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Shaping the Effects of Transcranial Direct Current Stimulation of the Human Motor Cortex

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Nitsche MA, Doemkes S, Karaköse T, Antal A, Liebetanz D, Lang N, Tergau F, Paulus W. Shaping the effects of transcranial direct current stimulation of the human motor cortex. *J Neurophysiol* 97: 3109–3117, 2007. First published January 24, 2007; doi:10.1152/jn.01312.2006. Transcranial DC stimulation (tDCS) induces stimulation polarity-dependent neuroplastic excitability shifts in the human brain. Because it accomplishes long-lasting effects and its application is simple, it is used increasingly. However, one drawback is its low focality, caused by *1*) the large stimulation electrode and *2*) the functionally effective reference electrode, which is also situated on the scalp. We aimed to increase the focality of tDCS, which might improve the interpretation of the functional effects of stimulation because it will restrict its effects to more clearly defined cortical areas. Moreover, it will avoid unwanted reversed effects of tDCS under the reference electrode, which is of special importance in clinical settings, when a homogeneous shift of cortical excitability is needed. Because current density (current strength/electrode size) determines the efficacy of tDCS, increased focality should be accomplished by *1*) reducing stimulation electrode size, but keeping current density constant; or *2*) increasing reference electrode size under constant current strength. We tested these hypotheses for motor cortex tDCS. The results show that reducing the size of the motor cortex DC-stimulation electrode focalized the respective tDCS-induced excitability changes. Increasing the size of the frontopolar reference electrode rendered stimulation over this cortex functionally inefficient, but did not compromise the tDCS-generated motor cortical excitability shifts. Thus tDCS-generated modulations of cortical excitability can be focused by reducing the size of the stimulation electrode and by increasing the size of the reference electrode. For future applications of tDCS, such paradigms may help to achieve more selective tDCS effects.

INTRODUCTION

Transcranial DC stimulation (tDCS) induces polarity-dependent excitability changes of the human motor cortex. These evolve during stimulation, but are stable for up to 1 h if stimulation lasts sufficiently long (Nitsche and Paulus 2000, 2001; Nitsche et al. 2003a). With appropriate pharmacological interventions, the duration of the aftereffects can be further extended (Nitsche et al. 2004a,b, 2006). Using adequate electrode positions, tDCS is also able to modify the excitability and functional properties of visual, somatosensory, and prefrontal cortices (Antal et al. 2003, 2004a ; Fregni et al. 2005a; Kincses et al. 2004; Matsunaga et al. 2004). Applied to the motor and visual cortices, tDCS was recently shown to improve learning processes (Antal et al. 2004b; Nitsche et al. 2003b). Thus this stimulation paradigm is an interesting tool for inducing neuroplastic changes of cortical functions. Moreover, recently conducted clinical pilot studies confirm its efficacy for reducing symptoms in chronic pain syndromes, depression, chronic stroke, and epilepsy (Fregni et al. 2005b, 2006a,b,c ; Hummel et al. 2005).

However, one important limitation of the technique is its low spatial focality. This is caused caused by *1*) the relatively large tDCS electrodes (35 cm^2). Moreover, in the standard protocols both the stimulation electrode and also the reference electrode are situated on the scalp over the brain. Thus *2*) anodal tDCS of one cortical area is combined with cathodal stimulation of another cortex and vice versa. Consequently a relatively widespread change of cortical excitability was demonstrated in the respective projection areas (Lang et al. 2005). The low focality of stimulation restricts its current application in basic and clinical research for two main reasons: the relatively large stimulation electrode covers in many cases not only the area of interest, but also adjacent cortices, and thus limits interpretation of the experimental results. Moreover, a functionally efficient reference electrode might increase the ambiguity of the interpretation of experimental results on the one hand but might also be dysfunctional in clinical studies, such as for the treatment of epilepsy. Here, excitability-diminishing tDCS of the primary epileptogenic focus is inevitably accompanied with an excitability enhancement of another cortical area, which could result in unwanted effects of tDCS. To overcome these limitations, it would be desirable to increase the focality of DC stimulation.

With respect to the *stimulation electrode*, the direct functional effects of tDCS are probably restricted to the area under the electrode, as suggested by simulation studies and animal experiments. It was shown that the electrical field strength is fairly homogeneous under the electrode but decreases very rapidly with distance from it (Miranda et al. 2006; Rush and Driscoll 1968). These data are supported by results of tDCS experiments in humans. Changing the position of the stimulation electrode a few centimeters distinctly alters its efficacy in an implicit motor-learning task (Nitsche et al. 2003b) and with regard to motor cortical excitability changes (Nitsche and Paulus 2000). Thus focality of the effects of the stimulation electrode can probably be increased by reducing its size. In doing so, however, current density (current strength/electrode size) must be kept constant because this parameter determines the efficacy of electrical stimulation (Agnew and McCreery 1987), as also directly demonstrated for tDCS (Nitsche and Paulus 2000).

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For the *reference electrode*, at first glance it seems that to eliminate its functional effects, an easy solution would be to position it further from the brain. However, the efficacy of tDCS in eliciting excitability changes critically depends on the position of the reference electrode because of the interdependence between current flow direction and neuronal orientation (Nitsche and Paulus 2000; Purpura and McMurtry 1965). Additionally, positioning the reference electrode somewhere on the body could result in brain stem stimulation, which might be dangerous because autonomous central nervous systems could be disturbed (Lippold et al. 1964). Alternatively, current density under the reference electrode can be diminished by increasing its size, but keeping current strength constant. In this case, as a result of the dependency of efficacy of tDCS on current density, the functional efficacy of the reference electrode could probably be eliminated without necessarily changing its position.

We explored whether *1*) reducing the stimulation electrode size and *2*) increasing the reference electrode size focuses the effects of tDCS in the human motor cortex tDCS protocol as a model. Here the stimulation electrode is fixed over the hand area of the primary motor cortex, whereas the reference electrode is situated contralaterally over the frontopolar cortex. Both the motor cortex and the frontopolar reference electrode induce functional effects in this protocol (Kincses et al. 2004).

We hypothesized *1*) that reducing the size of the motor cortex stimulation electrode, while holding current density constant, would focus the effects of tDCS without reducing its efficacy to elicit excitability changes in the cortical area under the electrode. We tested the effects of tDCS during a short stimulation, which elicits no aftereffects, and for a tDCS paradigm eliciting aftereffects that are stable for some minutes (*experiments* 1a and 1b).

For the reference electrode we hypothesized *2*) that an increase of its size while leaving current strength constant would eliminate its functional efficacy, while leaving the motor cortical excitability shifts unchanged (*experiments* 2a and 2b).

METHODS

Subjects

Twelve healthy subjects participated in each experiment. For details see Table 1. All gave written informed consent. The investigation was approved by the ethics committee of the University of Goettingen and we conformed to the Declaration of Helsinki.

Direct current stimulation of the motor cortex

Direct currents were transferred through a pair of saline-soaked surface sponge electrodes and delivered by a specially developed, battery-driven, constant-current stimulator (Schneider Electronic, Gleichen, Germany) with a maximum output of 2 mA. In each experiment, the motor-cortical electrode was fixed over the representational field of the right abductor digiti minimi muscle (ADM), as identified by transcranial magnetic stimulation (TMS), and the other electrode was fixed contralaterally above the right orbit. In *experiment* 1, the currents flowed continuously for 4 s (excitability shifts during tDCS, *experiment* 1a) or 7 min (short-lasting aftereffects, *experiment* 1b), to cover both intra- and aftereffects of tDCS (Nitsche and Paulus 2000, 2001; Nitsche et al. 2003a). For *experiment* 2, tDCS had to be extended to 10 min because this stimulation duration was applied in the original probabilistic classification learning protocol (Kincses et al. 2004). For *experiment* 1, motor cortex electrode size was 35 cm^2 in the "conventional electrode size" condition, whereas it was reduced to 3.5 cm^2 in the "diminished electrode size" condition. Here, the size

TABLE 1. *Stimulation paradigms, electrode sizes, and subject characteristics of the experiments*

Experiment	Electrode Size	DC-Stimulated Muscle	MEP Amplitude, mV, ± SD	tDCS Stimulation Duration per Cycle	Number of Subjects	Age of Subjects, yr, mean ± SD	Gender of Subjects (f/m)
1, Intra-tDCS	S: 35 cm^2	ADM	A: 0.995 ± 0.091 C: 1.054 ± 0.098	4 s A/C	12	25 ± 4	7/5
		FDI	A: 1.082 ± 0.070 C: 1.057 ± 0.098				
	S: 3.5 cm^2	ADM	A: 1.040 ± 0.056 C: 1.023 ± 0.072	4 s A/C	12	25 ± 4	7/5
		FDI	A: 1.071 ± 0.064 C: 1.064 ± 0.105				
1, Aftereffects	S: 35 cm^2	ADM	A: 0.986 ± 0.042 C: 0.991 ± 0.035	7 min A/C	12	24 ± 2	8/4
		FDI	A: 1.003 ± 0.048 C: 0.969 ± 0.046				
	S: 3.5 cm^2	ADM	A: 0.993 ± 0.060 C: 0.987 ± 0.059	7 min A/C	12	24 ± 2	8/4
		FDI	A: 0.963 ± 0.162 C: 0.986 ± 0.044				
2	R: 35 cm^2	ADM	A: 1.076 ± 0.139 C: 1.090 ± 0.813	10 min A/C	12	23 ± 2	10/2
	R: 100 cm^2	ADM	A: 1.045 ± 0.826 C: 1.075 ± 0.615	10 min A/C			

Experiment 1 refers to the focusing effects of diminished tDCS electrode size on motor cortex excitability changes during tDCS (a) or with regard to short-lasting aftereffects (b). The topic of *experiment* 2 concerns the effects of an increased size of the reference electrode on motor cortex excitability (*experiment* 2a) and the efficacy of frontopolar stimulation (*experiment* 2b). Mean TMS intensities to achieve non-tDCS (*experiment* 1a) or pre-tDCS (*experiments* 1b and 2a) MEP amplitude means of about 1 mV were calculated for each experimental condition. They did not differ between the respective conditions (Student's *t*-test, $P > 0.05$). tDCS duration was 4 s in *experiment* 1a. Here stimulation was repeated 15 times for each tDCS condition, whereas in the remaining protocols, only one DC stimulation per session was applied. As shown in the last columns, 12 subjects participated in each experiment; age and gender distribution were comparable between experiments. A, anodal tDCS; C, cathodal tDCS; f, female; m, male; R, reference electrode (frontopolar); S, motor cortex stimulation electrode; ADM, abductor digiti minimi muscle; FDI, first dorsal interosseus muscle.

of the reference electrode was kept constant (35 cm^2). Current strength was 1 mA for the 35-cm^2 motor cortex electrode, but reduced to 0.1 mA for the 3.5-cm^2 electrode condition to keep current density constant (about 0.03 mA/cm^2 in each condition). For *experiment* 2, the size of both electrodes was 35 cm^2 in the "conventional electrode size" condition, whereas it was enlarged to 100 cm^2 for the frontopolar reference electrode in the "reduced current density" condition, resulting in a current density of 0.01 mA/cm^2 under this electrode. Because a minimum current density of 0.017 mA/cm^2 was previously shown to be necessary to modify cortical excitability by tDCS in a former study in humans (Nitsche and Paulus 2000), this reduction of current density should suffice to make the reference electrode functionally inefficient.

Measurement of motor-cortical excitability

To detect current-driven changes of excitability, muscle-evoked potentials (MEPs) of the right abductor digiti minimi (ADM) and first dorsal interosseus muscle (FDI) (*experiment* 1 only) were recorded after stimulation of their motor-cortical representational fields by single-pulse TMS. These were induced using a Magstim 200 magnetic stimulator (Magstim, Whiteland, Dyfed, UK) and a figure-of-eight magnetic coil (diameter of one winding = 70 mm; peak magnetic field = 2.2 Tesla). The coil was held tangentially to the skull, with the handle pointing backward and laterally at 45° from midline, resulting in a posterior–anterior direction of current flow in the brain. The optimal position was defined as the site where stimulation consistently resulted in the largest MEP. Surface EMG was recorded from the right ADM and FDI (*experiment* 1 only) by use of Ag–AgCl electrodes in a belly-tendon montage. The signals were amplified and filtered with a time constant of 10 ms and a low-pass filter of 2.5 kHz. Signals were then digitized at an A/D rate of 5 kHz and further relayed into a laboratory computer using the Signal 1.62 software (CED, Cambridge, UK) and conventional averaging software. The intensity of the stimulator output was adjusted so that stimulation led to an average MEP amplitude of about 1 mV peak to peak during baseline recording (without or before DC stimulation).

Efficacy of frontopolar tDCS by the probabilistic classification task

The probabilistic classification learning task (PCL) was introduced as a promising tool to investigate implicit learning functions (Knowlton et al. 1994, 1996; Reber et al. 1996). In this task, subjects are asked whether a specific combination of different geometric forms predicts rainy or sunny weather. Each combination is probabilistically related to a particular weather outcome, although the relationship is not absolute: in different percentages the combinations are also associated with the opposite outcome. During the task, individuals learn gradually which of two outcomes would occur in each trial given the particular combination of cues that appears, although they have no conscious knowledge of the rule. Stimuli were four different geometrical shapes presented in one row on a computer screen. Each stimulus had a height of 120 pixels and a width of 120 pixels. In a given trial, a stimulus consisted of one, two, or three geometrical shapes. The exposure time of cues was 1,000 ms. In each trial, subjects were asked whether the given combination of geometrical shapes meant rainy or sunny weather. The response was given by pushing one of the two mouse buttons. After the subject's response, the correct answer was presented on the screen. The interstimulus interval (ISI) was 1,000 ms. Four cues were associated with the outcome sunshine either in 75, 57, 43, or 25%, and thus either 25, 43, 57, or 75% with rain. Fifty trials were presented in five blocks (ten trials each).

Experimental procedures

An overview of the experiments conducted is given in Fig. 1. All experiments were conducted in a repeated-measurement design. The order in which the experiments were conducted was randomized between subjects for *experiments* 1 and 2. The subjects were seated in a reclining chair.

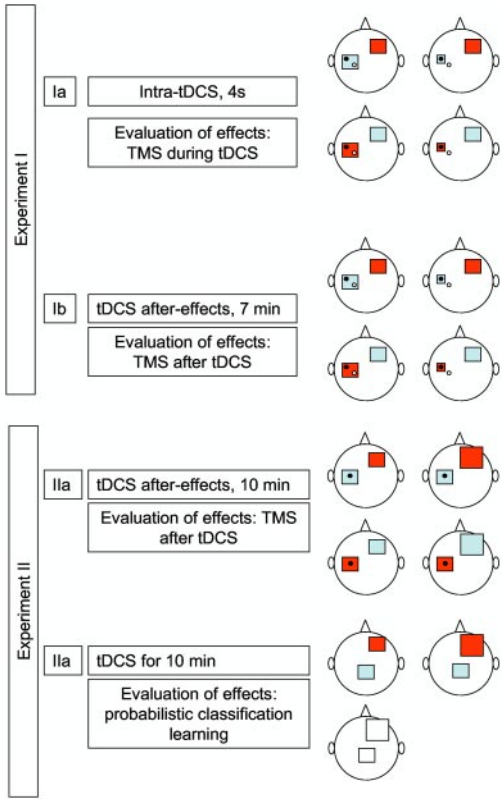

FIG. 1. Schematic overview of the experiments performed in this study. In *experiment* 1, we tested the effect of the size of the transcranial DC stimulation (tDCS) electrodes on the excitability of the motor cortex representation of 2 hand muscles, the first dorsal interosseus muscle (FDI) and the right abductor digiti minimi (ADM), for anodal and cathodal tDCS. In the conventional electrode size condition (35 cm^2), both muscle representations were situated under the electrode, whereas the small electrode (3.5 cm^2) covered the ADM representation only. In *experiment* 1a, effects of short-lasting tDCS (4 s) on cortical excitability during stimulation; in *experiment* 1b, effects of longer-lasting tDCS (7 min) on the aftereffects were tested with transcranial magnetic stimulation (TMS). In *experiment* 2a, we evaluated the effects of an enlarged (100 cm^2) frontopolar reference electrode, as compared with the conventionally sized (35 cm^2) electrode on the long-lasting aftereffects of 10-min anodal or cathodal tDCS of the motor cortex. In *experiment* 2b, the impact of the enlarged frontopolar tDCS electrode on performance of the probabilistic classification task was compared with the effect of the conventionally sized electrode and a placebo tDCS. Squares indicate tDCS electrodes. Red square, anodal tDCS; green squares, cathodal tDCS; white squares, placebo tDCS. Size of the squares refers to electrode size: small = 3.5 cm^2; medium = 35 cm^2; large = 100 cm^2. Filled circle indicates the motor cortical representation of the ADM, the white one the FDI representation.

EXPERIMENT 1. First, the left motor-cortical representational fields of the right ADM and FDI were identified by TMS (coil position that leads to the largest MEPs of ADM or FDI). One DC stimulation electrode, to which in the following the terms either "cathodal" or "anodal" stimulation refer, was fixed over the motor cortex representation of the ADM and also covered the representation of the FDI, when the 35-cm^2 electrode was used—but not if the 3.5-cm^2 electrode was used. The other DC electrode was fixed on the forehead contralaterally, above the orbit. In *experiment* 1a, TMS was administered with tDCS electrodes fixed on the head of the subjects. This was necessary because, in this experiment, TMS was performed during tDCS. For the remaining experiments, tDCS electrodes were fixed on

the head after baseline TMS recording and removed before TMS aftereffect recording.

In *experiment* 1a (intracurrent excitability changes), a randomized series (0.1 Hz) of 15 TMS-evoked MEPs 200 ms before the end of a 4-s DC stimulation and another 15 MEPs without preceding DC stimulation were recorded. Anodal and cathodal DC stimulations as well as TMS of the ADM or FDI motor cortex representations were performed on one day in randomized order using conventionally sized or small motor cortical electrodes for each of these conditions. Each of the altogether eight sessions (one session per tDCS polarity, electrode size, and TMS site) was separated from the next one by a break of ≥20 min. This break duration should be sufficient to avoid interference between the experimental sessions because 1 min of tDCS (cumulative tDCS duration of a single session) does not induce aftereffects (Nitsche and Paulus 2000). Moreover, it was previously shown that two sessions of 7-min tDCS, thus including much longer tDCS compared with the present experiment, do not interfere if these are separated by a break of 30 min (unpublished results of our group).

Because TMS-elicited electrical fields might be differently affected by TMS coil position and electrode size, we compensated for different efficacy of TMS by adjusting TMS intensity for eliciting MEP amplitude of about 1 mV in each non-tDCS stimulation condition.

In *experiment* 1b (short-lasting aftereffects), first, baselines of TMS-evoked MEPs (20 stimuli) were recorded at 0.25 Hz for ADM or FDI. Afterwards anodal or cathodal tDCS was administered for 7 min—using the conventionally sized or small tDCS electrodes—to elicit short-lasting aftereffects. After cessation of DC stimulation, 15 MEPs were recorded every fifth minute at 0.25 Hz ≤15 min after the end of tDCS. No more than two sessions per day were conducted with an interval of at least 1 h in between. This intersession break duration was chosen because it was shown to cause no interference between the respective tDCS sessions, as reported in a former study (Nitsche et al. 2005).

EXPERIMENT 2. In *experiment* 2a, we studied the dependency of the tDCS-elicited motor cortical excitability changes on the size of the reference electrode. The principal course of the experiment was identical to that of *experiment* 1b, with the following exceptions: tDCS was performed for 10 min; only excitability modifications of the ADM were recorded; and post-tDCS MEPs were elicited every fifth minute until 30 min after tDCS and 30, 60, and 90 min after the end of DC stimulation. Ten-minute tDCS was administered because this stimulation duration was necessary to obtain the intended cognitive effects in the companion study (*experiment* 2b) in a former experiment (Kincses et al. 2004). The size of the motor cortical electrode was kept constant (35 cm^2), but the size of the contralateral frontopolar electrode was 35 or 100 cm^2, and a break of at least 1 wk between each stimulation session was obligatory.

Experiment 2b included the probabilistic classification task. Before the test, each subject underwent a brief practice session, thus ensuring that all the subjects understood and were able to perform the task. Stimuli used in the practice session were not included in the task. Then the tDCS electrodes were fixed onto the head. The reference electrode (35 cm^2) was fixed at Cz and the frontopolar stimulation electrode (35 or 100 cm^2) at FP3. The reference electrode was positioned at Cz and not at the contralateral motor cortex because this electrode arrangement had effectively influenced performance in a previous study (Kincses et al. 2004). Because the critical question in this part of the study was whether the size of the frontopolar tDCS electrode would influence performance, a motor cortex reference was not essential. Anodal tDCS was applied for 10 min with an intensity of 1.0 mA in the real tDCS conditions. For sham tDCS, current flow was terminated after 10 s. After 5-min real or sham tDCS, the task was initiated and the fifth block was completed after precisely 10 min of stimulation. Every subject was tested three times (sham and anodal stimulation including the 35- or 100-cm^2 electrodes) with an interval of ≥1 wk between sessions in randomized order. The frequency values that indicated how many times the given geometric forms meant rain or sunshine were changed between the sessions and were randomized between the subjects.

Calculations and statistics

MEP MEASURES. Individual MEP amplitude means were calculated in *experiment* 1a for the DC and noncurrent conditions (15 stimuli each). In *experiments* 1b and 2a, individual MEP amplitude means were calculated for each time bin covering pre-tDCS baseline (20 stimuli) and post-tDCS time points (15 stimuli). The respective intra- or post-tDCS MEP amplitude means were normalized to non-tDCS MEPs in *experiment* 1a and to precurrent baselines in *experiments* 1b and 2a. In *experiment* 1, separate means were calculated for the respective ADM/FDI transcranial magnetic stimulation conditions under both electrode size conditions.

For *experiment* 1a, repeated-measures ANOVAs were calculated for absolute and standardized values, the independent variables being electrode size, tDCS, position of the TMS coil, and the dependent variable MEP amplitude. Then Student's *t*-test (paired samples, two-tailed, $P < 0.05$) were performed to determine whether tDCS modified MEP amplitudes within each tDCS/electrode size/TMS coil position condition and whether MEP amplitudes differed between these conditions. Furthermore, it was controlled for if the non-tDCS MEPs for each position of the TMS coil under all electrode size conditions were identical.

With regard to *experiment* 1b, a repeated-measures ANOVA (independent variables: time course, tDCS, electrode size, position of the TMS coil; dependent variable: MEP amplitude) was calculated, then Student's *t*-test (paired samples, two-tailed, level of significance <0.05) were performed to determine whether the MEP amplitudes before and after tDCS differed in each condition; whether these differences depended on the position of the TMS coil, electrode size condition, and tDCS polarity for each time bin; and whether the baseline MEP amplitudes were identical in all conditions. For *experiment* 2a, statistical testing was identical to that of *experiment* 1b with the exception that here the ANOVA encompassed only the independent variables of time course, tDCS, and electrode size.

PROBABILISTIC CLASSIFICATION TASK. In this task, the percentages of correct responses for each block were entered into a two-factorial ANOVA (independent variables: tDCS and time course). Moreover, performance in block 1 was compared with performance in the remaining blocks for each tDCS condition separately and the impact of tDCS on performance in each block was tested by Student's *t*-test. The level of significance was $P < 0.05$.

RESULTS

Effects of different motor cortex stimulation electrode sizes on intra-tDCS motor cortical excitability shifts (experiment 1a)

The ANOVAs for both the absolute and the standardized values revealed a significant main effect of tDCS and significant interactions between electrode size and tDCS and between TMS coil position, electrode size, and tDCS (Table 2). For the standardized values, additionally the interaction between TMS coil position and tDCS was significant. For the ADM, the *t*-test showed a significant excitability enhancement during anodal tDCS and a respective excitability reduction during cathodal tDCS in the 35- and in the 3.5-cm^2 tDCS electrode size condition (Fig. 2). The size of the respective excitability shifts did not differ significantly as a result of tDCS electrode size. For the FDI, anodal tDCS enhanced and cathodal tDCS significantly diminished excitability only in the 35-cm^2 electrode size condition. Consequently, for the FDI MEP amplitude

TABLE 2. *Results of ANOVAs conducted for the different experiments*

Experiment	Factor	df	F	*P*
1, Intra-tDCS (absolute values)	Electrode size	1	0.386	0.547
	TMS coil position	1	0.060	0.811
	tDCS	3	39.618	<0.001*
	Electrode size × TMS coil position	1	0.288	0.602
	Electrode size × tDCS	3	6.903	0.001*
	TMS coil position × tDCS	3	2.427	0.083
	Electrode size × TMS coil position × tDCS	3	7.152	0.001*
1, Intra-tDCS (standardized values)	Electrode size	1	0.336	0.574
	TMS coil position	1	0.011	0.918
	tDCS	1	88.800	<0.001*
	Electrode size × TMS coil position	1	1.624	0.229
	Electrode size × tDCS	1	15.438	0.002*
	TMS coil position × tDCS	1	4.949	0.048*
	Electrode size × TMS coil position × tDCS	1	8.258	0.015*
1, Aftereffects	Time course	4	3.702	0.011*
	TMS coil position	1	1.646	0.226
	Electrode size	1	4.235	0.064
	tDCS	1	75.721	<0.001*
	Time course × TMS coil position	4	1.224	0.341
	Time course × electrode size	4	4.870	0.002*
	TMS coil position × electrode size	1	4.718	0.053
	Time course × tDCS	4	61.248	<0.001*
	TMS coil position × tDCS	1	44.489	<0.001*
	Electrode size × tDCS		6.298	0.029*
	Time course × TMS coil position × electrode size	4	3.892	0.009*
	Time course × TMS coil position × tDCS	4	6.095	0.001*
	Time course × electrode size × tDCS	4	7.038	<0.001*
	TMS coil position × electrode size × tDCS	1	31.975	<0.001*
	Time course × TMS coil position × electrode size × tDCS	4	10.171	0.001*
2, MEP	Electrode size	1	1.703	0.218
	tDCS	1	274.059	<0.001*
	Time course	9	2.041	0.042*
	Electrode size × time course	9	1.642	0.114
	Electrode size × tDCS	1	0.989	0.341
	Time course × tDCS	9	30.840	<0.001*
	Electrode size × time course × tDCS	9	0.858	0.565
2, Learning	tDCS	2	12.148	<0.001*
	Block	4	4.100	0.007*
	tDCS × block	8	1.048	0.407

*Asterisks mark significant main effects and interactions. df, degrees of freedom; F, F-value; *P,* probability.

changes differed dependent on tDCS electrode size (Fig. 2). Comparing FDI and ADM, for the 35-cm^2 electrode size condition identical MEP amplitude changes were accomplished. However, for the 3.5-cm^2 tDCS electrode size, the excitability reduction accomplished by cathodal tDCS differed significantly between ADM and FDI. A similar trend can be seen for anodal tDCS. Without tDCS, MEP amplitudes did not differ between ADM and FDI in all conditions.

No perceptible tDCS-electrode heating occurred during TMS.

Impact of different motor cortex stimulation electrode sizes on tDCS aftereffects (experiment 1b)

For the influence of electrode size on the aftereffects of tDCS, the ANOVA revealed significant main effects of time course and tDCS as well as numerous significant interactions between the respective variables. These are shown in Table 2. In the anodal tDCS condition, tDCS resulted in a significant excitability enhancement lasting for 10 min after tDCS of the motor cortex representation of the ADM, which was identical for both electrode sizes. In the cathodal tDCS condition, these effects were reversed (Fig. 3*A*). Conversely, for the FDI only the 35-cm^2 tDCS electrode resulted in identical effects. In the 3.5-cm^2 electrode size condition, the MEP amplitudes of the FDI did not differ significantly from baseline values (Fig. 3*B*). This difference between the respective electrode size conditions was significant for ≤10 min after tDCS. Comparing FDI and ADM, for the 3.5-cm^2 electrode size condition MEP values differ ≤10 min after tDCS in the anodal and cathodal tDCS conditions. For the 35-cm^2 tDCS electrode condition, FDI and ADM differ significantly only immediately after tDCS. Here the excitability enhancement of the FDI representation is larger after anodal tDCS and the excitability diminution smaller after cathodal tDCS as compared with the ADM representation.

Baseline tDCS MEP amplitudes did not differ between ADM and FDI and for the different electrode sizes.

Impact of reference electrode size on long-lasting aftereffects of tDCS on MEP (experiment 2a)

In the ANOVA, the main effects of tDCS and time course, but not electrode size, were significant (Table 2). The interaction between these variables was also significant. As displayed in Fig. 4, anodal tDCS increased and cathodal tDCS decreased MEP amplitude significantly for 60 min after the end of stimulation regardless of reference electrode size.

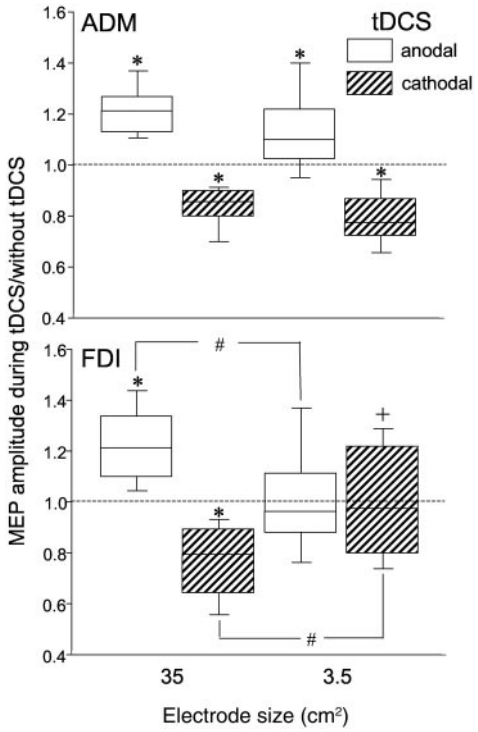

FIG. 2. Diminishing the size of the stimulation electrode focuses the effect of tDCS during current flow. Depicted are standardized mean muscle-evoked potential (MEP) amplitude sizes of the ADM and FDI during 4 s of anodal or cathodal tDCS. In each case, MEPs were recorded after TMS of the motor cortex representation of the ADM or FDI. In the 35-cm^2 stimulation electrode condition, anodal and cathodal tDCSs influence the MEP amplitude size of the ADM and the FDI to a similar extent. Here both muscle representations are situated under the area covered by the tDCS electrode. Using a smaller electrode covering only the representational field of the ADM eliminates the excitability changes of the cortical FDI representation. Asterisks indicate significant deviations between the current and the noncurrent MEPs; hash symbols indicate significant differences of MEP amplitudes resulting from electrode size for FDI and ADM, separately; and the plus symbol indicates differences between ADM and FDI for identical electrode size conditions (2-tailed *t*-test, paired samples, $P < 0.05$). Boxes cover the range of 25th to 75th percentiles, the error bars the 10th to 90th percentiles, the horizontal lines in the boxes indicate standardized mean MEP amplitudes.

Impact of reference electrode size on probabilistic classification learning (experiment 2b)

The ANOVA displays significant main effects of block and tDCS (Table 2). As shown by the results of the *t*-test, performance is significantly improved in blocks 3 and 5 for the anodal frontopolar tDCS condition with the 35-cm^2 electrode size relative to tDCS with the 100-cm^2 electrode size and relative to the sham tDCS condition. Moreover, only in the anodal frontopolar tDCS condition did the 35-cm^2 electrode size performance improve relative to block 1 in block 3, thus indicating a learning effect in this condition. The results of anodal tDCS with the 100-cm^2 electrode size do not differ significantly from those obtained with sham tDCS (Fig. 5).

DISCUSSION

Here we have shown that a modification of stimulation or reference electrode size can be used to focus the effects of tDCS. The primary motor cortex tDCS protocol served as a model. In *experiment* 1, we demonstrated that a reduction of stimulation electrode size—keeping current density constant—reduces the spatial extension of the relevantly stimulated area, while leaving its principal efficacy unchanged. In *experiment* 2, we have shown that an increase in reference electrode size—keeping current strength constant and thus reducing current density under this electrode—makes the reference electrode functionally inert, while not concomitantly influencing the effects under the motor cortex stimulation electrode, thus increasing the selectivity of tDCS by the motor cortex stimulation electrode.

Reduction of stimulation electrode size focuses its excitability-modifying effects during tDCS (experiment 1a)

The results of this experiment are important in two aspects. First, the intra-tDCS effects can indeed be focused by diminishing electrode size while keeping current density constant. TMS over the motor cortex representation of the FDI resulted in tDCS polarity-specific excitability changes in the 35-cm^2 tDCS electrode size condition, where this muscle representa-

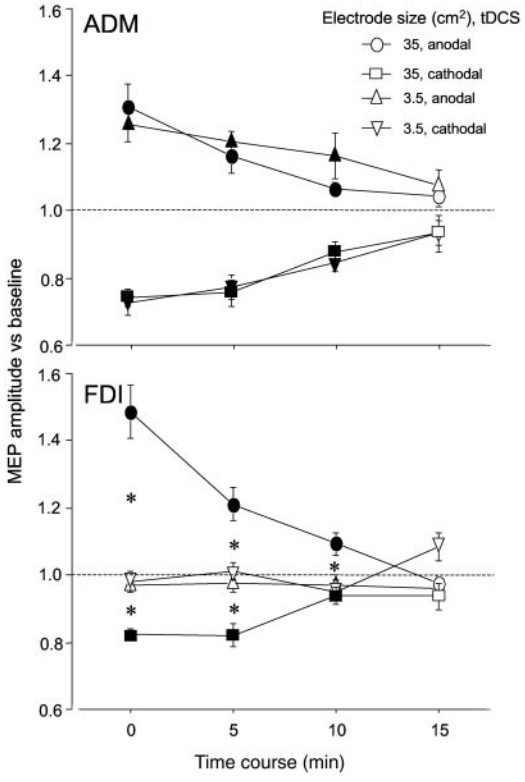

FIG. 3. Effects of different stimulation electrode sizes on the aftereffects of tDCS. Depicted are baseline-standardized mean MEP amplitude sizes of the ADM and FDI after 7 min of anodal or cathodal tDCS. In the 35-cm^2 electrode size condition, in which the tDCS electrode covers the motor cortex representation of the ADM and the FDI, anodal tDCS enhances and cathodal tDCS diminishes excitability of both areas for some minutes after the end of stimulation. As shown by the *t*-test, immediately after the end of anodal tDCS, the MEP amplitude change is larger for the FDI than for the ADM, but smaller immediately after cathodal tDCS. When tDCS is performed selectively over the motor cortex representation of the ADM (electrode size 3.5 cm^2), the excitability change for the ADM is identical to the former condition, but TMS over the representation of the FDI reveals no excitability changes as compared with baseline. Filled symbols indicate deviations of the post-tDCS MEP amplitudes relative to baseline; the asterisks mark differences between MEP amplitudes of the ADM or FDI obtained with different tDCS electrode sizes. Error bars represent SEs.

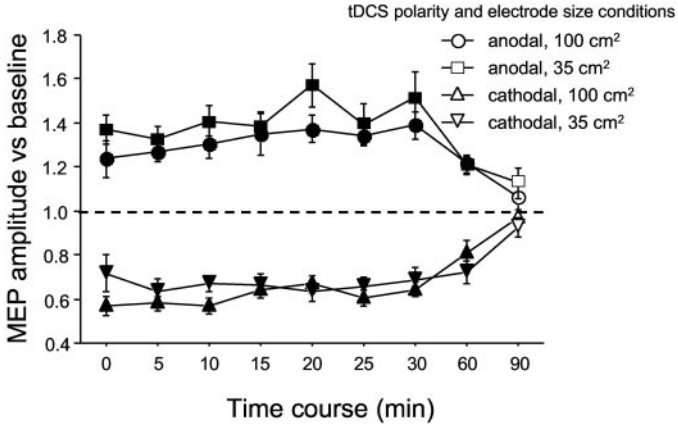

FIG. 4. Increasing the size of the reference electrode does not influence the effect of motor cortical tDCS. Depicted is the time course of baseline-standardized mean MEP amplitude changes after 10 min of anodal or cathodal tDCS of the motor cortex representation of the ADM. Anodal tDCS enhances whereas cathodal tDCS reduces excitability for ≤60 min after the end of stimulation. Effect is identical for both the 35- and the 100-cm^2 reference electrode size. Filled symbols indicate deviations of the post-tDCS MEP amplitudes relative to baseline; error bars represent SEs.

tion was situated under the electrode, but not in the 3.5-cm^2 tDCS electrode size condition (Fig. 2), where its representational field was outside the area covered by the electrode.

Second, the size of the tDCS electrode can be diminished without concomitantly reducing its excitability-modifying effects for the muscle representations under the electrode: For both tDCS polarities, the MEP amplitudes elicited from the ADM, which was situated under the tDCS electrodes in both electrode size conditions (conventional and small), were shifted to a similar degree. However, variability of the results was slightly larger for the small tDCS electrode, especially for anodal tDCS, which might have caused the nonsignificant difference between MEPs elicited from ADM and FDI for anodal tDCS under the small tDCS electrode condition.

The larger variability of the excitability shifts induced by the smaller stimulation electrode might be caused by two factors. First, a smaller stimulation electrode might be less effective in depth and thus a more superficial cortical volume may have been affected by tDCS. Second, the smaller electrode might have stimulated a smaller number of afferents of the ADM representation and thus reduced efficacy of stimulation in some subjects.

However, the reason for the similar efficacy of tDCS administered by both the small and the conventionally sized electrode is that current density was kept constant and that current density determines the efficacy of electrical stimulation (Agnew and McCreery 1987).

Reduction of stimulation electrode size focuses the aftereffects of tDCS (experiment 1b)

Also with respect to the aftereffects of tDCS, the experimental results demonstrate a more focal motor cortical effect of DC stimulation by diminished electrode size.

The 35-cm^2 tDCS electrode resulted in polarity-specific excitability modifications of both muscles tested that were in the range of those reported in former studies. With the exception of a slightly larger effect of anodal tDCS and a smaller effect of cathodal tDCS for the FDI, as compared with the ADM, immediately after the end of tDCS, tDCS elicited identical excitability shifts in both cortical movement representations.

Diminishing the electrode size to 3.5 cm^2 left the aftereffects of the ADM—whose motor cortical representational field was situated under the electrode in both conditions— unchanged. Conversely the MEP amplitude of the FDI, whose representational field lay outside the tDCS-covered area in this condition, was not changed by tDCS; thus diminishing electrode size restricted the excitability-modulating effect of tDCS to the motor cortex representation of the ADM.

The motor cortical representation of the FDI is situated posterior to the ADM. This means that by reducing the electrode size to the representation of the ADM, the representation of the FDI lies outside the current flow between stimulation and reference electrode. Thus it might be—and cannot be ruled out by the results of our experiment—that the specific position of the FDI representation might have contributed to the increased selectivity of tDCS by the decreased electrode size. In other words, a cortical area situated between the stimulation and reference electrode might have been modulated by tDCS arising from the current flow between the electrodes. However, because electrical field strength decreases very rapidly with distance from the electrode, most probably as a result of current spread (Miranda et al. 2006; Rush and Driscoll 1968), and we showed in a former study that a stimulation electrode position immediately posterior to the primary motor cortex is ineffective for motor cortex stimulation (Nitsche and Paulus 2000), this effect, if present at all, should be restricted to the immediate vicinity of the electrode.

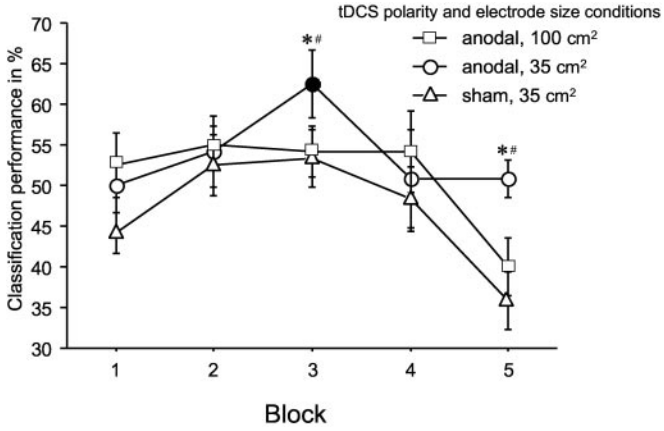

FIG. 5. Increasing the size of the frontopolar reference electrode eliminates its effects on probabilistic classification learning. Shown are the percentages of correct responses in the probabilistic classification task for anodal tDCS with 35- and 100-cm^2 frontopolar electrode size (current strength 1 mA in each condition) and sham tDCS. For blocks 3 and 5, anodal tDCS with the smaller electrode enhances performance relative to the other stimulation conditions. tDCS with the 100-cm^2 electrode size does not influence performance as compared with sham stimulation. Filled symbols indicate deviations of task performance relative to the first block; asterisks indicate differences between the 35- and the 100-cm^2 electrode size condition; hash symbols mark differences between the 35-cm^2 electrode size and the sham stimulation condition. Error bars represent SEs.

Selective elimination of functional efficacy of the reference electrode by increasing its size (experiments 2a and 2b)

Because current density is assumed to be the relevant factor for functional effects of electrical brain stimulation, it seems plausible that its reduction might diminish the efficacy of tDCS. This was previously shown to reduce current strength while keeping electrode size constant (Nitsche and Paulus 2000). Consequently, increasing the size of the reference electrode while keeping current strength constant should eliminate its functional efficacy and thus make possible a more selective tDCS. Our results are in accordance with this hypothesis. Enlargement of the frontopolar reference electrode from 35 to 100 cm^2 did not modify the motor cortical aftereffects of tDCS. In both conditions, 10-min anodal tDCS resulted in a motor cortex excitability increase lasting for 60 min after the end of stimulation, whereas cathodal tDCS resulted in reversed effects. Conversely, for the probabilistic classification task, which is known to be influenced by frontopolar anodal tDCS (Kincses et al. 2004), it was shown that, similar to the foregoing experiment, anodal tDCS of the left frontopolar cortex performed with an electrode size of 35 cm^2 did enhance probabilistic classification learning relative to sham stimulation, whereas an electrode size of 100 cm^2 did not modify performance. Compared with the foregoing experiment, it is noteworthy that there was a trend toward reduced performance in the last block of the experiment compared with the others. This might be caused by group characteristics or loss of attention. However, even in this block, anodal tDCS with a 35-cm^2 electrode improved performance relative to sham stimulation and tDCS with the 100-cm^2 electrode.

Specifically the reduction of current density should be responsible for the missing functional efficacy of the large reference electrode. Additionally the smaller distance between the stimulation and the large reference electrode might have contributed to the missing functional efficacy of the frontopolar electrode because a smaller distance between electrodes causes a larger amount of current shunted through the scalp. Although this effect was sufficiently small not to affect the efficacy of the motor cortical stimulation electrode, it might have contributed to the reduction of the functional effectiveness of the frontopolar electrode, specifically because in this case both electrodes were situated more closely than for the motor cortex stimulation.

Taken together, the results of this study demonstrate that it is possible to increase the focality of tDCS by increasing the size of the reference electrode and thus decreasing current density under this electrode, thereby eliminating the functional efficacy of tDCS for the cortical area under this electrode.

General remarks

In summary, tDCS has been evolving as a powerful tool to induce and to modulate neuroplasticity noninvasively and painlessly in humans during the last few years. The excitability changes produced by this technique were shown to be functionally relevant because they modify perception as well as cognition in healthy subjects (Antal et al. 2001, 2004a,b,c; Matsunaga et al. 2004; Nitsche et al. 2003) and were shown to improve clinical symptoms in patients with neuropsychiatric diseases (Fregni et al. 2005b, 2006a,b,c; Hummel et al. 2005). However, a current limitation of this technique is its relatively poor spatial and temporal resolution. The temporal characteristics of tDCS are inherent to the technique and can hardly be overcome because a critical stimulation duration is needed to induce relevant effects, although we have shown here that the effects of tDCS can be focalized by modifying the size of the stimulation or the reference electrode. Reducing stimulation electrode size to 10% of the original results in spatially more restricted, but not quantitatively diminished effects of tDCS, if current density is held constant. On the other hand, reduction of current density by increasing the size of the reference electrode makes it functionally inefficient and in this way increases the selectivity of tDCS.

These new features of tDCS might be relevant for upcoming studies using tDCS as a tool to modify cortical function in healthy subjects, where spatially restricted stimulation is needed to localize specific cortical functions. Here the original large stimulation electrodes in many cases do not allow a selective stimulation of the cortical areas of interest. Motor cortex stimulation with these electrodes, for example, will also inevitably cause stimulation of the adjacent somatosensory and premotor cortices and thus limit the interpretation of tDCS-induced shifts of performance in terms of cortical areas involved, such as in motor learning. Moreover, a functionally active reference electrode might further compromise interpretation of experimental results and thus require the performance of additional control experiments. Smaller stimulation electrodes and a functionally inert reference electrode enable a much more selective stimulation and are thus able to produce much less ambiguous results, especially because at least the direct effects of tDCS on cortical excitability seem to be restricted to the area under the electrode, as shown in the present experiments and in a recently conducted simulation study (Miranda et al. 2006). Also in the field of clinical applications, it could be advantageous to perform a functionally more selective stimulation. Here a small size of the stimulation electrode might in many cases be less important, compared with basic research, or even counterproductive because excitability modulation of a larger cortical area might result in larger beneficial effects. However, the oppositely directed effect of tDCS by the conventionally sized reference electrode on cortical excitability could be undesirable. In epilepsy or migraine, for example, where pathologically enhanced cortical excitability is to be diminished by cathodal tDCS, the conventionally sized reference electrode will result in an excitability enhancement of the cortices under this electrode and thus probably reduce the beneficial effects of an excitability diminution under the stimulation electrode. Making the reference electrode functionally inert by increasing its size will enable the desired excitability shift by the stimulation electrode without necessarily shifting excitability of another brain area in the opposite direction. It thus might enhance on the one hand the clinical efficacy of tDCS in patients and might on the other hand diminish the risk of inducing disadvantageous side effects of the stimulation. These new stimulation paradigms may thus appreciably improve the applicability of tDCS.

GRANTS

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