Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex

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Fricke K, Seeber AA, Thirugnanasambandam N, Paulus W, Nitsche MA, Rothwell JC. Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. J Neurophysiol 105: 1141–1149, 2011. First published December 22, 2010; doi:10.1152/jn.00608.2009.—Several mechanisms have been proposed that control the amount of plasticity in neuronal circuits and guarantee dynamic stability of neuronal networks. Homeostatic plasticity suggests that the ease with which a synaptic connection is facilitated/suppressed depends on the previous amount of network activity. We describe how such homeostatic-like interactions depend on the time interval between two conditioning protocols and on the duration of the preconditioning protocol. We used transcranial direct current stimulation (tDCS) to produce short-lasting plasticity in the motor cortex of healthy humans. In the main experiment, we compared the aftereffect of a single 5-min session of anodal or cathodal tDCS with the effect of a 5-min tDCS session preceded by an identical 5-min conditioning session administered 30, 3, or 0 min beforehand. Five-minute anodal tDCS increases excitability for about 5 min. The same duration of cathodal tDCS reduces excitability. Increasing the duration of tDCS to 10 min prolongs the duration of the effects. If two 5-min periods of tDCS are applied with a 30-min break between them, the effect of the second period of tDCS is identical to that of 5-min stimulation alone. If the break is only 3 min, then the second session has the opposite effect to 5 min tDCS given alone. Control experiments show that these shifts in the direction of plasticity evolve during the 10 min after the first tDCS session and depend on the duration of the first tDCS but not on intracortical inhibition and facilitation. The results are compatible with a time-dependent “homeostatic-like” rule governing the response of the human motor cortex to plasticity probing protocols.

transcranial magnetic stimulation

SYNAPTIC PLASTICITY, which leads to long-term changes in cortical excitability through structural or functional alterations of neuronal connectivity, provides the neurophysiological basis for most models of learning and memory (Hebb 1949; Abbott and Nelson 2000). However, the positive feedback nature of these neurophysiological alterations carries the risk of triggering an uncontrolled increase in synaptic effectiveness, which can be potentially destabilizing and overpower all other inputs in the system. This can be prevented by making the amount and direction of plasticity depend on the history of activation of the postsynaptic neuron and is formalized in the model of “homeostatic” plasticity originally described by Bienenstock et al. (1982) (Turrigiano and Nelson 2004). In recent years, several animal studies have empirically confirmed this homeostatic hypothesis (Huang et al. 1992; Wang and Wagner 1999; Abraham et al. 2001).

Homeostatic rules of plasticity have also been explored in humans. Iyer et al. (2003) were the first to demonstrate the existence of homeostatic plasticity mechanisms in humans. A brief pretreatment with repetitive transcranial magnetic stimulation (rTMS) of 5–6 Hz, which is known to increase cortical excitability, enhanced the inhibitory effect of subsequent 1-Hz stimulation. Siebner et al. (2004) and Lang et al. (2004) extended these findings by exploring bidirectional homeostatic effects in a transcranial direct current stimulation (tDCS)-rTMS protocol on the human motor cortex, whereas Müller et al. (2007) showed that they also applied to focal, associative plasticity [induced by paired associative stimulation (PAS)] in the human motor cortex. In contrast, homeostatic rules do not necessarily apply when different plasticity-inducing protocols are combined, such as tDCS and PAS. In this case, only simultaneous, not successive, application of the two protocols leads to homeostatic-like effects (Nitsche et al. 2007a). Another recently published study found that the efficacy of inhibitory cathodal tDCS was enhanced if a second period of stimulation was given during the aftereffects of the first one, but was reduced if the second stimulation was applied when the aftereffects of the first DC stimulation had vanished (Monte-Silva et al. 2010). Thus, taken together, the results of experiments on homeostatic plasticity in humans are heterogeneous and somewhat puzzling. Possible explanations for this nonuniformity of results might be differences in the mechanisms or the neurons affected by each experimental protocol, the timing of the protocols, and the duration of the induced shifts in excitability.

In this study we aimed to explore systematically how homeostatic plasticity depends on 1) the time interval between the application of two plasticity-inducing protocols and 2) the duration of plasticity induced by the first, preconditioning protocol. In the first experiment, we used 5-min periods of tDCS, which produce aftereffects on the motor cortex lasting for 5–10 min. In that experiment, two 5-min periods of anodal (experiment 1a) or cathodal tDCS (experiment 1b) were separated by an interval of 30 min (i.e., when the aftereffects of the first protocol had disappeared: 5-30-5 min), 3 min (when the aftereffects of the first protocol were still present: 5-3-5 min), or 0 min (5-0-5 min, corresponding to 10 min of continuous tDCS). The effects of the second period of tDCS were compared with those of a single session of 5-min tDCS. In a control
experiment (experiment 2), we aimed to explore the time course of interaction in more detail by applying a second period of anodal tDCS 1, 10, and 20 min after the first one. In a further control experiment (experiment 3), we explored the dependency of the aftereffects of repeated anodal tDCS on the duration of the first tDCS session by combining a second conditioning protocol of 5-min tDCS with a preconditioning stimulation of 7 min in duration. Finally, we explored whether homeostatic effects depend on changes of intracortical inhibition and facilitation to learn more about the physiological foundation of this phenomenon (experiment 4).

METHODS

Subjects

Six male and two female healthy subjects treated with anodal tDCS (mean age, 33.5 yr; age range, 23–49 yr) and six males and three females treated with cathodal tDCS (mean age, 32 yr; age range, 24–37 yr) participated in experiment 1. Four males and four females (mean age, 24.3 yr; age range, 21–29 yr) were included in experiment 2. Five males and seven females (mean age, 26.4 yr; age range, 22–30 yr) were included in experiment 3. Three males and five females (mean age, 31.0 yr; age range, 24–37 yr) took part in the paired-pulse experiment (experiment 4). Within experiments 1a (anodal tDCS), 1b (cathodal tDCS), 2, 3, and 4, a complete crossover design was applied. Subjects differed between the single experiments. Subjects recruited for experiment 4 were a subgroup of subjects who participated in experiment 1a (anodal stimulation). To avoid interference effects, a break of at least 1 wk was obligatory between experimental sessions. All participants were free of acute or chronic neurological, psychiatric, or medical diseases and did not take any medication. The procedures were approved by the Joint Ethics Committee of the National Hospital for Neurology and Neurosurgery, the Institute of Neurology (University College London), and the Ethics Committee of the University of Göttingen and were performed according to the ethical standards laid down in the Declaration of Helsinki.

Direct Current Stimulation of the Motor Cortex

Continuous direct currents were transferred by a saline-soaked pair of surface sponge electrodes (35 cm²) and delivered by a specially developed, battery-driven constant-current stimulator (Schneider Electronic, Gleichen, Germany) with a maximum output of 2 mA. The motor cortical electrode was fixed over the representational field of the right first dorsal interosseous muscle (FDI) as identified by TMS. The other electrode was placed contralaterally above the right orbit, since this arrangement is known to result in significant excitability changes of the primary motor cortex (Nitsche and Paulus 2000). The terms “anodal” or “cathodal” stimulation always refer to the polarity of the motor cortex tDCS electrode. Anodal and cathodal stimulation were applied with a current intensity of 1 mA (current density, ~0.03 mA/cm²), since this intensity has been shown to be painless but is strong enough to induce stable effects on motor cortex excitability (Nitsche and Paulus 2000).

Monitoring of Motor Cortex Excitability

Because TMS has been shown to be a reliable tool for the investigation of corticospinal excitability (Rothwell 1993), motor-evoked potentials (MEPs) of the right FDI were obtained by stimulation of its motor-cortical representational field by single-pulse TMS. Stimulation was induced by a Magstim 200 magnetic stimulator (Whiteland, Dyfed, UK) and a figure-of-eight magnetic coil (diameter of one winding, 70 mm; peak magnetic field, 2.2 T). The coil was held tangentially to the skull, with the handle pointing backward and laterally at 45° from midline. The optimal coil position was defined as the site where stimulation resulted consistently in the largest MEP. Surface electromyogram (EMG) was recorded from the right FDI using Ag-AgCl electrodes in a belly-tendon montage. Raw signals were amplified, band-pass filtered (3 Hz to 1 kHz), and digitized at a sample rate of 5 kHz using a CED 1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) controlled by Signal software (CED version 2.13). They were further relayed into a laboratory computer for off-line analysis.

In experiments 1–3, single-pulse TMS was used to test the influence of the tDCS protocol on global corticospinal excitability (Rothwell 1993). To test the specific influence of the protocol on intracortical inhibition and facilitation, we chose the paired-pulse paradigm introduced by Kujurai et al. (1993) for experiment 4.

Experimental Procedures

Each experiment was conducted in a repeated-measures design with the order of experimental sessions randomized between subjects. The volunteers were seated in a comfortable chair with a high back against which they could lean their heads. The left motor-cortical representational field of the right FDI was identified using TMS (the coil position that led to the largest MEPs of FDI). For all experiments, the intensity of the stimulator output was adjusted for baseline recordings so that the average stimulus led to an MEP of ~1 mV in 30 baseline sweeps. The motor cortical tDCS electrode was fixed on the FDI hot spot by means of a broad rubber band put around the head. The other electrode on the contralateral forehead, just above the orbit, was attached respectively. Both electrodes were covered by a thin wet sponge to improve conductance and to minimize uncomfortable (e.g., itching) sensations during stimulation. The current was ramped at the beginning and the end of the stimulation for 4 s. Nevertheless, some subjects felt a slight scalp itching sensation under the electrode contact points during current flow. The course of experiments 1–3 is depicted in Fig. 1. Throughout the experiments, as well as the intervals between DC stimulation, participants were resting and told to stay relaxed, awake, keep attention at the same level, and not think about things of major personal importance.

Experiment 1. The experiment consisted of four sessions with anodal (experiment 1a) or cathodal tDCS (experiment 1b), respectively, per subject group. First, baseline corticospinal excitability was measured by recording 30 TMS stimuli at 0.25 Hz with a stimulator output intensity that on average elicited MEP amplitudes of 1 mV. Afterward, tDCS was performed as follows: 1) 5-min single stimulation (5-0-0 min); 2) 5-min repeated stimulation, 30-min inter-tDCS break (5-30-5 min); 3) 5-min repeated stimulation, 3-min inter-tDCS break (5-3-5 min); and 4) 5-min repeated stimulation, 0-min inter-tDCS break (5-0-5 min).

It is known that 5 min of tDCS (condition 1 above) results in polarity-specific aftereffects lasting until approximately minute 5 after the end of stimulation (Nitsche and Paulus 2000). In condition 2, the second tDCS was performed well beyond the point where the aftereffects of the first tDCS session were expected to have vanished. In condition 3, an interval of 3 min between tDCS sessions was chosen. Thus, in this condition, we chose an inter-tDCS interval in which the aftereffects of the first tDCS are known to still be present when the second stimulation is performed. In condition 4, there was no break between the two 5-min sessions.

After DC stimulation was finished, MEPs at 0.25–Hz frequency were recorded continuously until minute 5 after tDCS, with baseline TMS intensity. From minute 10 up to minute 30, further 30 MEPs were measured at 0.25 Hz every 5th minute. Furthermore, 30 MEPs were recorded at minutes 60 and 90 after the end of tDCS.

Experiment 2. In this experiment, we aimed to explore the time course of homeostatic effects elicited by anodal tDCS to a larger extent. The course of the experiment was identical to that of experiment 1a with the exception that the tDCS repetition intervals
differed. tDCS was performed as follows: 1) 5-min single stimulation (5-0-0 min); 2) 5-min repeated stimulation, 20-min inter-tDCS break (5-20-5 min); 3) 5-min repeated stimulation, 10-min inter-tDCS break (5-10-5 min); and 4) 5-min repeated stimulation, 1-min inter-tDCS break (5-1-5 min).

Experiment 3. In this experiment, we aimed to explore whether the time course and duration of the aftereffects induced by repeated anodal tDCS depend on the duration of the first tDCS application, at least over the range of short-lasting aftereffects produced by short periods of tDCS. The design of the experiment was identical to that of experiment 1a

Experiment 4. For experiment 4, we applied the paired-pulse stimulation technique described by Kujirai et al. (1993) to explore homeostatic plasticity-related changes of intracortical inhibition and facilitation. Single test pulse TMS intensity was adjusted to achieve MEPs of 1 mV peak-to-peak amplitude. The conditioning pulse intensity was 70% of active motor threshold (AMT). AMT was defined as the minimum TMS intensity eliciting a MEP of a superior size compared with spontaneous moderate muscular activity (15% of maximum strength) in at least three of six trials and was obtained about 10 min before tDCS. This relatively weak intensity was chosen to prevent ceiling or floor effects of the double stimulation protocol and is sufficient to monitor tDCS-driven changes of intracortical facilitation and inhibition (Nitsche et al. 2005). The paired-pulse stimulation protocol included interstimulus intervals (ISI) of 2, 3, 7, 10, and 15 ms, with the first two ISIs representing inhibitory and the last two ISIs facilitatory intervals. The pairs of stimuli were organized in blocks in which each paired stimulation interval and an additional...
single test pulse were presented once. The blocks were repeated 12 times for each time bin of measurement (see below), and the order of the different pulses was pseudorandomized between blocks.

Because of the findings in experiments 1a and 1b, anodal tDCS was performed only for the two following protocols: 1) 5-min single stimulation (5-0-0 min); and 2) 5-min repeated stimulation, 3-min inter-tDCS pause (5-3-5 min). Twelve paired pulse blocks were obtained before as well as directly after and 20 min after tDCS. Because we aimed to test intracortical excitability directly after tDCS application, time restrictions meant we were not able to adjust test pulse intensity and AMT to post-tDCS conditions. However, according to the results of Nitsche et al. (2005), no significant change of AMT was expected.

Calculations and Statistics

**Experiment 1.** MEP amplitude means were calculated for each time bin covering baseline (Supplemental Table S1, available in the data supplement online at the Journal of Neurophysiology web site) and poststimulation time points. The post-tDCS MEPs were normalized intraindividually and are given as ratios of the pre-current baselines. A three-way repeated-measures ANOVA for all data with tDCS polarity as a between-subject factor (due to different subject groups in experiments 1a and 1b) and tDCS-protocol (5-0-0, 5-30-5, 5-3-5, and 5-0-5 min) and time course (1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 60, and 90 min after tDCS) as within-subject factors was calculated. A P value <0.05 was considered significant. Additional Student’s t-tests (paired samples, 2-tailed, level of significance <0.05) were performed to test whether the baseline MEP amplitudes differed significantly between tDCS conditions, whether the MEP amplitudes after tDCS differed significantly from the pre-tDCS amplitudes, and whether those differences depended on the tDCS ISI for anodal and cathodal stimulation.

**Experiment 2.** MEP amplitude means were calculated for each time bin covering baseline and poststimulation time points. The post-tDCS MEPs were normalized intraindividually and are given as ratios of the pre-current baselines. A two-way repeated-measures ANOVA with tDCS protocol (5-0-0, 5-30-5, 5-3-5, and 5-0-5 min) and time course (1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 60, and 90 min after tDCS) as within-subject factors was calculated. A P value <0.05 was considered significant. Additional Student’s t-tests (paired samples, 2-tailed, level of significance <0.05) were performed to test whether the baseline MEP amplitudes differed significantly between tDCS conditions, whether the MEP amplitudes after tDCS differed significantly from the pre-current amplitudes, and whether those differences depended on the tDCS intervals.

**Experiment 3.** The calculations performed for experiment 3 were identical to those for experiment 2 with the exception that the factor tDCS protocol differed (7-0-0, 7-1-5, 7-3-5, 7-10-5, 7-20-5, and 7-30-5 min). For experiments 1–3, we conducted additional analyses with nonstandardized MEP amplitude values, which are shown in the Supplemental Material (Table S2 and Figs. S1–S3).

**Experiment 4.** Means of the MEP amplitudes were calculated for each ISI and normalized to single test pulse amplitude for each time bin intraindividually. A three-way repeated-measures ANOVA including ISI, time point, and tDCS protocol as within-subject factors and MEP amplitude as a dependent variable was calculated. A P value <0.05 was considered significant. Additional Student’s t-tests (paired samples, 2-tailed, level of significance <0.05) were performed to test whether the paired pulse-elicted MEP amplitudes differed significantly from baseline. In addition, within each protocol we tested whether the results of the stimulation protocols after tDCS differed from the pre-tDCS protocol.

**RESULTS**

**Experiments 1a and 1b**

The results of the ANOVAs showed a significant three-way interaction of tDCS polarity × tDCS protocol × time course (Table 1). This was caused by tDCS polarity-specific effects, which differ for the inter-tDCS intervals, as shown in Fig. 2A for anodal and Fig. 2B for cathodal tDCS.

As expected from previous studies (Nitsche and Paulus 2000, 2001), 5-min (5-0-0 min) anodal stimulation facilitated MEPs for the next 5 min, whereas 10-min continuous stimulation (5-0-5 min) facilitated MEPs for 30 min. Aftereffects of the 5-30-5-min protocol were identical to those of the 5-0-5-min protocol for all time points. After 5-3-5-min conditioning, MEPs were facilitated for 3 min and then became significantly depressed from 10 to 30 min after tDCS. The difference between the 5-0-0-min and the 5-3-5-min protocols was significant at all time points from minute 5 to minute 30. Compared with the 10-min continuous stimulation (5-0-5 min), the MEP amplitude reduction was significant from minute 10 to minute 30 after tDCS.

Cathodal 5-0-0-min tDCS significantly suppressed MEPs for up to 5 min after tDCS, whereas in the 5-0-5-min protocol, MEPs were significantly suppressed for 30 min. Aftereffects of the 5-30-5-min protocol were identical to those of the 5-0-5-min tDCS. In contrast, 5-3-5-min conditioning did not suppress MEPs but resulted in significant facilitation of MEPs from 15 to 30 min after tDCS. The effect was significantly different from 5-0-0-min conditioning at all time points until minute 25. Compared with the 10-min continuous stimulation (i.e., the 5-0-5-min protocol), the difference was significant at all time points after tDCS up to minute 60. Baseline MEP amplitudes of

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**Table 1. Results of ANOVAs**

<table>
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<tr>
<th>Protocol</th>
<th>df</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time course</td>
<td>12</td>
<td>6.645</td>
<td>&lt;0.001*</td>
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<tr>
<td>Polarity</td>
<td>1</td>
<td>58.600</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Polarity × polarity</td>
<td>3</td>
<td>11.579</td>
<td>&lt;0.001*</td>
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<tr>
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<td>22.333</td>
<td>&lt;0.001*</td>
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<tr>
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<td>0.373</td>
</tr>
<tr>
<td>Protocol × time course × polarity</td>
<td>36</td>
<td>3.689</td>
<td>&lt;0.001*</td>
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<td></td>
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<tr>
<td>Protocol</td>
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</tr>
<tr>
<td>Time course</td>
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<tr>
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<td>Protocol</td>
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<td>0.038*</td>
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<tr>
<td>Time course</td>
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<td>0.843</td>
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<tr>
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<td>0.255</td>
<td>0.978</td>
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Data are results of the analyses of variance (ANOVA). In experiment 1, where anodal or cathodal transcranial direct current stimulation (tDCS) was applied for 5 min and repeated after 0 min without a break or after a 3- or 30-min break. In experiment 2, the second session of anodal tDCS followed the first after a 1-, 10-, or 20-min break. Experiment 3 included a slight prolongation of the preconditioning tDCS protocol (7-min stimulation instead of 5 min). In experiment 4, intracortical inhibition and facilitation were obtained before, immediately after, and 20 min after a single session of 5-min tDCS or two sessions of tDCS separated by a 3-min break. df, degrees of freedom; ISI, interstimulus interval.
all tDCS paradigms and polarities were identical (Student’s t-test, \( P > 0.05 \)).

Experiment 2

The ANOVA resulted in a significant main effect for time course. The impact of the different anodal tDCS protocols on MEP amplitudes is depicted in Fig. 3. Similar to experiment 1, 5-min (5-0-0 min) anodal stimulation facilitated MEPs during the first minutes after tDCS. However, the magnitude of the change was smaller, perhaps due to the fact that different individuals were tested. Aftereffects of the 5-20-3-min and the 5-1-5-min protocols did not differ significantly from baseline at any time point. Furthermore, they did not differ significantly from those obtained with the 5-0-0-min protocol. After 5-10-5 min conditioning, MEPs were not facilitated at all but became significantly depressed compared with baseline values for up to 90 min after stimulation. Compared with the 5-3-5-min protocol of experiment 1, this effect evolved without delay and had a longer duration.

Experiment 3

The ANOVA showed a significant main effect of tDCS protocol and a significant interaction of tDCS protocol \( \times \) time course (Table 1). Seven-minute anodal tDCS given alone increased corticospinal excitability for up to 20 min after stimulation. As in experiments 1 and 2, a 10-min break between consecutive tDCS protocols (7-10-5 min) reduced excitability significantly within the first 5 min after tDCS, with a smaller effect persisting to 30 min. In contrast to experiments 1 and 2, a 3-min break between tDCS protocols (7-3-5 min) also reduced excitability within the first 5 min after tDCS, and this persisted for up to 60 min. For the other conditions (7-1-5, 7-20-5, and 7-30-5 min), there was a tendency for the initial increase in excitability induced by tDCS to be reduced or abolished, but this recovered by 10-30 min after tDCS (Fig. 4). Note that due to the design of this experiment, the effects of the 5-min tDCS were compared statistically (in Table 1) with 7-min tDCS given alone, rather than with a single period of 5-min tDCS. The limitations of this are addressed in the Discussion.

Experiment 4

The three-way repeated-measures ANOVA showed significant main effects of the factors time course and ISI (Table 1).
There was no significant main effect or interaction involving tDCS protocols, indicating that the 5-0-0-min and the 5-3-5-min protocols had identical effects on paired-pulse interactions despite their different effects on single-pulse MEP amplitudes. In both protocols, ISIs of 2 and 3 ms reduced MEP amplitude relative to the single test pulse, whereas ISIs of 10 and 15 ms led to slight facilitation before tDCS. Immediately after both tDCS protocols, the amount of inhibition was significantly reduced and facilitation was enhanced relative to the pre-tDCS condition. For all ISIs, MEP amplitudes returned to baseline 20 min after tDCS with regard to both tDCS protocols. Taken together, the data indicate no significant difference at any ISI between the 5-0-0-min and the 5-3-5-min protocols (Fig. 5, A and B).

DISCUSSION

The main result of our study is that the effects of repeated short periods of motor cortex tDCS follow a time-dependent rule compatible with homeostatic mechanisms. As in previous experiments, a single tDCS session of 5-min duration increased excitability when anodal tDCS was applied and decreased it when cathodal tDCS was administered. If a second tDCS session followed the first one during a critical time interval of 3 or 10 min, but not 1, 20, or 30 min, the aftereffects of tDCS were reduced or even reversed. The results of the experiments are compatible with the suggestion that the duration of the first preconditioning DC stimulation has an effect on the time course of these changes. The homeostatic effect of a 3-min break after 5-min tDCS took 5 min to appear, whereas it occurred almost immediately after 7 min of tDCS. Alternatively, it cannot be ruled out that these differences are caused by the different duration of the delay between the start of the preconditioning and the end of the conditioning tDCS, which is longer in the 7-3-5-min condition, compared with the 5-3-5-min condition, due to the longer duration of the preconditioning stimulation. In this case, it might be speculated that the system needs some minutes to develop the respective homeostatic alterations. Interestingly, the homeostatic interactions seem not to be related to changes of intracortical inhibition or facilitation, as measured in the present study.

The time-dependent reversal of after effects is compatible with a homeostatic-like mechanism that regulates the ease and direction with which neuroplasticity can be induced according to the previous history of activity. Similar effects have been demonstrated in human motor cortex for different combinations of plasticity-inducing protocols (Iyer et al. 2003; Lang et al. 2004; Müller et al. 2007; Nitsche et al. 2007a; Siebner et al. 2004), such as repetitive TMS, paired associative stimulation, and paired-pulse TMS.
and combinations of tDCS, rTMS, and PAS. Moreover, homeostatic effects seem to have functional relevance for motor learning (Antal et al. 2008; Jung and Ziemann 2009).

Proposed Mechanisms of Action

There is little information on the physiological mechanisms of homeostatic plasticity effects in humans. Many of the mechanisms explored in animal experiments, such as changes in factors such as tumor necrosis factor (TNF)-α, brain-derived neurotrophic factor (BDNF), transsynaptic signaling molecules, and diverse intracellular pathways (Turrigiano 2008), work on a much longer timescale than that explored in the present study and thus may not be relevant.

Wankerl et al. (2010) recently suggested that L-type voltage-gated Ca\(^{2+}\) channels (L-VGCC) could be involved in short-term homeostatic plasticity studied in humans based on another noninvasive plasticity-inducing brain stimulation protocol, namely, theta-burst stimulation. This might be similar for tDCS, which has also been shown to induce calcium-dependent plasticity (Nitsche et al. 2003). Their proposal was based on two ideas: 1) that the direction [long-term potentiation (LTP)/long-term depression (LTD)] of synaptic plasticity depends on the magnitude and dynamics of different postsynaptic levels of Ca\(^{2+}\) induced by the presynaptic input, with high levels favoring LTP and lower levels LTD; and 2) that the history of activation of a neuron can affect the function of L-VGCC channels such that high preceding levels of activity would reduce their activity, whereas low levels would increase it (Sokolova and Mody 2008; Trasande and Ramirez 2007). In the present context, this would account for the reversal of the effects of 5-min anodal tDCS in the 5-10-5-min protocol. Thus the increase in neural activity produced by the initial 5-min conditioning might have reduced the effectiveness of L-VGCCs such that when the second 5-min stimulation was applied, levels of Ca\(^{2+}\) entering postsynaptic cells would have been less than normal and, rather than causing an LTP-like effect (high levels of Ca\(^{2+}\) influx), would instead have resulted in LTD. If this were correct, then the present results would imply that the effects on L-VGCCs take some minutes to build up, since immediate reversal only occurred after a 10-min break between 5-min tDCS sessions and was less clear at 1 min, but for the 7-min preconditioning protocol, reversal was complete after only a 3-min break immediately after conditioning tDCS. The theory may also be able to account for the slow reversal of aftereffects in the anodal and cathodal 5-3-5-min protocols. It might be that activity-dependent effects on L-VGCCs vary between different neurons in the population affected by tDCS. In the anodal case, we could imagine that after 3 min, L-VGCC in some neurons had been inactivated, whereas this was not complete in others. The latter population might produce a dominant short-lasting facilitatory aftereffect on corticospinal excitability, whereas the former could be responsible for the later suppression. For cathodal stimulation, the immediate alteration of the aftereffects could be caused by a slight enhancement of the activation of L-VGCC by both stimulation protocols, which together might enhance intracellular Ca\(^{2+}\) level to a degree that induces neuroplastic excitability enhancement, and not reduction. Since this additive effect would need no secondary mechanism, it is plausible that it would evolve immediately after the end of stimulation. Other mechanisms might also contribute to different temporal dynamics in the reduction/reversal of facilitatory and inhibitory plasticity mechanisms, which might in the present case be more quickly activated for inhibitory plasticity.

Although an attractive potential mechanism of action, this concept is hypothetical at present. Future experiments should explore the presumed physiological mechanisms more directly. In a first approach, we studied intracortical inhibition and facilitation by a double-pulse TMS protocol. We found no specific effect of the repeated anodal tDCS protocol on intracortical facilitation, which primarily probes the excitability of intracortical glutamatergic neurons (Paulus et al. 2008). On first sight, this contrasts with the proposed mechanism of action introduced above. However, it might be argued that a different population of glutamatergic neurons is important for homeostatic effects from that explored using the double-stimulation TMS protocol. Since TMS affects cortical neurons in a direction-specific manner, it most probably affects only subgroups of neurons. Moreover, the interpretability of the intracortical measures might be somewhat limited due to the fact that we were not able to adjust test pulse amplitude after tDCS because of temporal restrictions. Although intracortical inhibition and facilitation were not differentially affected by different sizes of single-pulse MEP amplitudes 20 min after tDCS, it cannot be excluded that single test pulse MEP amplitude had an impact on the results. On the other hand, a dissociation of the effects of TMS protocols on different parameters of cortical excitability has also been described in other studies (Huang et al. 2005). For exploring the suggested dependency of the effects on specific intracellular \(\text{Ca}^{2+}\) concentrations directly, animal experiments would be needed.

General Remarks

The results of the present study differ clearly from those of another tDCS study, which involved repeated cathodal tDCS with longer lasting stimulation protocols (9 min) that reduce excitability for up to 1 h. Repeated application of these protocols within 3–20 min led to prolongation of the inhibitory effect, rather than reversal, whereas ISIs of 3 and 24 h induced homeostatic effects (Monte-Silva et al. 2010). We suggest that these longer durations of tDCS allow time for other processes to develop, involving factors such as BDNF, TNF-α, and others (for an overview see Turrigiano 2008) that replace the much shorter lasting effects on VGCCs.

Some limitations of the present study should be taken into account. Ideally, all experiments should have been conducted in the same group of subjects, especially given the relatively large interindividual variability of short-lasting aftereffects of tDCS (Nitsche and Paulus 2001). Indeed, it is noteworthy that the absolute magnitude and duration (but not the overall direction) of the aftereffects of a single administration of 5-min anodal stimulation differ between the experiments. Age differs to some extent between the subexperiments and might be a possible reason for the variability, although in all subject groups the mean age was between 24 and 33 yr and would not be expected to have a major effect on plasticity. Another source of variability might have been that attention and cognitive activities during the experiments differed between subject groups. Although we tried to control for this by instructing the participants to stay awake, keep the same level of attention,
and avoid thinking about personally important things during the experiments, interindividual differences might have been present. In our opinion this would have resulted in an underestimation of the effects but not in qualitative differences, because the general direction of the effects did not differ between groups. The variation in baseline effects between subject groups should be taken into consideration, particularly when comparing the results of experiments 1 and 2, which explore how aftereffects of stimulation depend on the inter-tDCS interval. Thus we cannot exclude the possibility in experiment 2 that the lack of effect in the 5-1.5-min condition was due to the relatively low efficacy of the initial 5-min tDCS. It should also be noted that the presence of the aftereffects of the preconditioning tDCS during the application of the second DC stimulation is indirect and has to be derived from the single-tDCS stimulation conditions, because we did not measure MEP amplitudes during the break between stimulations. We chose this procedure because we wanted to avoid the theoretical possibility that TMS during the break affects cortical excitability. In experiment 3, we compared the repeated stimulation protocols with the 7-0-0-min stimulation protocol. A 5-0-0-min protocol was not available in this group. Although this might have an impact on the results, we think that this impact should be minor, because it has been shown that the aftereffect duration differs by only a few minutes between 5- and 7-min tDCS (Nitsche and Paulus 2001). Moreover, taking into account the distinction between short- and long-term plasticity, aftereffects lasting less than 30 min, as seen after 5- and 7-min tDCS in the present studies, are in the time range of short-term plasticity, whereas those lasting longer than 30 min are classified as long-lasting changes. Therefore, 5- and 7-min tDCS are likely to induce qualitatively similar effects. Furthermore, although in the present study homeostatic effects were induced by repeated stimulation with specific break durations, this does not mean that a break between two stimulation protocols is always necessary to induce homeostatic-like effects. Gentner et al. (2007) have demonstrated a reversal of the aftereffects of continuous theta-burst stimulation in the human motor cortex by prolongation of the stimulation duration without any break. Finally, it could be argued that the second electrode, which was positioned over the contralateral frontopolar cortex, might have affected motor cortex excitability. This is improbable, however, since both areas are not anatomically connected, and it has been shown that different sizes of the frontopolar electrode, which result in functionally effective or ineffective stimulation of this cortical area, do not affect the impact of tDCS on motor cortex excitability (Nitsche et al. 2007b).

**Conclusion**

Recently, it was suggested that disorders of homeostatic plasticity may underlie some diseases of the central nervous system. Thus patients with writer’s cramp, a form of focal dystonia, failed to show the normal homeostatic response pattern in a clinical study using the protocol of Siebner et al. (2004) (Quartarone et al. 2005). Noninvasive, painless methods like TMS and tDCS could turn out to be a promising tool in exploring the contribution of pathologically altered plasticity to diseases of the central nervous system, which display altered cortical excitability and activity.

Finally, the results of our study are important because a growing number of studies are beginning to explore the possibility of treating clinical symptoms with stimulation techniques in diseases known to have pathological altered cortical excitability. For example, in stroke patients, rTMS has been found to be a useful possible tool in neurorehabilitation (Khodr et al. 2005; Mansur et al. 2005). Recent studies with tDCS on patients suffering from stroke, epilepsy, depression, and central pain (Hummel et al. 2005; Fregni et al. 2005, 2006a,b,c) as well as models of cortical spreading depression (Liebetanz et al. 2006), point to the possibility of influencing the course of the disease by applying tDCS. As we have shown, the effect of a technique that influences plastic mechanisms might, at least under certain circumstances, depend not only on the excitability of the motor cortex at the time the stimulation is applied but also on its recent history, although this might not be the case for all kinds of plasticity and might not necessarily translate into respective behavioral or cognitive effects (Antal et al. 2008; Jung et al. 2009). This should be taken into account when techniques such as TMS and tDCS are used in neurorehabilitation and treatment of diseases of the nervous system.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


