Acute Changes in Motor Cortical Excitability During Slow Oscillatory and Constant Anodal Transcranial Direct Current Stimulation

Til Ole Bergmann, Sergiu Groppa, Markus Seeger, Matthias Mölle, Lisa Marshall, and Hartwig Roman Siebner

Department of Neurology, Christian-Albrechts University Kiel; Department of Neuroendocrinology, University of Lübeck, Lübeck, Germany; and Danish Research Center for Magnetic Resonance, Department of Magnetic Resonance, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark

Submitted 20 May 2009; accepted in final form 14 August 2009

Transcranial direct current stimulation (tDCS) was introduced into neurophysiology approximately a decade ago (Bindman et al. 1964; Purpura and McMurtry 1965), but was reintroduced to use time-varied tDCS with oscillatory fluctuations in the induced tissue current. Anodal tDCS (Marshall et al. 2004) as well as anodal slow oscillation stimulation (Marshall et al. 2006a) were applied in a bilateral prefrontal montage during human nonrapid eye movement (NREM) sleep. The slow oscillation stimulation protocol was designed to mimic slow oscillations during sleep with a repetition rate of 0.75 Hz (Marshall et al. 2006a). Both stimulation modes enhanced endogenous slow oscillatory activity and facilitated the mnemonic function of these oscillations (Marshall and Born 2007). Notably, slow oscillation stimulation did not only facilitate endogenous slow oscillations but also appeared to phase-lock them to the exogenous stimulation (Marshall et al. 2006a). Theta-like 5-Hz oscillation stimulation rather decreased slow oscillatory activity.

Recently, transcranial AC stimulation (tACS) with sinusoidally alternating polarities has been shown to modulate primary visual cortex excitability during wakefulness, as mea-
measured by ratings and thresholds of tACS-induced phosphenes (Kanai et al. 2008). Here, the effectiveness of several stimulation frequencies to induce phosphenes was dependent on the currently predominant endogenous oscillation and was thus most effective in the beta range when eyes were open and in the alpha range when eyes were closed (but see Schwiedrzik 2009 for a critical comment). However, when testing the effect of different frequencies (1–45 Hz; applied over M1HAND during wakefulness), neither oscillatory tDCS (fixed anodal or cathodal polarity) nor tACS altered subsequent (off-line) M1HAND corticospinal excitability (MEPs) or spontaneous motor cortical electroencephalographic (EEG) activity (Antal et al. 2008).

These studies suggest that oscillatory transcranial stimulation can noninvasively shape endogenous cortical rhythms, thereby modifying ongoing internal processing modes of the brain. However, the underlying neuronal mechanisms are yet unknown. One important question is whether oscillating DC shifts can be directly imposed on the membrane of cortical neurons, given that this is the mechanism proposed for constant tDCS. Shifting the membrane potential repeatedly back and forth in an oscillating manner might be the basic principle by which oscillating transcranial stimulation can also take effect on endogenous rhythms.

We therefore probed the immediate impact of oscillating tDCS on the excitability of the fast-conducting corticospinal output neurons in the human M1HAND. We applied either 0.8-Hz sinusoidally modulated anodal slow oscillatory tDCS (so-tDCS) or anodal c-tDCS to the left M1HAND and conversely measured phase-dependent changes in MEP amplitude (on-line tDCS effects) in the contralateral hand muscles. MEP amplitudes were additionally measured before and after tDCS interventions (off-line tDCS effects). The experiments were performed during quiet wakefulness to maintain comparability with the majority of previous studies probing the effects of conventional c-tDCS on cortical excitability during this state of vigilance. We did not expect to trigger full-blown sleep-like slow oscillations (strong hyperpolarization followed by a rebound of massive synchronous firing), but hypothesized that so-tDCS might induce slow oscillatory changes in the membrane depolarization of idling motor neurons and thus cause slow oscillatory fluctuations in corticospinal excitability.

**Methods**

**Participants**

Ten healthy right-handed male volunteers (mean age: 26.5 yr, range: 19–30 yr) participated in the experiments after they had given written informed consent. All participants were free of medication and had no history of neurological or psychiatric disease. Subjects were recruited from the student population of the University of Kiel. All of them were completely naive toward the techniques of tDCS and TMS as well as regarding the detailed purpose of the study. Experimental procedures conformed to the Declaration of Helsinki and were approved by the Ethics Committee of the University of Kiel.

**Experimental procedure**

In a within-subject design, each participant underwent both slow oscillatory (0.8 Hz) and constant anodal tDCS to the left M1HAND. The two stimulation modes were applied in separate experimental sessions performed ≥3 days apart and in randomized order. Participants were blinded to the type of tDCS and, despite the respective stimulation mode, experimental procedures were identical (Fig. 1). In both sessions, we repeatedly applied tDCS for 30 s. For monitoring and safety reasons the overall time of tDCS within a session was split into two consecutive blocks of 20.25 min. Each of the two on-line blocks consisted of thirty 30-s “tDCS trials” with anodal DC stimulation intermingled with five 30-s “tDCS-free trials” for use as a within-block baseline. Trials were separated by an intertrial interval (ITI) of about 5 s (Fig. 1).

**A Time line of a single experimental session**

- **Pre**
  - Offline MEP (30)
  - Online MEP during tDCS (105 MEP in 35 trials)
- **Block 1**
  - Online MEP during tDCS (105 MEP in 35 trials)
- **Inter**
  - Offline MEP (30)
- **Block 2**
  - Online MEP during tDCS (105 MEP in 35 trials)
- **Post**
  - Offline MEP (30)

**B Time line of a single tDCS trial**

- Current (mA)
  - 0 s to 30 s
  - 0–1.5 mA

**C Time line of a single tDCS cycle**

- Current (mA)
  - 0° to 360°
  - 0–1.5 mA
  - 0°, 60°, 120°, 180°, 240°, 300°

*FIG. 1.* Experimental procedure. A: slow oscillatory transcranial direct current stimulation (so-tDCS) and constant tDCS (c-tDCS) were applied in 2 experimental sessions on separate days. During one session 30 “off-line” motor-evoked potentials (MEPs) were measured before (“pre”), between (“inter”), and after (“post”) 2 consecutive stimulation blocks. Each block consisted of 35 randomized trials (duration = 30 s, intertrial interval [ITI] = 5 s), 30 tDCS trials, and 5 tDCS-free trials. B: tDCS trials consisted of 30 s of anodal tDCS either slow oscillatory (0.8 Hz, 24 cycles, current range between 0 and 1.5 mA; black line) or constant (ramped up before and down after each trial for 1 s, constant current = 0.75 mA; gray line). Dashed rectangles indicate time windows of “on-line MEP” measurements. C: single-pulse transcranial magnetic stimulation (TMS) was applied over the left primary motor cortex (M1) to measure MEPs at different phase angles of the slow oscillatory cycle (black line) or corresponding time points during c-tDCS (gray line). During each trial only one of the 6 angles was probed in all 3 time windows.
Acute on-line effects of tDCS on the excitability of the corticospinal pyramidal neurons in the M1<sub>HAND</sub> were assessed by MEPs measured concurrently to the application of tDCS. Within each trial excitability of the M1<sub>HAND</sub> was probed by three single MEPs roughly 10, 20, and 30 s after stimulation onset or corresponding time points in tDCS-free trials (Fig. 1B). In the so-tDCS session MEPs were measured at various precisely defined phases of the slow oscillation (Fig. 1C). In single-trial all three MEPs were evoked at one of six different phase angles (0, 60, 120, 180, 240, and 300°; i.e., every 0.208 s with respect to the cycle onset) or corresponding time points during the c-tDCS session. Trials of different phase angles were applied semirandomized, that is, in consecutive groups each comprising all possible phase angles in randomized order, to guarantee a uniform distribution of phase angles over time. Off-line effects of tDCS on the excitability of the corticospinal neurons were assessed with single-pulse TMS as during iDCS. These MEP measurements were performed off-line before ("pre-tDCS"), between ("inter-tDCS"), and directly after ("post-tDCS") the stimulation blocks (Fig. 1A).

**Transcranial DC stimulation**

Anodal tDCS was applied through a bipolar montage of saline-soaked sponge electrodes (12 cm<sup>2</sup>) using a battery-driven constant-current stimulator (Eldith DC-Stimulator, NeuroConn, Ilmenau, Germany). According to standard procedures (Nitsche and Paulus 2000), the center of the anode was placed directly above the "motor hot spot" of the first dorsal interosseus (FDI) muscle in the left M1<sub>HAND</sub> as identified by TMS before, whereas the cathode was fixed to the right forehead (Fig. 2A). In the c-tDCS trials the continuous-current strength of 0.75 mA (maximum current density: 0.0625 mA/cm<sup>2</sup>) was slowly ramped up for 1 s before and ramped down for 1 s after each 30-s iDCS-trial (Fig. 1B) to avoid unpleasant sensations and the occurrence of retinal phosphenes. In the so-tDCS trials, 24 consecutive sinus waves with a cycle duration of 1.25 s (0.8 Hz) were generated. Current strength ranged from a minimum of 0 mA to a maximum of 1.5 mA (maximum current density: 0.125 mA/cm<sup>2</sup>) (Fig. 1, B and C). Mean current density over time (0.0625 mA/cm<sup>2</sup>) and total charge density per 30-s trial (1.875 C/cm<sup>2</sup>) were thus equal for both stimulation modes (disregarding the short ramps in c-tDCS trials). The applied current is therefore both strong enough to be effective (Nitsche and Paulus 2000; Nitsche et al. 2007, 2008) and safe as being far below minimum values for inducing tissue damage (Nitsche et al. 2003b). Note, that the so-tDCS described earlier and the slow oscillation stimulation during sleep (Marshall et al. 2006b) differ in several parameters.

**Transcranial magnetic stimulation**

Motor cortical excitability was assessed using single-pulse TMS over the left M1<sub>HAND</sub> while recording a surface electromyogram (EMG) from the contralateral proximal hand muscles (Fig. 2B). Single-pulse TMS over the left M1<sub>HAND</sub> was performed using a figure-of-eight–shaped “MC-B70” coil, with an outer diameter of 70 mm, connected to a MagPro-100 stimulator (MagVenture, Farum, Denmark). The magnetic stimulus had a monophasic pulse configuration with a width of about 70 μs (from onset to peak). The coil was positioned tangentially to the skull above the left M1<sub>HAND</sub> with the handle pointing backward and laterally at an angle of about 45° to the sagittal plane (Fig. 2A), inducing an electrical current in the brain tissue with a posterior–lateral to anterior–medial direction roughly perpendicular to the central sulcus. This current orientation is known to be optimal for evoking a motor response in the contralateral hand (Mills et al. 1992). The location and exact orientation at which stimuli at slightly suprathreshold intensity consistently yielded maximal MEPs in the contralateral FDI muscle were considered to constitute the “motor hot spot” and used for TMS of the M1<sub>HAND</sub> as well as for placement of the tDCS anode directly below the coil. The tDCS electrode cables were kept orthogonal to the main direction of the magnetic field to avoid retinal phosphenes, caused by induced currents in the electrode cables. TMS intensity (expressed as a percentage of maximum stimulator output) was adjusted at the beginning of each experimental session after positioning the coil above the tDCS electrode to elicit a mean peak-to-peak MEP amplitude between 0.5 and 0.75 mV in the relaxed contralateral FDI muscle and then remained constant throughout all measurements. Pre-, inter-, and post-tDCS off-line measurements consisted of 30 MEPs each (interstimulus interval [ISI] = 10 s, 5-min duration). The within-block baselines consisted of 15 MEP measurements per iDCS block (3 trials × 3 MEPs; ISI within trial = 10 s). On-line measurements consisted of 30 MEPs per phase angle (2 iDCS blocks × 5 trials × 3 MEPs; ISI within trial = 10 s).

**Neuronavigation**

We used frameless stereotaxy (TMS-Navigator, Localite, Sankt Augustin, Germany) based on a coregistered individual T1-weighted magnetic resonance image to navigate the TMS coil and to maintain its exact location and orientation throughout an experimental session. T1-weighted images were acquired some days before on a 3-Tesla magnetic resonance tomograph (Philips Achieva, Philips Medical Systems, Best, The Netherlands) using a standard MPRAGE sequence (repetition time = 7.7 ms, time to echo = 3.6 ms, flip angle = 8°, 170 sagittal slices, 1 × 1 × 1-mm voxel size, field of view = 224 × 224 mm).

**Recordings**

EMG activity was recorded from the right first dorsal interosseus (FDI), abductor pollicis brevis (APB), and abductor digitii minimi (ADM) muscles with Ag/AgCl surface electrodes using a bipolar belly-tendon montage (Fig. 2B). The raw EMG signals were amplified by 1,000 (D360, Digitimer, Welwyn Garden City, Herts, UK), filtered between 2 and 2,000 Hz (plus 50-Hz notch), and digitized at 5,000 Hz per channel (CED Power1401, 16-bit ADC; Cambridge Electronic Design [CED], Cambridge, UK). The administration of TMS pulses and EMG data recording, storage, and analyses were performed with Signal software (CED). Peak-to-peak amplitudes of each MEP (mV) were measured and mean MEP amplitudes were calculated for each condition using NuCursor software (Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, Queen Square, London, UK).

Additionally, the tDCS-induced currents on the scalp were recorded with standard gold cup electrodes using a bipolar montage, with one
electrode placed between stimulation sites and the other one at the right lower lateral forehead (Fig. 2A) because this montage revealed the most stable signal (amplified by 300, filtered between 0.1 and 2,000 Hz).

**Questionnaires**

To additionally explore the potential effect of tDCS on subjective sleepiness, participants’ subjective reports were gathered using a German version of the Stanford Sleepiness Scale (SSS) (Hoddes et al. 1973) before, after, and, and three times within each stimulation block. SSS ratings before and after tDCS blocks were reported orally, whereas those within blocks were reported by hand sign with the left hand (after 10, 20, and 30 trials) in TMS-free intervals.

After each session, participants completed a questionnaire asking for the unpleasantness of the experimental procedures in general and of the TMS and tDCS application in particular (ten-point rating scales ranging from “neutral” to “absolutely intolerable”). Participants were also asked to report any unpleasant sensations such as burning, prickling, tugging, itching, pressure, and pulsation during the stimulation (and, if applicable, to rate the severity on four-point rating scales) as well as any discomforts during and after the experiment such as headache, dizziness, nausea, or a feeling of pressure.

**Data processing and statistical analyses**

Unless specified otherwise, MEP values refer to those measured at the FDI muscle. For analyses of off-line tDCS effects, peak-to-peak amplitudes of off-line-MEPs from pre-, inter- and post-tDCS measurements were first calculated for single trials and then averaged for each time point. Additionally, mean MEPs of TMS-free trials within blocks 1 and 2 served as in-block off-line MEP measurement. For analyses of on-line tDCS effects, on-line-MEPs were processed analogously, but averaged separately, dependent on stimulation form, phase angle (or time-matched values for c-tDCS), and stimulation duration, i.e., time relative to stimulation onset. Subsequently, percentage deviations from mean MEPs of TMS-free trials were calculated.

Repeated-measures ANOVAs were used to test for on-line and off-line tDCS effects on MEP amplitude. For on-line effects, the factors stimulation mode (so-tDCS, c-tDCS), phase angle (0, 60, 120, 180, 240, 300°), and stimulation duration (10, 20, 30 s) were included. Post hoc two-way ANOVA for stimulation duration was performed for tDCS-free trials only to exclude any within-trial baseline shifts (see Results for rationale). Off-line effects were tested on the factors stimulation mode and time (pre-tDCS, within-block baseline 1, inter-tDCS, within-block baseline 2, post-tDCS). Greenhouse–Geisser (GG) correction for nonsphericity was applied where necessary, and ANOVAs were followed by post hoc two-sided paired-sample t-tests where applicable. To test for facilitatory on-line effects of anodal tDCS using the within-block baseline-adjusted data (with test values set to zero by definition), one-sided, one-sample t-tests were applied. Two-sided paired t-tests tested differences between the facilitatory effect of both stimulation modes. To test whether the initial facilitation of corticospinal excitability during tDCS (i.e., on-line effect) predicts the lasting increase in corticospinal excitability beyond the time of tDCS (i.e., off-line effect), linear regression analyses were applied using individually averaged on-line MEPs of the first 12 trials (2 of each phase angle) as independent variable and off-line MEPs of either inter- or post-tDCS measures as dependent variable (all adjusted to pre-tDCS baseline). Values of $P \leq 0.05$ were considered significant. Group data are given as means $\pm$ SE if not specified otherwise.

**RESULTS**

Neither TMS intensities (so-tDCS 81.60 $\pm$ 3.14%; c-tDCS 83.5 $\pm$ 3.07%) nor baseline MEP amplitudes (so-tDCS 0.70 $\pm$ 0.51; c-tDCS 0.83 $\pm$ 0.54) were significantly different between stimulation conditions. Group data are given as means $\pm$ SE if not specified otherwise.

**FIG. 3.** On-line MEP effects. Means and SE are shown for percentage changes of on-line MEP amplitudes in the FDI muscle relative to TMS-free trials constituting the in-block baseline. A: effects are displayed separately for pre- and post-tDCS, with asterisks indicating significant ($P < 0.05$) differences from zero, and “n.s.” indicating a nonsignificant ($P > 0.5$) direct comparison of both stimulation modes. B: effects of so-tDCS (open circles) and c-tDCS (closed circles) tDCS relative to stimulation-free test trials are depicted separately for the different phase angles (so-tDCS or time-matched values (c-tDCS), respectively. C: facilitatory on-line tDCS effects (averaged over so-tDCS and c-tDCS) decreased within a 30-s trial relative to TMS-free trials. Asterisks indicate significant ($P < 0.05$) differences from zero.
angle, and during so-tDCS revealed any significant effects (and phase angle separately for all phase angles (or time-matched values in the acute on-line MEP facilitation of both stimulation modes ever, was a main effect of modulation by effect was evident for both stimulation modes but there was no size by means of so-tDCS might have needed some time to build up (e.g., due to slow phase-locking mechanisms), we additionally performed a three-way ANOVA for stimulation mode and phase angle during so-tDCS revealed any significant effects (P > 0.5). As can clearly be seen in Fig. 3B, a general facilitatory on-line effect was evident for both stimulation modes but there was no modulation by phase angle (i.e., acute current strength).

Because the presumed phase-dependent modulation of MEP size by means of so-tDCS might have needed some time to build up (e.g., due to slow phase-locking mechanisms), we additionally performed a three-way ANOVA for stimulation mode, phase angle, and stimulation duration. The only significant result, however, was a main effect of stimulation duration \( F_{(2,18)} = 4.49, P < 0.026 \), which can be explained by a general decrease in MEP size from 10 to 20 s (40 vs. 23%; \( T_9 = 2.30, P = 0.047 \)) as well as from 10 to 30 s (40 vs. 8%; \( T_9 = 2.86, P = 0.019 \)) after tDCS onset. An additional two-way ANOVA for stimulation mode and stimulation duration, calculated exclusively for tDCS-free trials, revealed that within-block baseline MEPs did not change with increasing duration of the tDCS-free interval for any of the stimulation modes (P > 0.3). Thus the observed decrement of the facilitatory on-line effect seems indeed due to the in-trial duration of tDCS application (Fig. 3) and cannot be accounted for by mere shifts in within-block baseline.

Off-line tDCS effects

We then aimed to test for the off-line effects of both stimulation modes (Fig. 4). The ANOVA for stimulation mode and time revealed a main effect of time only \( F_{(4,36)} = 6.62, P = 0.004, \text{GG} \), whereas the main effect of stimulation mode and the interaction remained nonsignificant (P > 0.4). Due to the missing interaction, post hoc comparisons were performed on time levels only, averaged across stimulation modes. They revealed a continuous increase in MEP size from pre- to inter- to post-tDCS measurements (inter–pre: \( T_9 = 0.31, P = 0.015 \); post–inter: \( T_9 = 8.98, P < 0.001 \); post–pre: \( T_9 = 4.92, P < 0.001 \)), and also within-block MEPs were significantly increased from baseline (block 1–pre: \( T_9 = 2.49, P = 0.034 \); block 2–pre: \( T_9 = 2.92, P = 0.017 \)).

Both anodal so-tDCS and c-tDCS had a strong facilitatory of off-line effect on MEP amplitude that did not significantly differ between stimulation modes (about 39% after the first and 54% after the second 20-min block; Table 2).

Linear regression analyses

At an individual level, the amount of on-line facilitation of MEPs at the beginning of tDCS predicted later off-line effects of tDCS. The increase in MEP amplitudes during the first 12 trials of tDCS application relative to pre-tDCS baseline corre-

![FIG. 4. Off-line MEP effects. Means and SE for off-line MEP amplitudes in the FDI muscle are shown for both stimulation modes: so-tDCS (open circles) and c-tDCS (filled circles). Off-line measurements before (pre), within (block1, block2), between (inter), and after (post) the 2 consecutive tDCS blocks are depicted.](http://jn.physiology.org/Downloadedfrom http://jn.physiology.org/)

### Table 1. Online MEPs

<table>
<thead>
<tr>
<th>Test Trial</th>
<th>0</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>so-tDCS</td>
<td>0.99 ± 0.16</td>
<td>1.04 ± 0.16</td>
<td>1.01 ± 0.16</td>
<td>1.04 ± 0.14</td>
<td>1.03 ± 0.16</td>
<td>0.99 ± 0.14</td>
</tr>
<tr>
<td>c-tDCS</td>
<td>0.86 ± 0.12</td>
<td>1.04 ± 0.17</td>
<td>0.98 ± 0.17</td>
<td>0.93 ± 0.13</td>
<td>0.97 ± 0.13</td>
<td>1.01 ± 0.21</td>
</tr>
</tbody>
</table>

**A. Absolute MEP amplitudes in mV**

**B. Percentage change from test-MEP amplitudes**

| so-tDCS | c-tDCS | | | | | |
|---------|-------| | | | | |
| 0 ± 0 | 20 ± 10 | 16 ± 8 | 23 ± 13 | 19 ± 11 | 16 ± 9 | 24 ± 7 |
| 0 ± 0 | 35 ± 12 | 28 ± 14 | 20 ± 6 | 27 ± 12 | 27 ± 11 | 27 ± 10 |

Values are means ± SE, with respect to percentage change in MEP amplitude from tDCS-free in-block baseline at the FDI muscle. Values are displayed separately for different phase angles (so-tDCS) or time-matched values (c-tDCS), respectively. Note that percentage changes were first individually calculated and averaged afterward and that percentages of mean values would thus differ from the depicted values.
Tolerability of experimental procedures in general (mean was questioned verbally and did not report any side effects). A survey of potentially unpleasant sensations across the entire experiment similarly in both tDCS conditions. As can be seen in Fig. 5, subjective sleepiness increased during stimulation modes (nine ratings: before, after, and three times within each of the two stimulation blocks) revealed a significant main effect of time only \([F(3,3,72) = 9.12, P < 0.001; \text{GG}]\) but no main effect of stimulation mode or interaction. As can be seen in Fig. 5, subjective sleepiness increased during tDCS blocks relative to tDCS-free periods as well as across the entire experiment similarly in both tDCS conditions.

### Table 2. Offline MEPs

<table>
<thead>
<tr>
<th></th>
<th>Pre-tDCS Offline-MEP</th>
<th>Block 1 Test-MEP</th>
<th>Inter-tDCS Offline-MEP</th>
<th>Block 2 Test-MEP</th>
<th>Post-tDCS Offline-MEP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. Absolute MEP amplitudes in mV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>so-tDCS</td>
<td>0.70 ± 0.08</td>
<td>0.98 ± 0.14</td>
<td>0.80 ± 0.12</td>
<td>1.00 ± 0.20</td>
<td>1.06 ± 0.17</td>
</tr>
<tr>
<td>c-tDCS</td>
<td>0.63 ± 0.07</td>
<td>0.76 ± 0.09</td>
<td>0.91 ± 0.11</td>
<td>0.96 ± 0.17</td>
<td>0.93 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>B. Percentage change from baseline MEP amplitudes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>so-tDCS</td>
<td>0 ± 0</td>
<td>42 ± 20</td>
<td>22 ± 18</td>
<td>48 ± 24</td>
<td>55 ± 22</td>
</tr>
<tr>
<td>c-tDCS</td>
<td>0 ± 0</td>
<td>29 ± 19</td>
<td>55 ± 22</td>
<td>55 ± 22</td>
<td>52 ± 23</td>
</tr>
</tbody>
</table>

Values are means ± SE, with respect to percentage change in MEP amplitude from baseline (pre-tDCS) MEPs at the FDI muscle. Note that percentage changes were first individually calculated and averaged afterward and that percentage of mean values would thus differ from the depicted values.

**Topographical specificity of tDCS effects**

Off-line MEP recordings in the APB and ADM muscles were available from only nine of ten subjects due to technical problems. To explore the topographical specificity of tDCS-effects, main analyses were performed independently for MEPs obtained from the APB and ADM muscles. The results of separate 2 × 5 two-way ANOVAs (stimulation mode × time) with appropriate post hoc t-tests mirrored the off-line tDCS effects in the FDI muscle (see Supplemental Table S1 for statistical details). Likewise, one-sided, one-sample t-tests revealed phase-independent on-line facilitation of both stimulation modes in these muscles, whereas again no phase-dependent modulation was observed in separate 2 × 6 two-way ANOVAs (stimulation mode × phase angle) (see Supplemental Table S2 for statistical details). Taken together, these analyses confirm the main results found for the FDI muscle and demonstrate the expectably low topographical specificity of tDCS effects.

**Sleepiness ratings**

Because both c-tDCS and slow oscillation stimulation have been shown to enhance the amount of endogenous slow oscillatory cortical activity when applied during natural sleep (Marshall et al. 2004, 2006a), we also tested for any changes in participants’ subjective sleepiness ratings. An ANOVA for stimulation mode and time (nine ratings: before, after, and three times within each of the two stimulation blocks) revealed a significant main effect of time only \([F(3,3,72) = 9.12, P < 0.001; \text{GG}]\) but no main effect of stimulation mode or interaction. As can be seen in Fig. 5, subjective sleepiness increased during tDCS blocks relative to tDCS-free periods as well as across the entire experiment similarly in both tDCS conditions.

**Survey of potentially unpleasant sensations**

Nine subjects completed the questionnaire (the first subject was questioned verbally and did not report any side effects). Tolerability of experimental procedures in general (mean ± SE: 2.89 ± 0.31) and of the TMS (mean ± SE: 2.11 ± 0.35) and tDCS (mean ± SE: 3.22 ± 0.49) application in particular was good and there was no difference between stimulation modes \((P > 0.16, \text{paired } t\text{-test})\). Participants also reported slight feelings of burning, pricking, tugging, itching, pressure, and pulsation during the tDCS application, which again did not differ between stimulation modes \((P > 0.2, \text{paired } t\text{-test})\). Two participants (one with both stimulation modes, the other with c-tDCS only) reported a light headache during the experiment, which had already decayed when they left the laboratory. Four participants (two with each stimulation mode) reported a feeling of pressure during the stimulation, which they ascribed to the fixation of sponge electrodes and neuronavigation markers on the head, not to the stimulation itself.

**Discussion**

We found that anodal so-tDCS and c-tDCS over M1HAND were equally capable of inducing an increase in motor cortical excitability both during tDCS (on-line effect) and beyond the time of tDCS (off-line effect). At an individual level, the initial on-line facilitation of corticospinal excitability was predictive of the off-line effect on corticospinal excitability. The amount of enhancement in motor cortical excitability measured during so-tDCS did not vary with the phase of the applied current—not even between the minima and maxima of current strength—suggesting that anodal so-tDCS failed to induce sleeplike slow oscillatory changes at 0.8 Hz in M1HAND during wakefulness. The implication is that oscillatory excitability changes in a frequency that is inappropriate for the present brain state cannot be exogenously imposed on the cortex by means of oscillatory tDCS.

**Off-line tDCS effects**

During ongoing stimulation both anodal so-tDCS and c-tDCS generally increased motor cortical excitability in the stimulated M1HAND relative to in-block tDCS-free trials and thus independently of any off-line effects developing concurrently. Although the effect of c-tDCS seemed to be slightly stronger (27% increase) than that of so-tDCS (19% increase), the difference was not significant (Fig. 3A).

Phase-locked analysis revealed that motor cortical excitability did not differ with respect to the phase angle of so-tDCS, not even between minimal (0 mA at 0°) and maximal (1.5 mA at 180°) current strength. Thus under the present experimental conditions exogenous DC currents with a sleeplike frequency of 0.8 Hz failed to induce measurable excitability changes in
corticospinal M1\textsubscript{HAND} neurons. However, this absence in regulation of motor corticospinal excitability could be limited to the state of quiet wakefulness. During NREM sleep, characterized by the occurrence of endogenous slow oscillations, corticospinal motor neurons in M1\textsubscript{HAND} might be more receptive for a slow oscillatory stimulation frequency of 0.8 Hz. This interpretation closely ties in with the findings of previous studies indicating a dependence of tDCS efficiency on the relationship between predominant endogenous EEG brain oscillations and frequency modulation of applied current (Kanai et al. 2008; Marshall et al. 2006a).

Spontaneous EEG rhythms reflect reverberating activity within reentrant neuronal circuits (such as the thalamocortical system). In addition, certain intrinsically defined properties of individual neurons may crucially contribute to the generation of membrane-voltage oscillators within a specific frequency (Hutcheon and Yarom 2000). These membrane properties are not fixed but subject to neuromodulatory modification (e.g., Lawrence 2008). Since slow oscillatory cortical activity at frequencies around 0.8 Hz is typically absent during wakefulness, but a dominant feature of NREM sleep (Steriade 2006), it is likely that wakefulness does not provide the appropriate neuronal milieu for the generation of slow oscillations. This explains why, in the present study, the cortical circuits in M1\textsubscript{HAND} showed no tendency to respond with excitability fluctuations to the externally imposed so-tDCS. Yet it remains to be tested whether time-varied exogenous DC currents that are tuned to the “preferred frequency” of the brain state at time of stimulation may induce phase-locked undulations in motor cortical excitability. Testing this hypothesis would require the measurement of phase-locked fluctuations in motor cortical excitability while applying 1) so-tDCS during NREM sleep or 2) “\(\mu\) rhythm”-like 10-Hz oscillatory tDCS during relaxed wakefulness. Both approaches are challenging because 1) TMS during sleep and EEG sleep monitoring during tDCS are technically highly demanding and 2) 10-Hz oscillatory tDCS during wakefulness may be unpleasant and induce strong retinal phosphenes.

Interestingly, the facilitatory on-line tDCS effect was strongest after 10 s of stimulation and then strongly decayed within the subsequent 20 s of acute tDCS (Fig. 3C). This finding was somewhat surprising because the assumed shift in membrane potential was expected to build up with time. However, Priori and colleagues (1998) already hypothesized that neuronal elements in the motor cortex might adapt to and actively compensate for scalp DC-induced changes in the membrane potential. This potential compensatory mechanism, although sustained for the duration of stimulation, is quite short-lived because facilitatory effects recovered immediately after the 5-s ITI. The underlying sources of this regulatory mechanism apparently occurring at the cellular or network level are completely unknown, stressing the need for further research into...
the physiological responses acutely elicited by tDCS in the human cortex. This might include a thorough investigation of inhibitory processes across the time course of acute tDCS application, although Nitsche and colleagues (2005) reported no c-tDCS–induced changes in intracortical inhibition within 4-s epochs. In this context, the duration of a single tDCS epoch also appears to be of particular relevance. Although a similar on-line facilitation of MEP amplitudes (ranging from 20 to 50%) already emerges after 4 s of c-tDCS (Nitsche and Paulus 2000; Nitsche et al. 2003a, 2007), very brief c-tDCS epochs with a duration of 100 ms without ramps produce a less-consistent facilitation and require very high current densities to be efficient (≤0.33 mA/cm²) (Furubayashi et al. 2008).

Furthermore, early tDCS-induced on-line facilitation during the first dozen trials proved to be a good predictor for individual aftereffects after prolonged tDCS application. The predictive power of the initial on-line response to tDCS could be helpful in future studies to screen for responsive subjects or to probe stimulation efficacy before therapeutic application. Yet the neuronal underpinnings of this relationship are to be determined.

**Off-line tDCS effects**

Repeated application of tDCS also facilitated motor cortical excitability beyond the time of actual stimulation. Repeated measurements before, between, and after both stimulation blocks revealed a steady increase in excitability (about 39 and 54% after the first and second 20-min blocks, respectively), which was also roughly reflected by the within-block tDCS-free trials (Fig. 4). There was no significant difference in the amount of MEP facilitation between anodal so-tDCS and c-tDCS in the present study, indicating that both stimulation modes were equally effective in producing an excitability increase in corticospinal output neurons. In a separate series of experiments (S Groppa, TO Bergmann, C Siems, M Mölle, L Marshall, HR Siebner, unpublished data), we identified the mean current density over time (ma/cm²) as an important variable determining the efficacy of so-tDCS to induce after-effects in cortical excitability. Therefore we matched the mean current density over time between c-tDCS and so-tDCS in the present study. We wish to stress that this study was explicitly designed to compare the acute (on-line) effects of so- versus c-tDCS. Therefore variables influencing the efficacy of so-tDCS to induce long-lasting (off-line) effects on corticospinal excitability remain to be investigated.

Previous studies have always applied tDCS continuously (i.e., for several minutes) to induce lasting shifts in corticospinal excitability (Nitsche et al. 2008). In this study, tDCS was given continuously for only 30 s, with short breaks between consecutive tDCS applications. We also applied single-pulse TMS, both during and between tDCS trials. Despite the intermittent mode of tDCS and intermingled TMS measurements, the conditioning effect of tDCS gradually built up over time and reached a magnitude similar to that of conventional continuous tDCS. This implies that tDCS can be shortly interrupted or paired with occasional single TMS pulses without affecting the plasticity-inducing effects of tDCS. Since we applied single TMS pulses concurrently with tDCS, it is possible that the two types of stimulations had an additive influence on the off-line changes in MEP amplitude, for instance through a gating mechanism (Ziemann and Siebner 2008). This possibility needs to be explored more systematically in future studies.

A recent study by Antal and colleagues (2008) did not find any significant off-line effects in motor cortical excitability or spontaneous EEG recordings after application of oscillatory anodal tDCS to M1/HAND. We attribute this discrepancy with the present study to the significantly shorter stimulation durations (2–4 vs. 2 × 20 min) and lower current densities (maximum current density: 0.0156 vs. 0.125 mA/cm²; mean current density: 0.0078 vs. 0.0625 mA/cm²) that have been used by Antal and colleagues because it is unknown whether c-tDCS with these comparably weak parameters is able to induce plastic changes in the M1/HAND either.

**Subjective sleepiness**

Subjective sleepiness ratings revealed that there were no stimulation-specific changes in subjective sleepiness. This was tested for because so-tDCS occurred in a frequency corresponding to that endogenously arising during NREM sleep. Independent of the stimulation mode, sleepiness apparently increased with session time as well as within stimulation blocks (Fig. 6). However, this effect is likely due to the participants’ reduced motor activity and the monotonous sensory input associated with the experimental setting, rather than due to the stimulation itself. Because there was no sham stimulation, an unspecific tDCS effect on sleepiness cannot be ruled out in the present study.

**Conclusions**

To summarize, we found that acute motor cortical excitability is appreciably facilitated during both anodal so-tDCS and c-tDCS in general, but that there is no phase-locking of cortical excitability to the exogenously applied slow oscillation. With respect to the current literature we attribute this lack in phase dependence to the incompatibility of sleeplike 0.8-Hz stimulation and the present state of wakefulness. Moreover, acute facilitation effects rapidly decayed within 30 s of stimulation, but were totally renewed after a 5-s pause, suggesting the presence of fast regulatory mechanisms counteracting the induced DC shift.

**GRANTS**

This work was funded by the Deutsche Forschungsgemeinschaft Project A6/SFB 654 “Plasticity and Sleep.” H. R. Siebner was supported by Bundesministerium für Bildung und Forschung Structural Grant 01GO0511 to NeuroImageNord.

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