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Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans

G. Batsikadze[1], V. Moliadze[1,2], W. Paulus[1], M.-F. Kuo[1] and M. A. Nitsche[1]

[1] *Department of Clinical Neurophysiology, University of Göttingen, Robert-Koch-Straße 40, 37075 Göttingen, Germany*
[2] *Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Goethe-University of Frankfurt am Main, Deutschordenstraße 50, D-60528 Frankfurt am Main, Germany*

Key points

- Application of 2 mA cathodal transcranial direct current stimulation for 20 min results in cortical excitability enhancement instead of inhibition.
- Longer or more intensive stimulation does not necessarily increase its efficacy.
- Short intracortical inhibition and facilitation are shifted towards excitability enhancement after both 2 mA anodal and cathodal stimulation.
- I-waves, input–output curves and cortical silent period are unaffected immediately after 2 mA stimulation.

Abstract Transcranial direct current stimulation (tDCS) of the human motor cortex at an intensity of 1 mA with an electrode size of 35 cm^2 has been shown to induce shifts of cortical excitability during and after stimulation. These shifts are polarity-specific with cathodal tDCS resulting in a decrease and anodal stimulation in an increase of cortical excitability. In clinical and cognitive studies, stronger stimulation intensities are used frequently, but their physiological effects on cortical excitability have not yet been explored. Therefore, here we aimed to explore the effects of 2 mA tDCS on cortical excitability. We applied 2 mA anodal or cathodal tDCS for 20 min on the left primary motor cortex of 14 healthy subjects. Cathodal tDCS at 1 mA and sham tDCS for 20 min was administered as control session in nine and eight healthy subjects, respectively. Motor cortical excitability was monitored by transcranial magnetic stimulation (TMS)-elicited motor-evoked potentials (MEPs) from the right first dorsal interosseous muscle. Global corticospinal excitability was explored via single TMS pulse-elicited MEP amplitudes, and motor thresholds. Intracortical effects of stimulation were obtained by cortical silent period (CSP), short latency intracortical inhibition (SICI) and facilitation (ICF), and I wave facilitation. The above-mentioned protocols were recorded both before and immediately after tDCS in randomized order. Additionally, single-pulse MEPs, motor thresholds, SICI and ICF were recorded every 30 min up to 2 h after stimulation end, evening of the same day, next morning, next noon and next evening. Anodal as well as cathodal tDCS at 2 mA resulted in a significant increase of MEP amplitudes, whereas 1 mA cathodal tDCS decreased corticospinal excitability. A significant shift of SICI and ICF towards excitability enhancement after both 2 mA cathodal and anodal tDCS was observed. At 1 mA, cathodal tDCS reduced single-pulse TMS-elicited MEP amplitudes and shifted SICI and ICF towards inhibition. No significant changes were observed in the other protocols. Sham tDCS did not induce significant MEP alterations. These results suggest that an enhancement of tDCS intensity does not necessarily increase efficacy of stimulation, but might also shift the

M.-F. Kuo and M. A. Nitsche contributed equally to this work.

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direction of excitability alterations. This should be taken into account for applications of the stimulation technique using different intensities and durations in order to achieve stronger or longer lasting after-effects.

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Corresponding author M. A. Nitsche: Department of Clinical Neurophysiology, University of Göttingen, Robert-Koch-Straße 40, 37075 Göttingen, Germany. Email: mnitsch1@gwdg.de

Abbreviations AMT, active motor threshold; CSP, cortical silent period; FDI, first dorsal interosseus; I–O, input–output; I-wave, indirect wave; ICF, intracortical facilitation; LTD, long-term depression; LTP, long-term potentiation; MEP, motor-evoked potential; MT, motor threshold; RMT, resting motor threshold; SICI, short latency intracortical inhibition; tACS, transcranial alternating current stimulation; TBS, theta burst stimulation; tDCS, transcranial direct current stimulation; TMS, transcranial magnetic stimulation; tRNS, transcranial random noise stimulation.

Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that is able to induce polarity-dependent shifts of cortical excitability, which can last for approximately up to a few hours after stimulation with conventional protocols. Anodal tDCS depolarizes cortical neurons and increases their excitability, whereas cathodal tDCS is presumed to hyperpolarize neuronal membranes and decrease neuronal excitability. Pharmacological studies have shown that the long-lasting after-effects involve *N*-methyl-D-aspartate (NMDA) receptors and the GABAergic system (Liebetanz *et al.* 2002; Nitsche *et al.* 2003*a*, 2004*b*). The duration and strength of tDCS after-effects depend on duration and intensity of the applied current. The interdependency between these factors has been shown to be linear for a current strength of up to 1 mA (electrode size 35 cm^2) and a stimulation duration of up to 13 min (Nitsche & Paulus, 2000, 2001; Nitsche *et al.* 2003*b*).

In recent years tDCS has been increasingly used in functional studies in healthy humans, as well as clinical applications in patients suffering from neuropsychiatric diseases (Nitsche *et al.* 2008; Nitsche & Paulus, 2011). In these studies, stimulation duration and intensity has often been increased above the routine stimulation parameters based on an implicit assumption that longer stimulation duration or higher intensities will enhance efficacy of stimulation. Although these more intensive protocols have been shown to be effective in numerous studies (Fregni *et al.* 2006*a*; Ferrucci *et al.* 2009; Brunoni *et al.* 2011; Bueno *et al.* 2011), knowledge about their physiological effects is limited.

As non-linear effects of stimulation parameters on alterations of cortical excitability were demonstrated recently for other non-invasive brain stimulation protocols, such as theta burst stimulation (TBS), transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS) (Doeltgen & Ridding, 2010; Gamboa *et al.* 2010; Moliadze *et al.* 2012), here we aimed to explore if increased intensity and prolongation of tDCS results in enhanced efficacy of stimulation with regard to polarity-dependent excitability alterations. We therefore administered 2 mA cathodal and anodal tDCS for 20 min to the primary motor cortex of healthy subjects, which is a frequently used stimulation protocol in cognitive and clinical studies (Iyer *et al.* 2005; Fregni *et al.* 2006*b*; Ferrucci *et al.* 2009; Brunoni *et al.* 2011; Ladeira *et al.* 2011). We explored the impact of these stimulation protocols on various parameters of corticospinal and intracortical excitability. The global change of corticospinal excitability in the motor cortex was measured by motor evoked potentials (MEPs) elicited by single-pulse transcranial magnetic stimulation (TMS), active and resting motor thresholds (MTs) and input–output (I–O) curves (Chen, 2000; Abbruzzese & Trompetto, 2002). Short latency intracortical inhibition (SICI) and facilitation (ICF) of motor cortex were explored by a paired-pulse TMS stimulation protocol, where a subthreshold conditioning stimulus is followed by a suprathreshold test pulse. The resulting increase or decrease of the MEP amplitude elicited by the test stimulus is determined by the respective interstimulus interval (ISI) (Kujirai *et al.* 1993). To monitor indirect waves (I-waves) generated by motor cortex stimulation as a parameter of the interaction between corticocortical circuits, another paired-pulse TMS protocol was used. Here a suprathreshold TMS test pulse was followed by a subthreshold one (Ziemann *et al.* 1998; Ziemann & Rothwell, 2000). The resulting change of MEP amplitude is specific for certain ISIs, reflecting cortical interactions between the interneuronal circuits. To study changes of cortical inhibition, furthermore the cortical silent period (CSP) was obtained (Fuhr *et al.* 1991; Bertasi *et al.* 2000; Romeo *et al.* 2000). Thus, ICF is determined by the glutamatergic system, whereas CSP and I-wave facilitation depend primarily on GABA (Paulus *et al.* 2008).

For 1 mA stimulation (stimulation duration 13 min anodal, 9 min cathodal tDCS), anodal DC stimulation enhanced single-pulse MEP amplitudes, slope of the I–O curve, intracortical facilitation and I-wave facilitation,

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Table 1. Subject characteristics

Experimental session	Subjects *n*	Sex (M/F)	Age	RMT (%)*	AMT (%)*	SI_{1mv} (%)*	Baseline MEP amplitude (mV)
Experiment 1							
2 mA anodal	14	9 F/5 M	25.8 ± 3.7	40.1 ± 8.1	31.4 ± 7.2	49.2 ± 9.8	0.96 ± 0.13
2 mA cathodal	14	9 F/5 M	25.8 ± 3.7	40.1 ± 7.4	32.7 ± 7.6	49.4 ± 8.7	0.99 ± 0.07
Experiment 2							
1 mA cathodal	9	6 F/3 M	26 ± 4.5	43.6 ± 8.5	33.3 ± 7.8	53.1 ± 9.5	1.005 ± 0.15
Experiment 3							
Sham	8	6 F/2 M	26.9 ± 2.6	—	32.1 ± 9.4	51.6 ± 12.7	0.93 ± 0.03

Data are presented as mean ± SD; *n* = number of participants; F = female; M = male; RMT = resting motor threshold; AMT = active motor threshold; SI_{1mv} = TMS intensity adjusted to elicit ∼1 mV peak-to-peak amplitude of motor evoked potentials (MEPs).
*Percentage of maximum stimulator output.

while cathodal tDCS had grossly antagonistic effects in previous studies (Nitsche *et al.* 2005). Because 2 mA cathodal tDCS applied for 20 min resulted in excitability-enhancing effects, we added two control experiments with 1 mA and sham stimulation for the same duration to explore the dependency of this effect from stimulation intensity, and rule out any unspecific effects depending on the time course of the study or tDCS-related arousal.

Methods

Subjects

Twenty-one healthy subjects aged 26.28 ± 3.4 years (7 males/14 females) (for details see Table 1) were recruited. All subjects were right-handed according to the Edinburgh handedness inventory (Oldfield, 1971). None of them took any medication, or had a history of neurological diseases, pregnancy or metallic head implants. They all gave written informed consent and were compensated for participation. Subjects were blinded for stimulation conditions. The investigation was approved by the Ethics Committee of the University of Göttingen, and conforms to the principles laid down in the Declaration of Helsinki.

tDCS

Direct current was applied through a pair of saline-soaked surface sponge electrodes (100 and 35 cm^2) and delivered by a battery-driven constant current stimulator (neuroConn GmbH, Ilmenau, Germany). The motor cortex electrode (35 cm^2) was fixed over the area representing the right first dorsal interosseus (FDI) muscle as identified by TMS, and the other electrode (100 cm^2) was placed contralaterally above the right orbit. tDCS was applied for 20 min, with current ramped up and down to and from 2 mA or 1 mA over 8 s. The intensities correspond to current densities of 0.057 mA cm^{-2} (2 mA/35 cm^2) and 0.029 mA cm^{-2} (1 mA/35 cm^2) under the active electrodes and 0.02 mA cm^{-2} (2 mA/100 cm^2) and 0.01 mA cm^{-2} (1 mA/100 cm^2) under the reference electrodes for 2 and 1 mA conditions, respectively. During sham stimulation, the current was ramped up for 20 s, followed by 30 s of 2 mA stimulation, and then it was ramped down for 10 s. The polarity for sham stimulation was randomized (Ambrus *et al.* 2012). Twenty minutes after the beginning of sham tDCS, the stimulation electrodes were removed and TMS measurements were taken. The minimum period between sessions for a single subject was 7 days, and sessions were applied in randomized order.

Monitoring of motor cortical excitability

MEPs were induced in the right FDI by single-pulse TMS over the left primary motor cortex, conducted by a Magstim 200 magnetic stimulator (Magstim, Whiteland, Dyfed, UK) with a figure-of-eight magnetic coil (diameter of one winding, 70 mm; peak magnetic field, 2.2 T). For the paired-pulse TMS protocols, the coil was connected to two Magstim 200 stimulators via a bistim module. The coil was held tangentially to the skull, with the handle pointing backwards and laterally at 45° from the midline. The optimal coil placement (hotspot) was defined as the site where TMS resulted consistently in the largest MEPs of the contralateral FDI. Surface MEPs were recorded from the right FDI with Ag-AgCl electrodes in a belly-tendon montage. The signals were amplified, and band-pass filtered (2 Hz to 2 kHz; sampling rate, 5 kHz). Signals were digitized with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK), controlled by Signal Software (Cambridge Electronic Design, v. 2.13) and stored for offline analysis. A waterproof pen was used to mark the positions of TMS coil and FDI electrodes to ensure that they were positioned at the same spot during the whole experimental session.

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Motor threshold determination

Resting motor threshold (RMT) was determined as the minimum stimulator output needed to elicit an MEP response of 50–100 μV in the relaxed FDI muscle in at least three of six consecutive trials. The active motor threshold (AMT) was the minimum intensity needed to elicit an MEP response of ~200–300 μV during moderate spontaneous background muscle activity (~15% of the maximum muscle strength) in at least three of six consecutive trials.

Single-pulse MEPs (1 mV)

Single-pulse MEPs were recorded with the TMS intensity adjusted to elicit ~1 mV peak-to-peak amplitude (SI_{1mV}) at baseline. Stimulation intensity was kept constant for the post-stimulation assessment.

Input–output curve

The I–O curve was determined using TMS intensities of 100, 110, 130 and 150% RMT (15 stimuli per block).

Intracortical inhibition and facilitation

Intracortical inhibition and facilitation were obtained by a TMS paired-pulse protocol including ISIs of 2, 3, 5, 10 and 15 ms (Kujirai *et al.* 1993). The first three ISIs represent inhibitory and the last two ISIs facilitatory intervals. The exact interval between the paired pulses was randomized (4 ± 0.4 s). In this protocol a subthreshold conditioning stimulus was applied (determined as 70% of AMT), followed by a second suprathreshold test stimulus. The test stimulus was adjusted to achieve a baseline MEP of ~1 mV and readjusted during the respective stimulation protocols, if needed, to compensate for the effects of tDCS-caused corticospinal excitability changes on test pulse amplitude. The pairs of stimuli were organized in blocks in which each ISI and one test pulse was represented once and were pseudorandomized. These blocks were repeated 15 times. Blocks of MEPs in which the muscle was not relaxed were excluded from the analysis.

I-wave facilitation

I-wave facilitation was measured using a TMS paired-pulse protocol including ISIs of 1.1, 1.3, 1.5, 2.3, 2.5, 2.7, 2.9, 4.1, 4.3 and 4.5 ms (Ziemann *et al.* 1998). In this protocol the TMS test stimulus precedes the conditioning stimulus (determined as 70% of RMT). The test stimulus was adjusted to achieve a baseline MEP of ~1 mV and readjusted during the respective stimulation protocols, if needed, to compensate for the effects of corticospinal excitability changes on test pulse amplitude. The pairs of stimuli were organized in blocks in which each ISI and one test pulse was represented once and were pseudorandomized. These blocks were repeated 15 times. Blocks of MEPs in which the muscle was not relaxed were excluded from the analysis.

Cortical silent period

CSP was measured in the voluntarily contracted (~15% of the maximum muscle strength) FDI muscle. For eliciting CSP, TMS was applied at an intensity of SI_{1mV} and 120% RMT, each for 10 consecutive recordings. Latency and duration of CSP were calculated from the time of the stimulus onset to the reappearance of voluntary muscle activity (Fuhr *et al.* 1991; Bertasi *et al.* 2000; Romeo *et al.* 2000).

Experimental procedures

Experiment 1. The volunteers were seated in a comfortable chair with head and arm rests. First, the hotspot (the coil position that produced the largest MEPs of the right FDI) was identified by TMS. Then the stimulation intensity was adjusted to elicit single-pulse MEPs with peak-to-peak amplitudes of an average of 1 mV and 20 MEPs were recorded. After determination of SI_{1mv}, RMT and AMT were obtained. After measuring AMT, a 15 min break followed to avoid an effect of muscle contraction on the next measurements. After this break the following parameters were measured: I–O curves, I-waves, intracortical inhibition and facilitation, and CSP. The order of measurement of these parameters was randomized, except of that of CSP, which was obtained always at the end of this block, as it required a consecutive ~20 min break because of long-lasting voluntary muscle contraction. After this break, 2 mA cathodal or anodal tDCS was administered for 20 min and immediately after removal of the tDCS electrodes single-pulse MEPs were recorded, and resting and active MTs were obtained. The other parameters (I–O curves, I-waves, SICI-ICF, CSP) were then measured. For the latter protocols, TMS intensity was readjusted to obtain single test pulse amplitudes of 1 mV, if needed. Further TMS measurements (MEPs at SI_{1mV}, motor thresholds and SICI-ICF only) were conducted every 30 min up to 2 h after the end of tDCS, in the evening of the same day (SE), the next morning at ~09:00 h (NE), next noon at ~12:00 h (NN) and next evening at ~18:00 h (NE) (Fig. 1).

Experiment 2. Due to results of Experiment 1, we decided to conduct a control experiment using the identical study design with 1 mA cathodal tDCS. Nine of 14 subjects from

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Experiment 1 participated in Experiment 2. In this session, no TMS measurements were performed on the second day. Results of this experiment were compared with the results from 2 mA cathodal tDCS of Experiment 1.

Experiment 3. A control experiment was conducted using sham tDCS. Eight subjects were recruited for this session. Single-pulse MEPs, AMTs and SICI-ICF were measured before tDCS, immediately after, and 30 and 60 min after the end of tDCS.

Analysis and statistics

Experiment 1. To compare MTs, the inter-individual means of the TMS intensity at AMT and RMT were calculated for the before and after-stimulation conditions separately. A repeated measures analysis of variance (ANOVA) was performed on the above-mentioned data using AMT/RMT value as the dependent variable, and polarity of stimulation and time course as independent within-subject factors. For significant ANOVA results, for all conditions values before tDCS were compared with those after tDCS using *post hoc* Student's *t* tests (paired samples, two-tailed, $P < 0.05$).

For the single-pulse TMS conditions, the individual means of 20 MEP amplitudes were calculated for all subjects and the after-stimulation mean MEP amplitudes were normalized to the respective mean baseline MEP amplitudes. Grand averages for each time point were then calculated. A repeated-measures ANOVA was performed on the above-mentioned data using MEP amplitude as the dependent variable, and polarity of stimulation and time course as within-subject factors. For I–O curves, TMS intensity served as an additional within-subject factor. For significant ANOVA results, *post hoc* comparisons were performed using Student's *t* tests (paired samples, two-tailed, $P < 0.05$).

For the paired-pulse stimulation protocols, the resulting mean values were normalized to the respective single-pulse condition. First intra-, and then inter-individual means were calculated for each condition. To determine significant changes, repeated measures ANOVAs were performed (ISIs, polarity of stimulation and time course as independent within-subject factors and MEP amplitude as dependent variable) (Table 2). In case of significant results of ANOVA, *post hoc* comparisons were performed using Student's t tests (paired samples, two-tailed, $P < 0.05$) to compare mean MEP amplitudes at time points after plasticity induction *vs.* the respective baseline values for the respective ISIs.

For the CSP protocol, individual means of CSP durations were calculated for all subjects both at the intensity of SI_{1mV} and at 120% RMT and the after-stimulation CSP values were normalized to respective mean baseline CSP durations. A repeated-measures ANOVA was performed on the above-mentioned data using CSP duration as the dependent variable, and polarity of stimulation, TMS

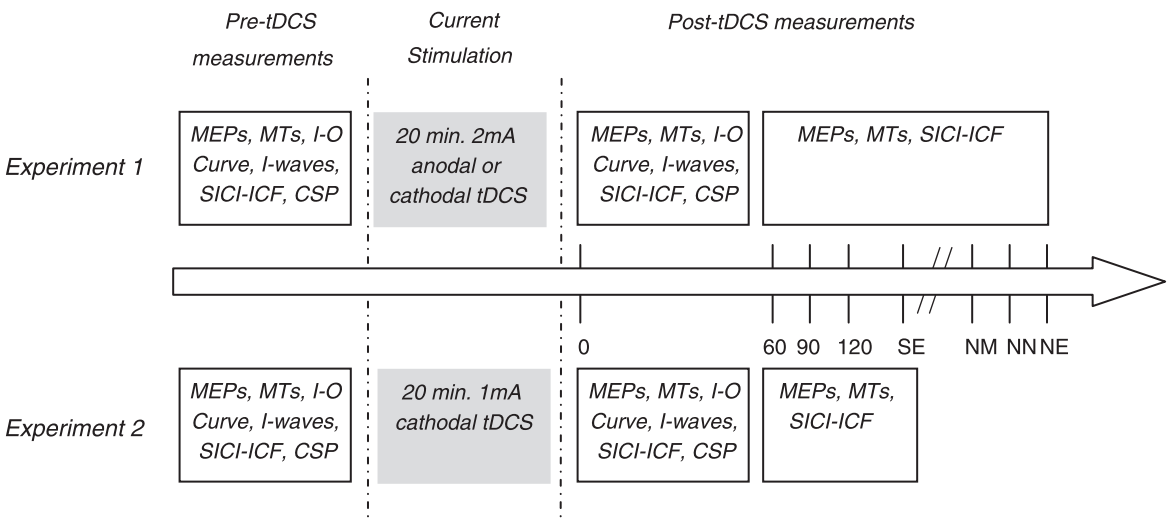

Figure 1. Course of the study
In the beginning of each session, 20 baseline single-pulse MEPs of SI_{1mv} intensity, resting motor threshold (RMT), active motor threshold (AMT), input–output (I–O) curve, I-waves, short-latency intracortical inhibition, intracortical facilitation (SICI-ICF) and cortical silent period (CSP) were recorded. Afterwards, 2 or 1 mA tDCS over 20 min was administered and then the above-mentioned parameters were recorded again. From 60 min after the stimulation, single- and double-pulse TMS parameters were recorded as follows: single-pulse MEPs of SI_{1mv} intensity, RMT, AMT and SICI-ICF 60, 90 and 120 min after the end of tDCS and at the evening on the same day (∼18:00; SE = same evening). For Experiment 1 we also performed these measurements on the next morning (∼9:00; NM), next noon (∼12:00; NN) and next evening (∼18:00; NE).

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Table 2. Repeated-measures ANOVA results for single- and paired-pulse protocols

Measurement	Factor	d.f.	*F*	*P*
Experiment 1				
MEP	TDCS	1	0.155	0.702
	Time	8	5.394	<0.001*
	TDCS × Time	8	1.761	0.096
RMT	TDCS	1	1.792	0.204
	Time	8	0.782	0.620
	TDCS × Time	8	0.971	0.463
AMT	TDCS	1	0.001	0.975
	Time	8	1.335	0.234
	TDCS × Time	8	0.694	0.696
I–O curve	TDCS	1	1.239	0.286
	Time	1	0.340	0.570
	Intensity	3	52.650	<0.001*
	TDCS × Time	1	0.013	0.909
	TDCS × Intensity	3	1.442	0.245
	TIME × Intensity	3	0.237	0.870
	TDCS × Time × Intensity	3	2.385	0.084
SICI-ICF	TDCS	1	0.378	0.549
	time	8	1.929	0.063
	ISI	4	20.949	<0.001*
	TDCS × Time	8	2.102	0.042*
	TDCS × ISI	4	1.310	0.279
	Time × ISI	32	1.141	0.277
	TDCS × Time × ISI	32	1.005	0.463
I-wave facilitation	TDCS	1	1.911	0.190
	Time	1	0.334	0.573
	ISI	9	17.574	<0.001*
	TDCS × Time	1	0.207	0.657
	TDCS × ISI	9	0.343	0.959
	TIME × ISI	9	0.460	0.899
	TDCS × Time × ISI	9	0.894	0.533
CSP	TDCS	1	0.590	0.456
	Intensity	1	0.115	0.740
	Time	1	0.034	0.856
	TDCS × Intensity	1	0.696	0.419
	TDCS × Time	1	0.590	0.456
	Intensity × Time	1	0.115	0.740
	TDCS × Intensity × Time	1	0.696	0.419
Experiment 2				
MEP	TDCS	1	19.018	**0.003***
	Time	5	1.329	0.275
	TDCS × Time	5	2.657	**0.039***
RMT	TDCS	1	4.659	0.063
	Time	5	1.804	0.134
	TDCS × Time	5	0.904	0.488
AMT	TDCS	1	0.620	0.454
	Time	5	1.894	0.117
	TDCS × Time	5	0.924	0.476

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Table 2. Continued

Measurement	Factor	d.f.	*F*	*P*
I–O curve	TDCS	1	1.356	0.257
	Time	1	1.239	0.298
	Intensity	3	38.440	< **0.001***
	TDCS × Time	1	0.790	0.400
	TDCS × Intensity	3	0.549	0.654
	Time × Intensity	3	1.126	0.358
	TDCS × Time × Intensity	3	0.575	0.637
SICI-ICF	TDCS	1	1.051	0.339
	Time	5	4.106	**0.005***
	ISI	4	9.853	< **0.001***
	TDCS × Time	5	0.981	0.443
	TDCS × ISI	4	0.502	0.735
	Time × ISI	20	0.787	0.726
	TDCS × Time × ISI	20	1.273	0.207
I-wave facilitation	TDCS	1	0.895	0.372
	Time	1	2.200	0.176
	ISI	9	20.922	< **0.001***
	TDCS × Time	1	0.014	0.909
	TDCS × ISI	9	1.115	0.364
	Time × ISI	9	1.347	0.229
	TDCS × Time × ISI	9	0.691	0.715
CSP	TDCS	1	3.679	0.091
	Intensity	1	1.561	0.247
	Time	1	0.360	0.565
	TDCS × Intensity	1	3.596	0.094
	TDCS × Time	1	3.679	0.091
	Intensity × Time	1	1.561	0.247
	TDCS × Intensity × Time	1	3.596	0.094
Experiment 3				
MEP	Time	3	0.142	0.934
AMT	Time	3	0.237	0.870
SICI-ICF	Time	3	0.123	0.945
	ISI	4	3.225	**0.027***
	Time × ISI	12	1.358	0.203

MEP = motor-evoked potential; RMT = resting motor threshold; AMT = active motor threshold; SICI-ICF = short-latency intracortical inhibition and intracortical facilitation; CSP = cortical silent period.
*$P < 0.05$.

intensity and time course as independent within-subject factors.

To exclude differences between baseline values of different tDCS conditions, for both single- and double-pulse protocols, we compared the respective values using Student's *t* tests. The Mauchly test of sphericity was performed and the Greenhouse–Geisser correction was applied when necessary.

Experiment 2. For Experiment 2, calculations were identical to those of Experiment 1, the only exception being that stimulation intensity was used as independent within-subject factor instead of polarity of stimulation.

Experiment 3. For Experiment 3, calculations were identical to those of Experiment 1, the only exception being that stimulation polarity was not used as an independent within-subject factor.

Results

Subjects reported similar itchy sensations at the skin during both 2 mA cathodal and anodal trials, but these sensations were weaker during 1 mA cathodal tDCS. Baseline values of MEPs, MTs and CSPs did not differ significantly between stimulation conditions.

Experiment 1

Motor thresholds. Baseline RMT was 40.1 ± 8.1% (all values are reported as means ± standard error of the mean

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(SEM)) of maximum stimulator output for 2 mA cathodal and 40.1 ± 7.4% for 2 mA anodal stimulation; AMT was 31.4 ± 7.2 and 32.8 ± 7.6%, respectively. Baseline values did not differ between stimulation conditions. For the after-tDCS conditions, the ANOVA results were not significant (results of respective ANOVAs of Experiment 1 and 2 are shown in Table 2).

Single-pulse MEPs (1 mV). Baseline MEP values were 0.96 ± 0.13 mV for 2 mA anodal and 0.99 ± 0.07 for 2 mA cathodal stimulation obtained by 49.4 ± 8.7 and 49.2 ± 9.8% of maximum stimulator output, respectively. Baseline values did not differ between stimulation conditions. ANOVA revealed a significant main effect of time after stimulation ($F_8 = 5.378$, $P < 0.001$). The results of the *post hoc* tests showed a significant increase of MEP amplitudes at 60 and 90 min after 2 mA anodal and 90 and 120 min after 2 mA cathodal stimulation ($P < 0.05$) (Fig. 2, for the results obtained for non-standardized MEP amplitudes, see supplementary Fig. S1 and Table S1).

Input–output curve. The slope of the I–O curve was not changed by either cathodal or anodal 2 mA stimulation. ANOVA showed a significant effect of TMS Intensity ($F_3 = 52.650$, $P < 0.001$), but no significant interaction between tDCS, Time and TMS Intensity. Baseline values did not differ between stimulation conditions.

Intracortical inhibition and facilitation

ANOVA showed significant effects of ISI ($F_4 = 20.929$, $P < 0.001$) and tDCS × Time ($F_8 = 2.102$, $P = 0.042$). *Post hoc* Student's *t* tests (paired, two-tailed, $P < 0.05$) show that both 2 mA cathodal and anodal stimulation shifted cortical excitability towards an enhancement of excitability. At 2 mA, anodal tDCS increased facilitation for an ISI of 10 ms immediately after stimulation and decreased inhibition for an ISI of 5 ms both immediately, and 60 and 90 min after stimulation. A similar increase of facilitation for an ISI of 10 ms and decrease of inhibition for an ISI of 5 ms was observed 90 and 120 min after 2 mA cathodal stimulation (Fig. 3*A* and *B*). Baseline values did not differ between stimulation conditions.

I-wave facilitation. ANOVA revealed a significant main effect of ISI ($F_9 = 17.574$, $P < 0.001$), but no significant interaction between tDCS, Time and ISI. Both 2 mA anodal and cathodal stimulations resulted in no change of the respective I-wave peaks. Baseline values did not differ between stimulation conditions.

Cortical silent period. Average baseline CSP durations were 0.136 ± 0.027 and 0.141 ± 0.032 s for 2 mA anodal, and 0.14 ± 0.025 and 0.147 ± 0.031 s for 2 mA cathodal

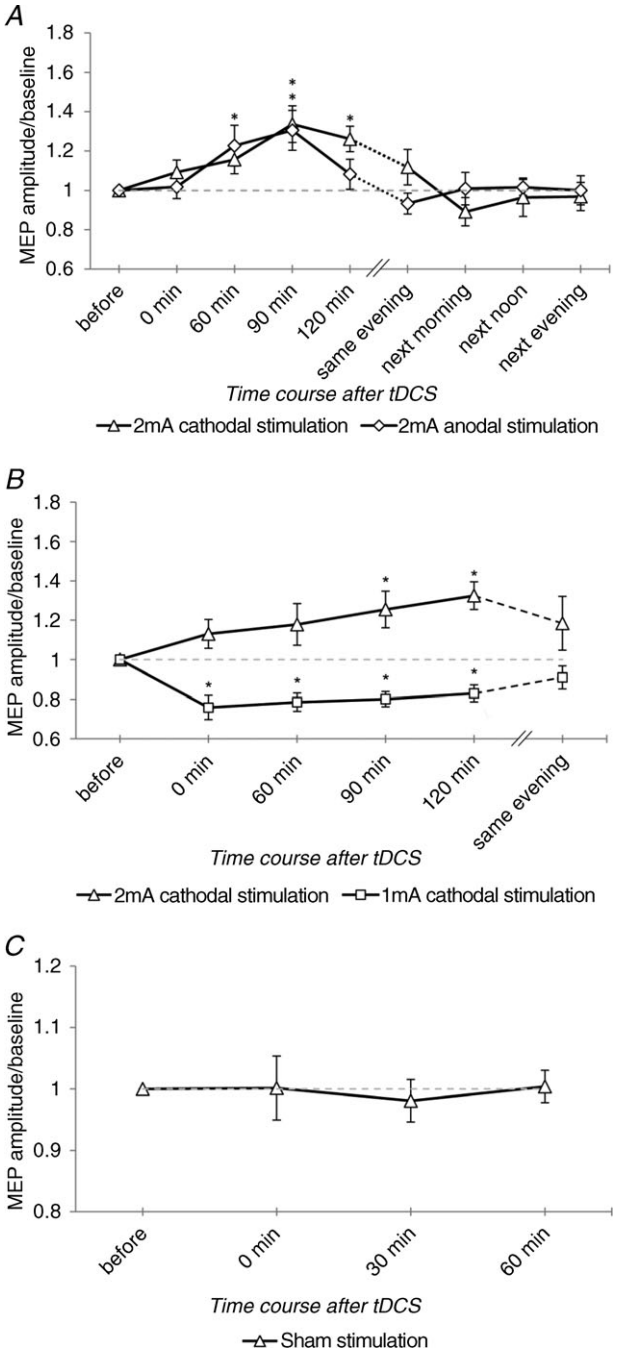

Figure 2. After-effects of anodal and cathodal tDCS on single-pulse MEP amplitudes
A–C, after-effects of (*A*) 2 mA anodal and 2 mA cathodal tDCS (number of participants = 14), (*B*) 2 mA cathodal and 1 mA cathodal tDCS (number of participants = 9) and (*C*) sham tDCS (number of participants = 8) on the single-pulse MEP amplitudes (means ± SEM) at the TMS intensity which elicited 1 mV MEP amplitudes at baseline. Asterisks indicate significant differences of MEP amplitudes from baseline values ($P < 0.05$). Anodal stimulation at 2 mA shows a significant increase of MEP amplitudes 60 and 90 min after stimulation, compared with 2 mA cathodal stimulation 90 and 120 min after tDCS. Cathodal stimulation at 1 mA shows a significant decrease in MEP amplitudes at 0–120 min after stimulation. Sham tDCS did not induce any significant changes.

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stimulation for 120% RMT and SI_{1mV} TMS intensities, respectively. ANOVA showed no significant change in CSP duration and also no interaction between TDCS, Intensity and Time. Baseline values did not differ between stimulation conditions.

Experiment 2

Motor thresholds. Baseline MTs in this experiment did not differ significantly from the respective values of Experiment 1. RMT was $43.6 \pm 8.5\%$ and AMT was $33.3 \pm 7.8\%$ of maximum stimulator output. ANOVA for the 2 and 1 mA cathodal stimulation conditions was not significant.

Single-pulse MEPs (1 mV). The average baseline MEP value was 1.005 ± 0.15 mV obtained by $53.1 \pm 9.5\%$ of maximum stimulator output. MEP amplitude and stimulation intensity did not differ significantly from that of Experiment 1. ANOVA for the 2 and 1 mA cathodal stimulation conditions revealed a significant main effect of tDCS ($F_1 = 23.691$, $P < 0.001$) and tDCS × TIME

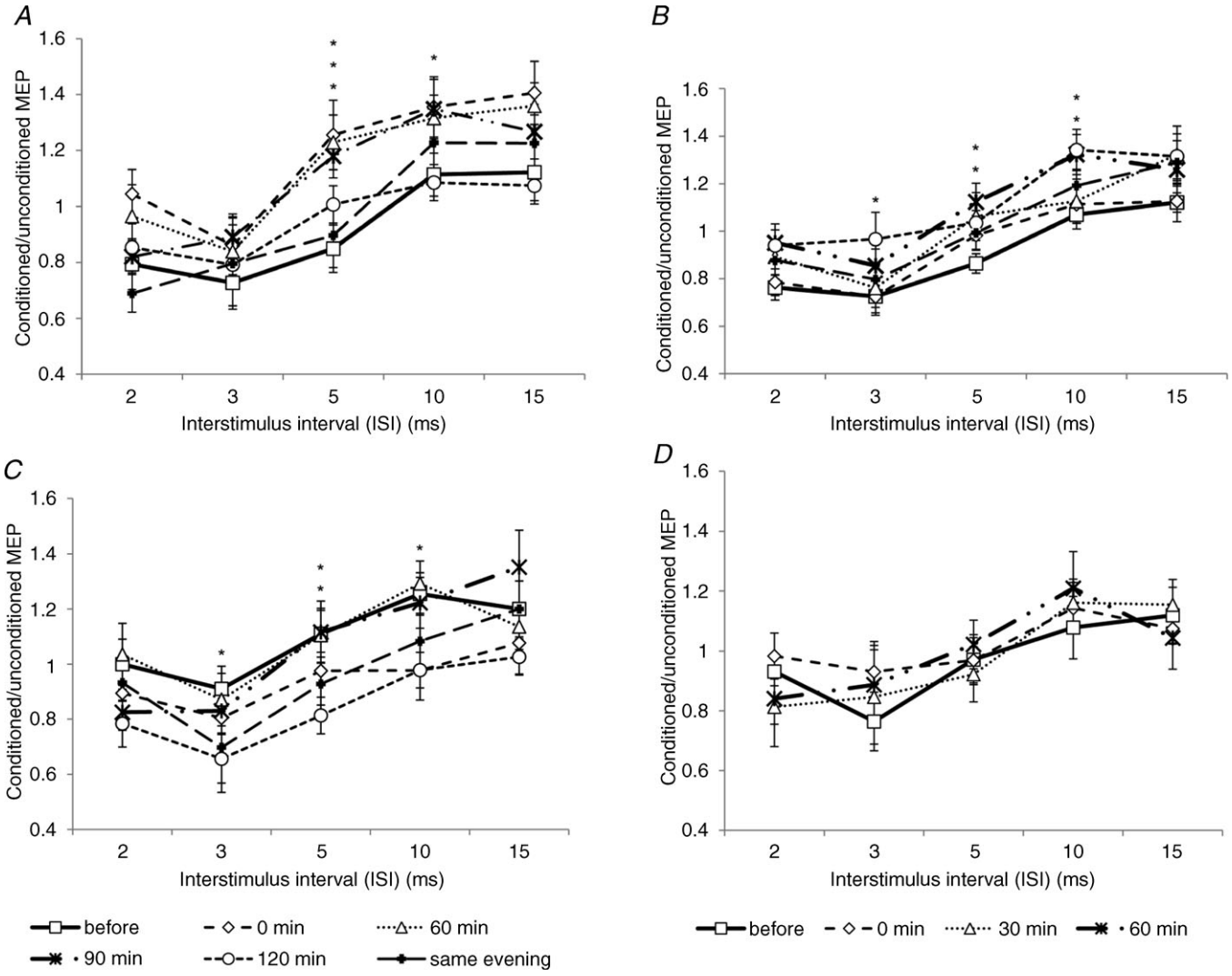

Figure 3. Intracortical inhibition and facilitation is modulated by tDCS
A–D, single-pulse standardized double stimulation MEP amplitude ratios ± SEM are depicted for ISIs revealing inhibitory (ISIs of 2, 3 and 5 ms) and facilitatory (ISIs of 10 and 15 ms) effects for (*A*) 2 mA anodal, (*B*) 2 mA cathodal, (*C*) 1 mA cathodal and (*D*) sham tDCS. Anodal tDCS at 2 mA decreases inhibition and increases facilitation immediately after stimulation for ISIs of 5 and 10 ms and 60 and 90 min after stimulation for an ISI of 5 ms; similar effects were observed 90 and 120 min after 2 mA cathodal tDCS. After 1 mA cathodal tDCS, facilitation is decreased for an ISI of 10 ms immediately after stimulation and inhibition is increased for ISIs of 5 ms at 90 min and 3 and 5 ms at 120 min after stimulation. Sham tDCS did not induce any significant changes. Asterisks indicate significant differences of standardized double stimulation MEP amplitudes from respective before stimulation values ($P < 0.05$).

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interaction ($F_5 = 4.141$, $P < 0.003$). The results of the *post hoc* Student's *t* tests showed a significant decrease of MEP amplitudes after 1 mA cathodal stimulation as compared to baseline and an excitability increase after 2 mA cathodal stimulation for 120 min after tDCS ($P < 0.05$) (Fig. 2).

Input–output curve. ANOVA for 2 and 1 mA cathodal tDCS showed a significant effect for TMS intensity ($F_3 = 38.440$, $P < 0.001$), but no significant interaction between tDCS, Time and TMS Intensity. A non-significant tendency towards a decrease of the I–O curve slope can be observed for the 1 mA condition (Fig. 4). MEP amplitudes were 1.91 ± 1.33 and 2.73 ± 1.83 mV before stimulation and 1.55 ± 0.77 and 2.32 ± 1.27 mV after stimulation at intensities of 130 and 150% of RMT, respectively.

Intracortical inhibition and facilitation. ANOVA showed a significant effect of ISI ($F_4 = 9.853$, $P < 0.001$) and Time ($F_5 = 4.106$, $P = 0.005$). For 1 mA cathodal tDCS, intracortical facilitation (ISI 10 ms) decreased immediately after stimulation and inhibition (ISIs of 3 and 5 ms) increased significantly 90 and 120 min after the end of stimulation, compared to the respective baseline values, as shown by the *post hoc* Student's *t* tests ($P < 0.05$) (Fig. 3*C*). Baseline values did not differ between stimulation conditions.

I-wave facilitation. ANOVA for the 2 and 1 mA cathodal stimulation showed a significant main effect for ISI ($F_9 = 18.068$, $P < 0.001$), but no significant interaction between tDCS, Time and ISI. Baseline values did not differ between stimulation conditions.

Cortical silent period. Average baseline CSP values were 0.138 ± 0.03 and 0.146 ± 0.027 s for 120% RMT and SI_{1mV} TMS intensities, respectively, and did not differ significantly from those of Experiment 1. ANOVA for the 2 and 1 mA cathodal tDCS showed no significant change in CSP duration and no interaction between tDCS, Intensity and Time.

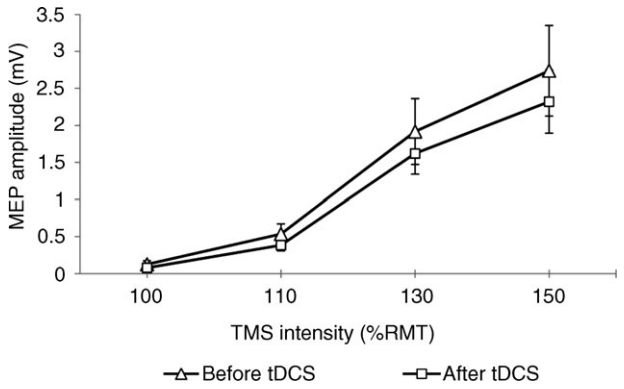

Figure 4. Effect of 1 mA cathodal tDCS on input–output curve MEP amplitudes (means ± SEM) are displayed before and after application of 1 mA cathodal tDCS. A trend towards a decrease of MEP amplitudes after tDCS can be observed, in line with a previous study of our group (Nitsche *et al.* 2005).

Experiment 3

Active motor threshold. Baseline AMT values in this experiment did not differ significantly from the respective values of Experiment 1. AMT was $32.1 \pm 9.4\%$ of maximum stimulator output. The ANOVA results were not significant.

Single-pulse MEPs (1 mV). The average baseline MEP value was 0.93 ± 0.03 mV obtained by $51.6 \pm 12.7\%$ of maximum stimulator output. MEP amplitude and stimulation intensity did not differ significantly from that of Experiment 1. ANOVA did not reveal significant main effect of Time ($F_3 = 0.142$, $P = 0.93$) (Fig. 2*C*).

Intracortical inhibition and facilitation. ANOVA showed a significant effect of ISI ($F_4 = 3.225$, $P = 0.027$) but no significant interaction between Time and ISI (Fig. 3*D*).

Discussion

Cathodal stimulation, so far thought to be the cornerstone in producing cortical inhibition by tDCS, loses this property with double intensity and instead induces excitation. The results of the present study show that opposing directions of plasticity are no longer warranted at 2 mA tDCS for 20 min. As this stimulation has recently become increasingly used in clinical studies and some positive effects have been achieved, it is important to study its physiological effects. Based on previous experiments with 1 mA stimulation (Nitsche & Paulus, 2001; Nitsche *et al.* 2003*b*) we expected a direct correlation between stimulation intensity and time. In contrast, the increase of intensity and duration of stimulation did not uniformly produce a stronger effect. To rule out the possibility that this effect was due to the specific subject group explored, we performed 1 mA cathodal stimulation for 20 min in nine subjects of the same group. Here the results were similar to those described in previous studies, where application of 1 mA cathodal tDCS for 18 min resulted in a decrease of single-pulse MEP amplitudes lasting for up to 120 min after stimulation and a SICI–ICF shift towards reduced intracortical excitability (Nitsche *et al.* 2005; Monte-Silva *et al.* 2010). Furthermore, sham tDCS did not induce significant MEP alterations, which ruled out an unspecific effect of 2 mA tDCS on motor cortex excitability.

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Effects of tDCS on corticospinal excitability

Effect of tDCS on single-pulse MEPs. Anodal stimulation at 2 mA resulted in an excitability enhancement which lasted up to 90 min after stimulation, comparable to the after-effects of 13 min 1 mA anodal stimulation (Nitsche & Paulus, 2001). In contrast, 2 mA cathodal tDCS induced qualitatively different effects, as compared to previous studies with 1 mA cathodal tDCS (Nitsche *et al.* 2003*b*; Monte-Silva *et al.* 2010). Interestingly, other recently conducted studies applying different plasticity-inducing stimulation protocols also show a non-linear association between stimulation intensities and the direction of the resulting after-effects (Doeltgen & Ridding, 2010; Moliadze *et al.* 2012). For theta burst transcranial magnetic stimulation (TBS) it was demonstrated that a short duration continuous TBS applied with an intensity of 65% of RMT induced cortical inhibition, whereas the same technique at an intensity of 70% RMT resulted in an excitability enhancement (Doeltgen & Ridding, 2010). Another study demonstrated that tACS and tRNS reduced cortical excitability at an intensity of 0.4 mA and enhanced it at an intensity of 1 mA (Moliadze *et al.* 2012). One possible mechanism for these reversed effects might be the dependency of the direction of plasticity from the amount of neuronal calcium influx caused by the respective stimulation protocol, as shown primarily in animal models so far. Thereby, low postsynaptic calcium enhancement causes long-term depression (LTD), whereas large calcium increases result in long-term potentiation (LTP; Cho *et al.* 2001; Lisman, 2001). Thus, it might be speculated that the larger stimulation intensity in the case of 2 mA cathodal tDCS, and the stronger TBS, tACS and tRNS protocols increase calcium level to an amount that induces LTP-like plasticity, whereas lower stimulation intensity results in a lower, LTD-like plasticity-generating calcium level. Accordingly, the after-effects of tDCS are caused by calcium-dependent mechanisms (Nitsche *et al.* 2003*a*). It has also been demonstrated that doubling the stimulation duration from 13 to 26 min shifts the 1 mA anodal tDCS-induced after-effects to excitability diminution, and that this effect is calcium-dependent (Monte-Silva *et al.* in press). Further evidence for non-linear effects of tDCS, which might be calcium-dependent, originates from pharmacological studies, where a serotonine reuptake inhibitor and a D2/D3 receptor agonist at high dosage switched the 1 mA cathodal tDCS-induced after-effect to excitation (Nitsche *et al.* 2009). Another possible mechanism explaining excitatory after-effects of 2 mA cathodal tDCS could be that DC stimulation induces de- and hyperpolarization via hyperpolarizing the soma and depolarizing dendrites, respectively, with cathodal stimulation (Jefferys, 1981; Ghai *et al.* 2000; Bikson *et al.* 2004). Moreover, the resulting neuronal excitability change is determined by the axonal orientation relative to the electric field vector, from which it follows that tDCS-induced homogenous electric fields do not uniformly modulate all neurons in the stimulated area (Kabakov *et al.* 2012). Doubling current intensity in the case of 2 mA cathodal tDCS could therefore have increased dendritic depolarization to a level which has an impact on neuronal excitability or resulted in polarization of structures with different neuronal orientation, therefore producing plasticity different from that of 1 mA tDCS. Furthermore, due to modelling and imaging studies the current injected by tDCS with conventional electrode montages affects several regions of the brain (Datta *et al.* 2009), also beyond the target area, and changes functional connectivity between them (Polania *et al.* 2011*a*,*b*). An increase in the intensity of injected current should proportionally increase the electric field in every affected brain region, and might lead to recruitment of other non-target brain regions, which could indirectly affect and change the direction of plasticity in the target regions. In accordance, it has been demonstrated that 1 mA anodal tDCS over the premotor cortex decreases intracortical inhibition and increases facilitation in the primary motor cortex (Boros *et al.* 2008). Moreover, it was shown that the inhibitory ventral premotor to primary motor cortex pathway can be changed to excitatory in a state-dependent manner after paired associative stimulation of premotor and motor cortices (Davare *et al.* 2009; Buch *et al.* 2011). At present, however, all of these explanations are speculative, and should be explored in future studies directly.

Interestingly, in contrast to the conventional 1 mA stimulation protocols, 2 mA stimulation induces after-effects with a delay. There is no clear explanation for this delayed effect so far, although it has been observed in animal studies, and also for other non-invasive plasticity induction protocols in humans (Bindman *et al.* 1964; Bi & Poo, 1998; Stefan *et al.* 2000) and under lorazepam-reinforcing GABAergic contribution for tDCS (Nitsche *et al.* 2004*b*). Possible reasons for this delay might be transient homeostatic counter-regulation, alterations of intracellular calcium, and different neuronal populations affected by 2 mA, as compared to the 1 mA stimulation protocols, because stronger protocols should affect deeper cortical layers, and might also generate plasticity in other types of neurons (Purpura & McMurtry, 1965).

No effect on MTs. Both 2 mA cathodal and anodal, as well as 1 mA cathodal tDCS did not change motor thresholds, just as after application of 1 mA tDCS in a former study (Nitsche *et al.* 2005), which was explained by major tDCS effects on cortical neurons, while MTs depend primarily on corticospinal neurons. Moreover, the spatial disparity between the large tDCS electrode, which should affect many more neurons than those are affected by motor threshold determination, might have prevented significant effects. Only one study reported an RMT increase after

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1.5 mA cathodal tDCS for 10 min (Ardolino *et al.* 2005), but still inducing inhibitory after-effects at this amplitude.

No effect on I–O curve. Both 2 mA cathodal and anodal stimulation resulted in no change of the I–O curve slope, which was obtained only once immediately after the end of tDCS. These results are not in accordance with those of a previous study with 1 mA protocols (Nitsche *et al.* 2005). This discrepancy is most probably caused by the fact that tDCS in the current study induced delayed after-effects evolving not immediately after stimulation, as can be seen also by the other parameters obtained in the present study. In the 1 mA cathodal stimulation condition, which induced after-effects without a prominent delay, we saw a tendency towards the decrease of I–O curve slope, which is similar to the results of the above-mentioned study. The non-significant trend after 1 mA cathodal tDCS is most probably a result of the higher variability in the present as compared to the previous study caused by the lower number of subjects and randomized order of measurements before and after stimulation.

Effects of tDCS on intracortical excitability

SICI and ICF are affected by tDCS. For 2 mA anodal tDCS, short latency intracortical inhibition and facilitation are shifted towards an excitability enhancement immediately after stimulation lasting for at least 90 min (Fig. 3*A*). For cathodal tDCS with 2 mA, a gradual increase of facilitation, reaching the peak 120 min after tDCS and returning to baseline values after 6–8 h, can be observed (Fig. 3*B*). In contrast, 1 mA cathodal tDCS resulted in a significant enhancement of intracortical inhibition, and a respective reduction of facilitation (Fig. 3*C*). The effects of 2 mA cathodal tDCS are qualitatively different from those of 1 mA stimulation (Nitsche *et al.* 2005), and are more similar to that of 2 mA anodal stimulation. Thus, it might be speculated that 2 mA anodal and cathodal tDCS have similar mechanisms of action on intracortical systems, which might be mediated by a calcium increase in the LTP range for both stimulation protocols.

No effect on I-wave facilitation and cortical silent period. For the 2 mA stimulation protocols, the results show no effect on either polarity of I-wave facilitation or the cortical silent period. Essentially the same holds true for the effects of 1 mA cathodal tDCS. These missing effects differ from those of previous studies with regard to I-wave facilitation, where 1 mA stimulation had an effect (Nitsche *et al.* 2005; Lang *et al.* 2011). For the 2 mA conditions, the missing effect in the present study might be due to the fact that both parameters were solely obtained immediately after tDCS, when the stimulation might have had only minor effects on cortical excitability, as can be derived from the missing effect on single-pulse MEP amplitudes. Furthermore, in contrast to the above-mentioned TMS protocols, which are influenced by glutamatergic mechanisms, I-wave facilitation and CSP are primarily controlled by the GABAergic system (Paulus *et al.* 2008), on which tDCS might have no major impact (Nitsche *et al.* 2004*b*). At first sight, this seems to contradict the results of a recently published magnetic resonance spectroscopy (MRS) study, which showed a decrease in free GABA concentration within the stimulated area after 10 min of both anodal and cathodal 1 mA tDCS (Stagg *et al.* 2009). Reasons for the opposing results might be the differences of stimulation protocols with regard to stimulation intensity and duration. Furthermore, the amount of free GABA concentration might not translate one to one to TMS-induced activity of GABAergic synapses. Moreover, it cannot be excluded that the longer and stronger protocols in the present study have different effects on GABAergic neurons (e.g. due to depth of the induced electrical field). Finally, CSP and I-wave facilitation were obtained only immediately after stimulation due to temporal restrictions, and at this time point the excitability alterations were not significant as well with regard to other stimulation protocols.

General remarks

Taken together, the results of our study show that the enhancement or prolongation of tDCS intensity or stimulation duration is not always accompanied by an increase of its efficacy, but might even change the direction of effects. This leads to the assumption that in healthy subjects a 'ceiling effect' of single stimulation protocols might exist, which cannot be overcome with simply more intensive stimulation. However, repeated stimulation protocols might be candidates to enhance the efficacy of stimulation (Monte-Silva *et al.* 2010), and also pharmacological interventions have been shown to prolong the after-effects of tDCS for up to about 24 h after the end of stimulation (Nitsche *et al.* 2004*a*; Kuo *et al.* 2008; Monte-Silva *et al.* 2009).

It is not self-evident that the results of this study, which was conducted in healthy young subjects, translate one-to-one to the effects in neuropsychiatric patients. In neuropsychiatric diseases, transmitter availability and other features of brain function might be different, and have a prominent impact on the efficacy of non-invasive brain stimulation to alter cortical excitability. Moreover, in clinical protocols often repetitive stimulation is performed, which might have an impact on the resulting plasticity. Finally, it is not completely clear if the neuroplastic effects of tDCS determine the clinical efficacy in each case. Nevertheless, the results of the present study argue for the importance to probe the physiological effects of extended stimulation protocols, and not to take enhanced efficacy of stronger protocols for granted.

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Author contributions

The experiments were conducted at the University Medical Center, Dept. Clinical Neurophysiology, Georg-August-University, Goettingen. M.A.N., M.-F.K., G.B., V.M., and W.P. contributed to the design and conception of the experiments. G.B., V.M., and M.-F.K., and M.A.N. contributed to the collection, analysis, and interpretation of the data. G.B. drafted the paper, and M.A.N., M.-F.K., V.M., and W.P. revised it critically for important inellectual content. All authors approved the final version of the manuscript.

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