleep experiment. Average sleep stages were determined by associating values of 1, 2, 3, and 4 to sleep stages 1–4 and 0 and −1 to REM sleep and wakefulness, respectively. Significant differences between the time courses are indicated at the bottom. The gray area represents the stimulation interval.

periods of stage 2 sleep. The effect was most pronounced at central (C3, C4) and parietal (P3, P4) electrode sites (Fig. 4). During periods of SWS, tDCS suppressed frequencies around the θ and lower α range (4–10 Hz) (Fig. 4). During the stimulation interval, visual spindle counts per sec in the tDCS versus placebo condition were 0.11 ± 0.01 versus 0.13 ± 0.01 ($p < 0.05$).

The comparison of 15 sec epochs of acute anodal polarization with the intermittent epochs when stimulation was discontinued indicated most consistent differences for the slow oscillatory and δ frequencies <3 Hz (Fig. 5). During acute anodal stimulation, power in this low frequency range was increased over the frontal cortex, most consistently ~2 Hz, compared with intervals of discontinued stimulation. At parietal sites, anodal stimulation acutely increased slow oscillatory activity <1 Hz (Fig. 5). Comparisons of the time course of short-term effects across the 15 sec epochs of acute anodal polarization versus intermittent epochs did not yield consistent effects.

Hormones

Average plasma levels of norepinephrine, cortisol, and growth hormone were not affected by tDCS (compared with placebo for both sleep and wake experiments; $p > 0.4$ for cortisol and growth hormone; $p > 0.1$ for norepinephrine).

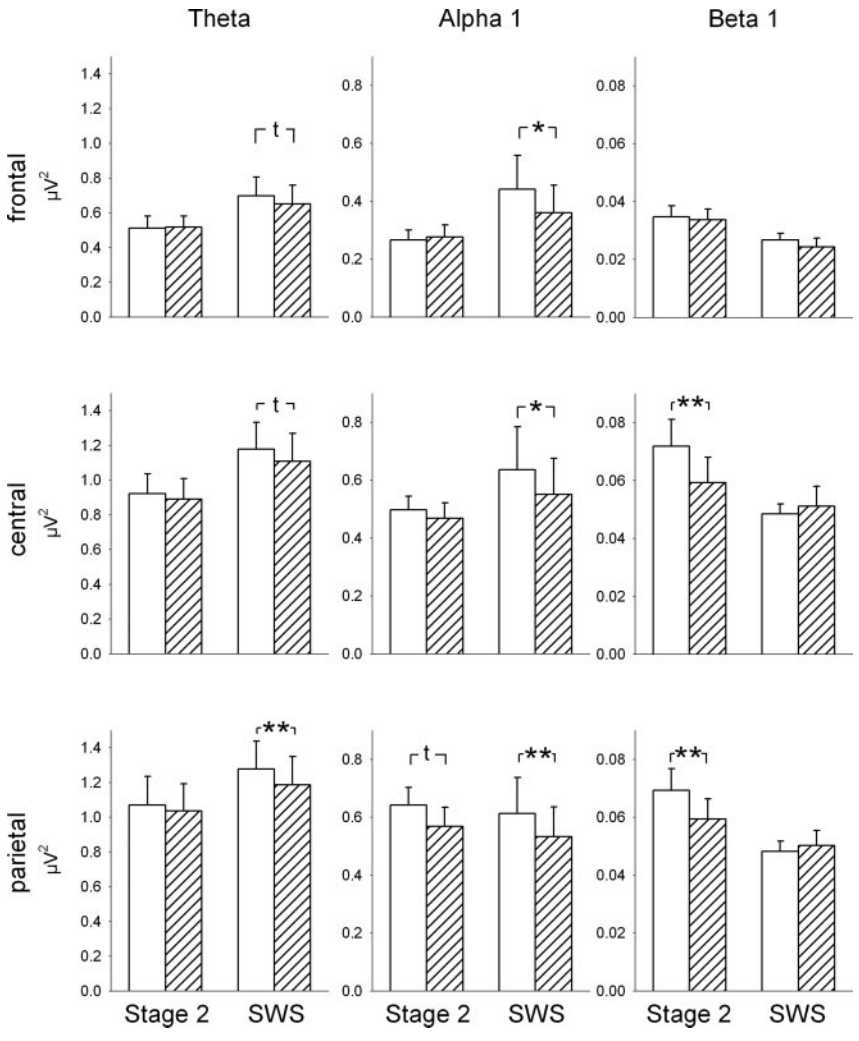

Figure 4. Average EEG power for periods of stage 2 sleep and SWS during the 30 min interval of tDCS (hatched bars) and a corresponding interval during the placebo condition (white bars) of the Sleep experiments. θ (4–8 Hz), lower α (8–10 Hz), and lower β (15–20 Hz) bands are averaged for frontal (F7, Fz, F8), central (C3, Cz, C4), and parietal (P3, Pz, P4) electrode locations. $^{**}p < 0.01$; $^{*}p < 0.05$; $^{t}p < 0.1$, for differences between tDCS and placebo (stage 2 sleep, $n = 14$; SWS, $n = 16$). Error bars represent SEM.

Discussion

Our study examined the influence of anodal tDCS, inducing extracellular potentials of negative polarity in underlying tissue, on processes of declarative memory formation known to be enhanced during periods rich in SWS (Plihal and Born, 1997, 1999). Results indicate that tDCS repeatedly applied during deep NonREM sleep improved declarative memory retention, whereas performance was unaffected during wakefulness. Retention of procedural memories, in contrast, was not affected by tDCS but was also not enhanced by sleep. Electrophysiological modification of the cortex by weak anodal polarization during sleep consisted of an acute increase in slow oscillatory activity <3 Hz, accompanied by diminished power in the faster θ, lower α, and lower β EEG frequency bands across the 30 min polarization period. The shift toward enhanced slow oscillatory activity during the period of tDCS expressed itself also as an increase in the depth of average sleep stage, which per se represents a mere descriptive measure that cannot account for enhanced retention performance. Duration of the stage SWS was not enhanced by tDCS. Finally, there were signs of improved mood after tDCS in the Sleep and also in the Wake experiments, a finding that may have some implications for treatment of mood disorders.

Effects on memory

The central effect of this study was the improvement in declarative memory for word pairs after tDCS during sleep. This improvement is remarkable because it was

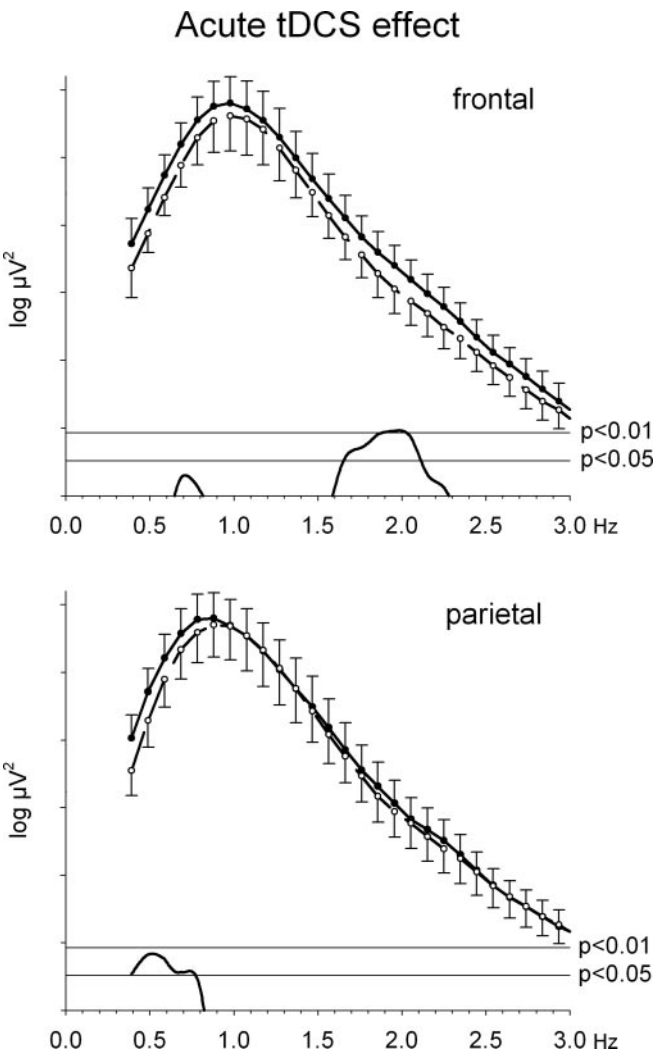

Figure 5. Average EEG power within the slow <3 Hz frequency band for the 60 15 sec stimulation periods of acute anodal polarization (solid line) and the 60 intermittent 15 sec periods (dotted line) in which stimulation was discontinued in the tDCS condition of the Sleep experiment. Data are shown for the mean of frontal (F7, Fz, F8) and parietal (P3, Pz, P4) electrode locations, where most consistent increases in power were observed during acute anodal stimulation. Significant differences between the spectra are indicated at the bottom of each diagram ($n = 16$). Error bars represent SEM.

found in healthy young students performing already at a high level on memory tasks, and it was found after a period of SWS-rich early nocturnal sleep, which per se is known to optimize declarative memory (Ekstrand et al., 1977; Plihal and Born, 1997; Born and Gais, 2003; Gais and Born, 2004). The present study confirmed previous studies in showing generally enhanced declarative memory for word pairs across sleep compared with the Wake experiments. The moderate size of this effect can be explained by the rather short period of intervening sleep, averaging only 90 min, compared with the intervals of 3–4 hr examined in foregoing studies. Also in line with those previous studies, we did not find any beneficial effect of early SWS-rich sleep on procedural memory for mirror tracing, which probably benefits strongest from periods rich in REM sleep (Smith, 2001; Mednick et al., 2003). Here, we focused on the first nocturnal NonREM–REM sleep cycle.

tDCS was applied during retention sleep, which speaks for an immediate effect on processes of memory consolidation taking place during the retention interval. However, effects of anodal tDCS on measures such as amplitude of motor-evoked potentials after transcranial magnetic stimulation have been observed to survive the period of acute stimulation by up to 90 min (Nitsche and Paulus, 2001). On this background, additional proactive effects of tDCS on recall starting ~1 hr after tDCS cannot be excluded. However, it is unlikely that such proactive effects alone explain the memory effects of tDCS for the following reasons. If the effects of tDCS were primarily on recall, they should have occurred also in the Wake experiments, but a significant enhancement in word pair retention performance after tDCS occurred only for the Sleep experiments. Moreover, psychometric tasks did not provide evidence for enhanced attention or concentration after tDCS that could have generally facilitated recall performance. Likewise, performance on the mirror tracing task being comparable between tDCS and placebo conditions at recall testing argues against the sole influence of proactive effects of tDCS on cortical excitability that might have contaminated retention performance of declarative memories.

There were signs of improved mood after tDCS, after both sleep and wake intervals. However, it is unlikely that this factor promoted enhanced retention of emotionally neutral word pairs (Blaney, 1986; Singer and Salovey, 1988), and if so, this also should have happened in both the Sleep and Wake experiments. Together, the pattern of changes justifies concluding that tDCS effects processes during retention rather than during recall of declarative memories. A more delayed recall testing might have enabled a clearer distinction between effects on consolidation and recall in this context. However, we chose the relatively short time of 1 hr intervening between tDCS and recall to minimize the amount of sleep subsequent to stimulation that may have posed as another nonspecific interference.

Neurophysiological aspects

Considering the neuroanatomy of the neocortex and the induction of an extracellular negative sink in underlying neural tissue by anodal polarization, the primary effect of the applied stimulation involves membrane depolarization of the most superficial neocortical layers. In fact, the tips of apical and basal dendrites are highly susceptible to changes in membrane polarization induced by application of weak extracellular DC fields (Bikson et al., 2004). The question, therefore, is how this influence of tDCS translates into an enhancement of declarative memory consolidation, as observed here.

The most superficial layer I of the neocortex contains distal apical dendrites of deeper laying pyramidal cells and represents the site of synaptic terminations from corticocortical projections (Wong-Riley, 1978; Rockland and Pandya, 1979), especially from higher-order cortical areas (Pandya and Yeterian, 1985; Zeki and Shipp, 1988; Felleman and Van Essen, 1991), suggesting the particular relevance of this layer (compared with corticocortical connections of the middle layers) for associative cortical processing. Membrane depolarization induced in these apical dendrites may thus enhance this type of associative cortical processing in general, as by increasing excitability, postsynaptic dendrite potentials, and dendritic spike activity, which can be generated independently from the somatic response (Shepherd et al., 1985; Kim and Connors, 1993; Cauller and Connors, 1994; Schiller et al., 1997; Sourdet and Debanne, 1999; Roland, 2002; Bikson et al., 2004). Additionally, tDCS may influence local dendritic protein synthesis subserving (e.g., synaptic plasticity) (Islam et al., 1994, 1995; Bradshaw et al., 2003).

Notably, the processes of memory consolidation invoked by anodal tDCS are dependent on the state of the brain, because they

were selectively observed during the early NonREM sleep period and not during wakefulness. A hallmark of electrophysiological activity during this state is the slow oscillatory activity. Slow oscillatory activity exerts a grouping influence on faster EEG frequencies such that the appearance of these frequencies becomes restricted to the depolarizing up phase of these oscillations. Accordingly, the development of slow oscillations accompanying the deepening of sleep is typically found to coincide with the decrease of faster frequencies under natural conditions (Marshall et al., 2003). The examination of cellular processes occurring during the synchronized depolarization of the slow oscillation up phase indicate that the cortical network possesses the intrinsic ability to generate persistent activity (McCormick et al., 2003). This persistent activity of the up phase of slow oscillations in the sleeping brain is attributed to the recurrent corticocortical excitatory activities alone, compared with the wake state in which the additional influence of neuromodulatory systems is required for maintaining the state-specific neocortical activity. Moreover, this depolarizing phase has been considered to set the stage for processes of neocortical plasticity, in which neocortical networks become particularly sensitive to afferent inputs resulting from reactivation of acutely acquired memory traces as stored in hippocampal regions (Buzsaki, 1989; Steriade et al., 2001; Huber et al., 2004). Integration of these representations into neocortical networks could be a mechanism underlying retention of declarative memories. In this way, the acute enhancement of activity in slow oscillatory bands in the present study by tDCS indeed supports the concept that tDCS enhances retention performance by facilitating the slow oscillatory corticocortical network activity. The enhancement of slow oscillatory power during acute anodal polarization also corroborates the concept that endogenous negative DC potentials arising at the transition into SWS and accompanying associated shifts in extracellular ionic concentration play a supportive role in the generation of slow oscillatory activity. A factor adding to the facilitation of slow oscillations during tDCS could be our 15 sec on–15 sec off stimulation protocol (McCormick et al., 2003; Shu et al., 2003).

At the synaptic level, acute influences of anodal polarization possibly reflect the facilitation of specific cationic currents (Amzica and Steriade, 2000; Bazhenov et al., 2002). The generation of δ and slow oscillations relies particularly on Ca^{2+}-mediated K^+ currents and a persistent Na^+ current, with the latter proposed to reexcite the depolarizing phase of the slow oscillation (Buzsaki et al., 1988; Steriade et al., 1991, 2001; Timofeev et al., 2001). Depletion in extracellular Ca^{2+} concentration coincides with the depolarizing phase of the slow oscillation (Massimini and Amzica, 2001; Amzica et al., 2002). Interestingly, pharmacological blocking of Na^+ and Ca^{2+} channels has been consistently found to suppress effects of anodal stimulation in humans (Liebetanz et al., 2002; Nitsche et al., 2003b).

Spindle activity triggered by the depolarizing phase of slow oscillations has been considered another sign of processes that enhance plasticity within neocortical networks via increased Ca^{2+} flow into pyramidal cells (Sejnowski and Destexhe, 2000; Steriade and Timofeev, 2003). The failure to see here, in conjunction with enhanced slow oscillatory power, increased spindle power during anodal tDCS is difficult to interpret within this line of reasoning. However, once SWS has been established, slow oscillatory activity might primarily exert a grouping influence on the occurrences of spindle activity without necessarily changing average power in this frequency band. Alternatively, the decrement in lower β power (15–20 Hz) during stage 2 sleep in the tDCS condition in this context could be even taken to infer a decrease in spindle activity overlapping with this frequency range. In fact, suppressed spindle counts during the tDCS interval suggest spindle activity in the present study was not a mediator for the enhanced declarative memory retention (Gais et al., 2002).

Together, our data show that anodal tDCS over frontocortical areas repeatedly applied during a period of SWS-rich early sleep improves declarative memory consolidation. The effect of tDCS might involve slow oscillatory activity, which has been considered to favor plastic processes in neocortical networks and which is acutely enhanced by anodal polarization, presumably as a consequence of a global increase in excitability of the underlying cortex.

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