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Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability

David Liebetanz, Michael A. Nitsche, Frithjof Tergau and Walter Paulus

Department of Clinical Neurophysiology, Georg-August University Goettingen, Goettingen, Germany

Correspondence to: W. Paulus, Department of Clinical Neurophysiology, Georg-August University Goettingen, Robert-Koch Strasse 40, 37075 Goettingen, Germany
E-mail: wpaulus@med.uni-goettingen.de

Summary

Weak transcranial direct current stimulation (tDCS) induces persisting excitability changes in the human motor cortex. These plastic excitability changes are selectively controlled by the polarity, duration and current strength of stimulation. To reveal the underlying mechanisms of direct current (DC)-induced neuroplasticity, we combined tDCS of the motor cortex with the application of Na^+-channel-blocking carbamazepine (CBZ) and the *N*-methyl-D-aspartate (NMDA)-receptor antagonist dextromethorphan (DMO). Monitored by transcranial magnetic stimulation (TMS), motor cortical excitability changes of up to 40% were achieved in the drug-free condition. Increase of cortical excitability could be selected by anodal stimulation, and decrease by cathodal stimulation. Both types of excitability change lasted several minutes after cessation of current stimulation. DMO suppressed the post-stimulation effects of both anodal and cathodal DC stimulation, strongly suggesting the involvement of NMDA receptors in both types of DC-induced neuroplasticity. In contrast, CBZ selectively eliminated anodal effects. Since CBZ stabilizes the membrane potential voltage-dependently, the results reveal that after-effects of anodal tDCS require a depolarization of membrane potentials. Similar to the induction of established types of short- or long-term neuroplasticity, a combination of glutamatergic and membrane mechanisms is necessary to induce the after-effects of tDCS. On the basis of these results, we suggest that polarity-driven alterations of resting membrane potentials represent the crucial mechanisms of the DC-induced after-effects, leading to both an alteration of spontaneous discharge rates and to a change in NMDA-receptor activation.

Keywords: carbamazepine; dextromethorphan; neuroplasticity; transcranial direct current stimulation; transcranial magnetic stimulation

Abbreviations: ADM = abductor digiti minimi muscle; CBZ = carbamazepine; DC = direct current; DMO = dextromethorphan; LTD = long-term depression; LTP = long-term potentiation; MEP = motor evoked potential; NMDA = *N*-methyl-D-aspartate; tDCS = transcranial direct current stimulation; TES = transcranial electric stimulation; TMS = transcranial magnetic stimulation

Introduction

Neuronal activity is coded by the frequency of spike firing, which in turn is controlled by the level of the neuronal membrane potential. While more positive potentials lead to increased discharge rates, more negative potentials are associated with reduced firing rates. Transcranial direct current stimulation (tDCS) modulates these processes selectively, depending on the current polarity, duration or strength, and is capable of inducing after-effect excitability changes in the human motor cortex (Nitsche and Paulus, 2000, 2001; Baudewig *et al*., 2001). These features favour the evolution of the tDCS technique as a promising tool in neuroplasticity research as well as a therapeutic instrument in neurological disorders associated with hyper- or hypoexcitable cortex.

Indeed, the therapeutic employment of tDCS is not a new idea: Lippold and colleagues reported in the 1960s on mental changes in human subjects and beneficial effects in psychiatric patients caused by weak direct current (DC) stimulation (Costain *et al*., 1964; Lippold and Redfearn, 1964; Redfearn *et al*., 1964). However, until recently it remained the domain of animal studies to relate these DC effects to acute and

Table 1 *Pharmacokinetics of the studied drugs and some details of the study protocol*

Drug	Single oral dose (mg)	P_{max} (h)	No. of subjects	Age (years)	Weight (kg)
CBZ	600	6–24	8	28.0 ± 6.1	68.6 ± 8.2
DMO	150	1.2–2.2	8	28.0 ± 6.1	68.6 ± 8.2

CBZ = carbamazepine; DMO = dextromethorphan; P_{max} = plasma peak time.

Table 2 *Side effects related to drug intake*

Drug	Side effect	No. of subjects
Dextromethorphan	Sedation	2
	Mild vertigo	3
Carbamazepine	Tiredness	4
	Vertigo	3
Placebo	–	–

lasting changes of neuronal activity. By assessing spontaneous discharge rates or the amplitude of evoked potentials, particular modes of action at cortical neuronal membranes have been proposed as being responsible for the changes in neuronal activity during the DC stimulation: (i) the modulation of resting membrane potential by cathodal stimulation hyperpolarizes neurones, whereas anodal stimulation causes depolarization (Bishop and O'Leary, 1950; Terzuolo and Bullock, 1956; Creutzfeldt *et al*., 1962; Bindman *et al*., 1964; Landau *et al*., 1964; Purpura and McMurry, 1965); and (ii) alterations of membrane potentials in turn cause alterations of spontaneous neuronal discharge rates (Bindman *et al*., 1962, 1964; Purpura and McMurry, 1965; Gorman, 1966).

In contrast to the excitability changes during DC stimulation, those mechanisms, which are responsible for the prolongation of excitability changes beyond the period of DC stimulation, still remain poorly understood. If the current flow is applied for a sufficiently long period (10–30 min), after-effects lasting up to several hours have been achieved in animals (Bindman *et al*., 1962, 1964; Gartside, 1968) as well as in humans (Nitsche and Paulus, 2001). As a likely mechanism for this, DC-related changes in discharge rates have been linked to enduring transformations of synaptic strength (Hattori *et al*., 1990; Moriwaki, 1991; Islam *et al*., 1995).

To obtain indirect information about possible mechanisms of DC-induced after-effect excitability changes, we employed two different classes of CNS-active drugs: the membrane acting ion-channel blocker carbamazepine (CBZ) and the *N*-methyl-D-aspartate (NMDA)-receptor blocker dextromethorphan (DMO). CBZ was chosen to investigate the role of the membrane potential shift for the DC-induced after-effects. CBZ stabilizes the membrane potential in a voltage-dependent manner, i.e. the drug is active when the membrane potential is reduced (McLean and Macdonald, 1986). Thus, CBZ should interfere selectively with the excitability changes after anodal tDCS. DMO represents a blocker of the NMDA receptor (Wong *et al*., 1988; Tortella *et al*., 1989; Franklin and Murray, 1992; Netzer *et al*., 1993), which mediates neuroplastic changes of the motor cortex, such as long-term potentiation (LTP) or long-term depression (LTD) (Artola and Singer, 1987; Iriki *et al*., 1989; Kirkwood *et al*., 1993; Hess *et al*., 1994, 1996). Consequently, the NMDA blockade has been demonstrated to prevent plastic changes of the motor and somatosensory cortex in animals and humans (Kano and Iino, 1991; Garraghty and Muja, 1996; Ziemann *et al*., 1998*b*).

Another significant reason for testing the NMDA receptor blocker on the DC-induced after-effect excitability changes originates from a striking feature of the NMDA receptor, namely that it depends on the membrane potential when mediating synaptic plasticity (see, for example, Nayak and Browning, 1999). Assuming that altered spontaneous discharge rates consecutive to polarity-specific shifts of resting membrane potentials induce lasting synaptic changes promoted by NMDA receptors, a blockade of these receptors should prevent the induction of persisting excitability changes.

Materials and methods

Studies were performed on 11 right-handed volunteers (eight men, three women) with a mean age of 31 years (range 25–48 years), who all gave their written informed consent. Potential subjects who were ill, pregnant, suffering from drug abuse or had received any medication (including oral contraceptives) were excluded by an interview and a short clinical examination. Also excluded were people who had metallic implants or who had undergone neurosurgery. All experimental procedures were approved by the ethics committee of the University of Goettingen.

Experimental design

To assess the pharmacological condition, eight subjects were each tested in six separate sessions. Two different pharmacological interventions and a placebo control session were combined with both cathodal and anodal tDCS and the pharmacological interventions. The volunteers were subjected to cathodal or anodal tDCS in a repeated measurement design. Two hours prior to 5 min of anodal or cathodal tDCS, each subject received a single oral dose of either 150 mg DMO, 600 mg CBZ or placebo tablets (Table 1). The dosages were chosen according to the pharmacokinetics of DMO (Silvasti *et al*., 1987) and CBZ (Geradin *et al*., 1976; Pynnonen, 1979) to ensure a sufficient plasma level at the time of the DC stimulation. However, blood levels were not measured, which is a limitation of this study. None of the

fully reversible side effects interfered with the subjects' ability to comply with the requirements of the experiments (Table 2). In order to minimize tiredness, all sessions were performed during the morning hours. The sequence of the sessions was randomized and sessions were separated by 1 week to avoid a potential drug interaction between each session. Additionally, in three subjects (M.A.N., F.T. and W.P.), motor cortex excitability after 5 min tDCS was measured by transcranial electric stimulation (TES). The subjects, as well as the experimenter analysing the data, were unaware of the experimental conditions.

Transcranial DC stimulation

During the sessions, subjects were seated in a reclining chair and were observed continuously. tDCS was applied for 5 min to the left human motor cortex at a current strength of 1 mA. The direct currents were transferred via a saline-soaked pair of surface sponge electrodes (35 cm^2), which were connected to a battery-driven constant-current stimulator (Schneider Electronics, Gleichen, Germany). One electrode was placed over the representational field of the right abductor digiti minimi muscle (ADM), as determined by transcranial magnetic stimulation (TMS), was the other electrode placed above the contralateral orbita. This electrode position was found to be optimal for stimulation of the motor cortex in a previous study (Nitsche and Paulus, 2000). The polarity refers to the electrode of the left motor cortex. All subjects experienced a slight itching under the electrodes during both cathodal and anodal tDCS.

Monitoring of DC effects

Induced cortical excitability changes were assessed by the motor potentials evoked by single-pulse TMS using a Magstim 200 magnetic stimulator (Magstim Company, 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 backwards and laterally at 45° from midline. The optimal position was defined as the site where stimulation resulted consistently in the largest motor-evoked potential (MEP). Surface EMG was recorded from the right ADM. 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 analogue-to-digital rate of 5 kHz, and further relayed into a laboratory computer using the Neuroscan software collection (Neuroscan Inc., Herndon, VA, USA) and conventional averaging software.

The intensity of the stimulator output was adjusted for baseline recording so that the average stimulus led to an MEP of ~1 mV, 5 min prior to DC stimulation. A baseline sequence of 20 stimuli was recorded at a rate of 0.25 Hz. Before the first session, subjects were trained to acquire complete muscle inactivity. During this phase and during the recording sessions acoustic feedback was given through a loudspeaker. In addition, subjects were requested to rest 30 min before the DC stimulation in order to acquire stable baseline levels. Immediately after the termination of the DC stimulation, MEPs were recorded at the same stimulator output intensity at a frequency of 0.25 Hz during the following 10 min. The DC stimulation itself did not produce any muscle activity as controlled by permanent EMG monitoring.

TES was performed with the anode placed above the representational field of the ADM and the cathode above the vertex. The MEPs of the ADM were recorded using Ag–AgCl electrodes in a belly-tendon montage and a laboratory computer, using the Neuroscan system (Neuroscan Inc., Herndon, VA, USA). A randomized series of each 20 TMS- and TES-stimuli (frequency 0.25 Hz) was applied before and after 5 min tDCS.

Statistical analysis

For statistical calculation, the MEP amplitudes after DC stimulation were averaged over 1 min and normalized to baseline: they are given as a percentage of the respective antecedent baseline. The distribution of data was tested for normality by applying the Kolmogorov–Smirnov test. Three-way-factorial repeated measurements ANOVAs (analyses of variance) were performed with drug, polarity and time course after current stimulation as independent variables, and the MEP-amplitude serving as the dependent variable. *Post hoc* tests [Fisher's PLSD, (protected least significant difference), level of significance $P < 0.05$] were then performed to compare the baseline MEP amplitudes before current stimulation with those afterwards, as well as to compare the post-tDCS values of each drug condition with the corresponding time bin of the placebo condition. Differences in the stimulator output intensities that were required to produce MEPs of 1 mV in the different drug conditions were assessed using Student's *t*-tests (level of significance $P < 0.05$). Likewise, the TES/TMS values before and after tDCS were compared using Student's *t*-tests (paired samples, level of significance $P < 0.05$).

Results

We recorded MEPs for 10 min immediately after the end of 5 min of anodal or cathodal tDCS of the motor cortex, after the subjects had taken either CBZ, DMO or placebo tablets 2 h previously. The MEP amplitudes were compared with those obtained directly before the DC stimulation (Fig. 1). The Kolmogorov–Smirnov test revealed normality of distribution for the data set ($P < 0.1$).

Three-way factorial ANOVA

The results of the ANOVA showed a significant main effect of polarity and drug on the MEP size ($P < 0.05$; Table 3). Furthermore, significant interactions of time and polarity, as

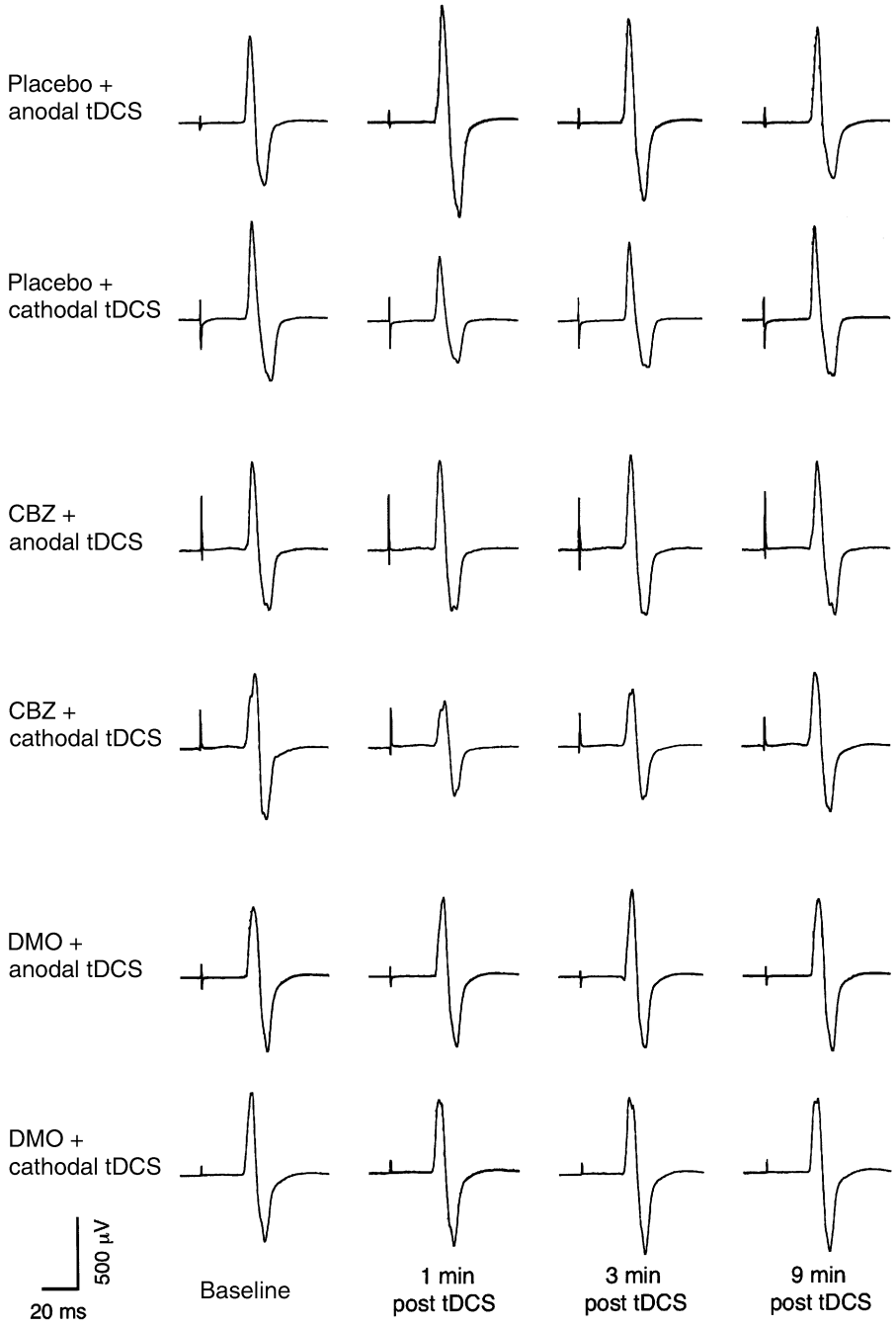

Fig. 1 TMS-elicited MEP amplitudes before and after 5 min of anodal and cathodal tDCS, under different pharmacological conditions. Shown are EMG recordings from the right ADM muscle of one subject, illustrating the effects of CBZ and DMO on the after-effect excitability changes induced by tDCS. All MEPs are averages of a sequence of 15 single recordings. Note the selective prevention of an MEP increase after anodal DC stimulation by CBZ as compared with the placebo. In addition, DMO suppressed the DC-induced after-effects, irrespective of the polarity of stimulation.

well as of polarity and drug, and of polarity, time and drug intake were revealed ($P < 0.05$).

The results of the three-way-factorial repeated measurements ANOVA (Table 3) show that the effects of the current flow depend on the drug condition (CBZ, DMO and placebo) and the polarity of current flow. Significant interactions of time and polarity, as well as of polarity and drug, indicate that the time course of DC after-effects depends on stimulation polarity, and that each drug interacted differently with respect to the direction of the

current flow. Furthermore, the interaction of polarity, time and drug intake demonstrates that drug intake changes the time course of tDCS-induced excitability changes depending on the polarity.

Drug-free condition

As in previous experiments (Nitsche and Paulus, 2000), pure anodal tDCS caused an elevation in MEP sizes for several minutes following 5 min of tDCS. The initial maximum reached nearly 140% of baseline level (Fig. 2A; Fisher's PLSD, paired samples, $P < 0.05$). During the following minutes the MEP size attenuated to baseline level, where it remained stable. After cathodal tDCS, the MEP size was decreased for several minutes following tDCS. The initial reduction to nearly 60% disappeared within the following minutes. Similar to the course of the excitability changes after anodal tDCS, the baseline level was re-established after 5 min (Fig. 2B).

Drug interactions

As compared with the placebo condition, the stimulator output intensity had to be increased by 5–8% of the maximum output to achieve baseline MEP sizes of ~1 mV when CBZ was administered ($P < 0.05$) (Table 4). Conversely, DMO had no discernible effects on the baseline MEP amplitudes when compared with the drug-free condition, and the stimulator output intensity did not have to be adjusted.

According to the results of the *post hoc,* test (Fisher's PLSD), CBZ intake abolishes the after-effects of anodal tDCS completely. The MEPs did not differ significantly in size from the baseline level over the entire time-course ($P > 0.05$) (Fig. 2A). In contrast, CBZ altered neither amplitude nor time-course of the after-effect decrease of MEP sizes after cathodal tDCS as compared with the placebo condition (Fig. 1B).

DMO, in contrast to CBZ, prevented both anodal and cathodal tDCS after-effects completely; it hindered any

Table 3 *Results of the three-way factorial repeated measurements ANOVA*

Variables	Degrees of freedom	*F* values	*P* values
Polarity of current stimulation	1	50.292	<0.0001*
Time course	9	1.196	0.311
Drug	2	4.552	0.012*
Time course × polarity	9	17.969	<0.0001*
Time course × drug	18	1.279	0.171
Drug × polarity	2	4.750	0.01*
Drug × polarity × time course	18	4.414	<0.0001*

*$P < 0.05$.

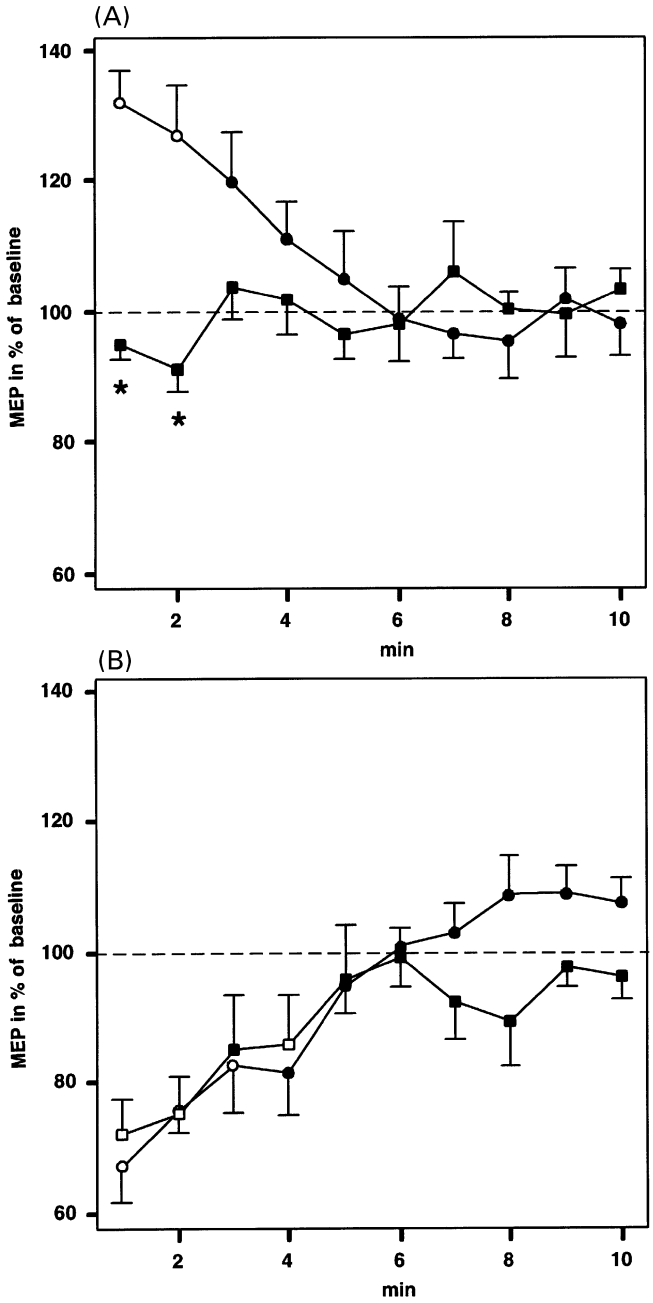

Fig. 2 Comparison of post-stimulation MEP amplitudes after intake of CBZ or placebo. The time-course of TMS-assessed persisting changes in motor cortical excitability is shown after 5 min tDCS with 1 mA. In the placebo condition (circles), the MEP amplitudes return to baseline within 5 min. CBZ applied prior to anodal tDCS (**A**, squares) prevents any significant post-stimulation excitability changes, while CBZ does not interfere significantly with the after-effect hypoexcitability produced by cathodal tDCS (**B**, squares). Significant MEP size alterations from baseline are represented with open symbols; asterisks indicate significant differences between the drug conditions in the corresponding time bin of the placebo condition (Fisher's PLSD, $P < 0.05$; bars show the standard error of the MEP amplitudes).

Table 4 *Stimulator output intensities required producing a preset MEP size of 1 mV*

Drug	Stimulator output in per cent (standard error)	*t*-test
Placebo	53.25 (1.53)	–
Carbamazepine	57.00 (0.88)	0.001*
Dextromethorphan	54.56 (0.80)	0.228

*$P < 0.05$.

significant change in post-anodal or post-cathodal MEP sizes from baseline level (Fisher's PLSD, $P < 0.05$) (Fig. 3A and B).

TES versus TMS

TES-elicited MEP amplitudes did not rise following anodal tDCS, at the same time as TMS-elicited MEP amplitudes increased in the very same experimental session. Also after cathodal tDCS, only the TMS-elicited MEPs decreased, while the TES-evoked MEPs remained stable (*t*-test, $P < 0.05$) (Fig. 4).

Discussion

The principle finding of this study is that both drugs tested, each with different mechanisms of action, interfered in a specific manner with the capability of the motor cortex to react with sustained excitability changes to tDCS.

The question of possible underlying mechanisms of tDCS after-effects includes that of the locus of action of the applied currents. There is strong evidence in the available animal literature that DC stimulation directly affects the underlying cortex and that cortical neurones alter their activity state during, as well as after, the current flow (Bishop and O'Leary, 1950; Terzuolo and Bullock, 1956; Bindman *et al*., 1962, 1964; Creutzfeldt *et al*., 1962; Landau *et al*., 1964; Purpura and McMurry, 1965; Gorman, 1966; Hattori *et al*., 1990; Kano and Iino, 1991; Moriwaki, 1991; Islam *et al*., 1995; Weiss *et al*., 1998). Further indication for the site of action of tDCS originates from our control experiments using a sequence of TES- and TMS-elicited MEPs. In contrast to TMS, TES produces mainly a direct activation of cortico-spinal neurones (Day *et al*., 1987; Rothwell *et al*., 1991, 1997). Therefore our results with the TES-evoked MEPs, which in contrast to the TMS-evoked MEPs remained stable after 5 min of tDCS, show that the TMS-assessed changes in motor cortical excitability (as a general measure of both membrane excitability and intra-motor-cortical synaptic transmission) are most probably localized in the motor cortex and not at the level of the corticospinal tract.

Additionally, the fact that no peripheral muscle activation has been recognized in the completely relaxed muscle during the DC stimulation, as tested by high gain surface EMG recordings, indicates that tDCS did not produce an above-threshold activation of the pyramidal tract neurones or spinal motor neurones.

By binding to sodium channels in the inactive state and by slowing the rate of recovery of these channels, CBZ produces a limitation of high frequency repetitive firing (Schwarz and Grigat, 1989; Mcdonald, 1995). It is of particular interest for our results that this action of CBZ is voltage-dependent. That means that the CBZ effect is enhanced when the membrane potential is reduced and remains absent when the membrane is hyperpolarized (McLean and Macdonald, 1986). CBZ specifically enhances the TMS threshold, which is considered to reflect the membrane excitability (Ziemann *et al*., 1996). Using the double pulse TMS paradigm, CBZ has been excluded from interfering with intracortical facilitation or inhibition, both of which are assumed to correspond to the condition of intracortical synaptic transmission (Ziemann *et al*., 1996). As a consequence of the threshold-increasing properties in the present study, CBZ caused a reduction in the resting MEP size in comparison to the placebo condition. We therefore had to increase the mean stimulator output by 4.75% of the maximum output to maintain the preset MEP sizes of 1 mV. By applying the drug 2 h before the DC stimulation and by recording the baseline values immediately before the DC stimulation, we assume similar TMS threshold conditions during both baseline and post-DC recordings. The changes in the MEP sizes after CBZ intake relative to placebo imply effective CBZ levels in all subjects.

The results on the complete suppression of anodal after-effects by the ion channel blocker CBZ in our study now suggest that, in addition to the acute effects, the induction of after-effects by anodal DC stimulation depends on its action at the neuronal membrane also. Moreover, in respect to the voltage dependency of the CBZ effect, the polarity-specific interaction of CBZ indicates that a lowering of the resting membrane potential towards depolarization may be essential for the development of anodal tDCS-induced after-effect excitability changes. The fact that cathodal after-effects have been left unchanged by CBZ would be in line with a membrane hyperpolarization by cathodal tDCS, as shown in the animal experiment (Purpura and McMurry, 1965). However, CBZ also increases the release of acetylcholine (Mizuno et al., 2000). Yet, these effects in rat hippocampus and striatum were achieved at a dose that is 2.5–3.2 times the dose we gave, even where total absorption had occurred. CBZ, in addition, has been shown to raise dopamine levels via activation of adenosine A2 receptors (Okada *et al*., 1997). However, a relevant dopaminergic action of CBZ on our results is unlikely since CBZ at a similar dose as used in our study had no effect on intracortical inhibition, which according to a previous TMS study is enhanced by dopaminergic drugs (Ziemann *et al*., 1997).

DMO intake prevented both anodal and cathodal tDCS-induced after-effects, demonstrating that DMO critically interferes with the functionality of tDCS irrespective of the

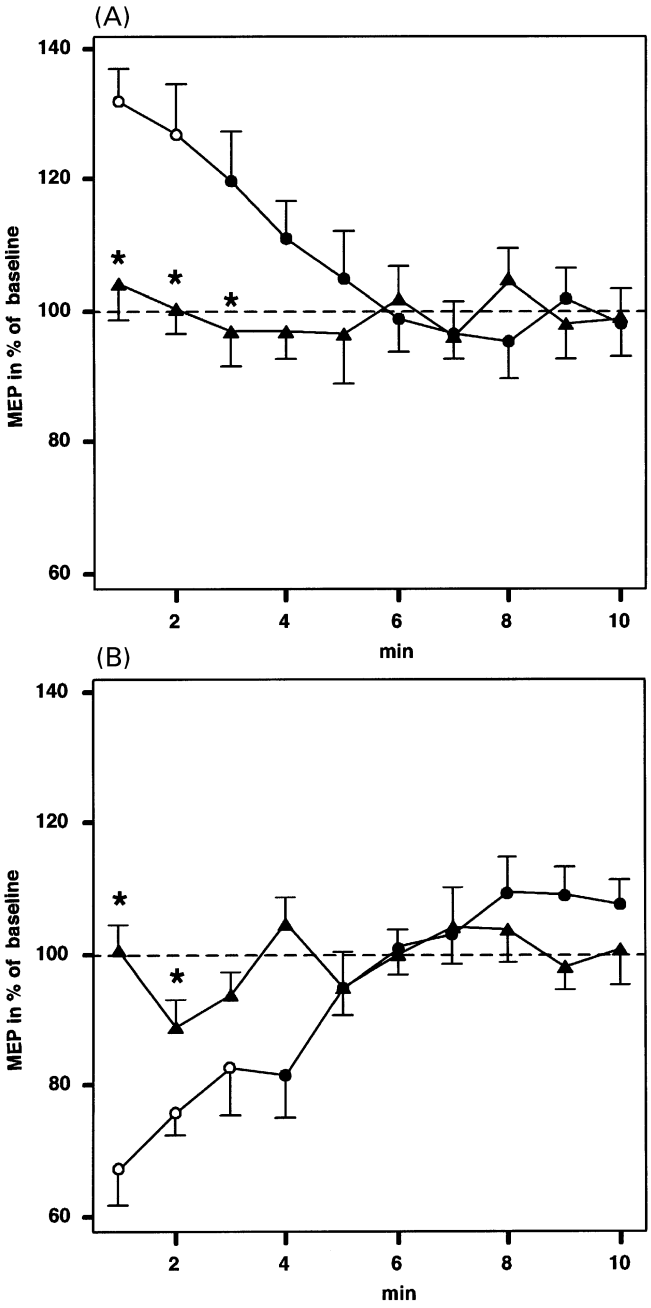

Fig. 3 Comparison of post-stimulation MEP amplitudes after intake of DMO or placebo. The time course of TMS-assessed persisting changes in motor cortical excitability is shown after 5 min tDCS with 1 mA. DMO (triangles) 2 h before tDCS hinders significant post-stimulation excitability changes after both anodal (**A**) and cathodal tDCS (**B**), which are present in the placebo condition (circles). Significant MEP size alterations from baseline are represented with open symbols; asterisks indicate significant differences between the drug conditions in the matching time bin of the placebo condition (Fisher's PLSD, $P < 0.05$; bars show the standard error of the MEP amplitudes).

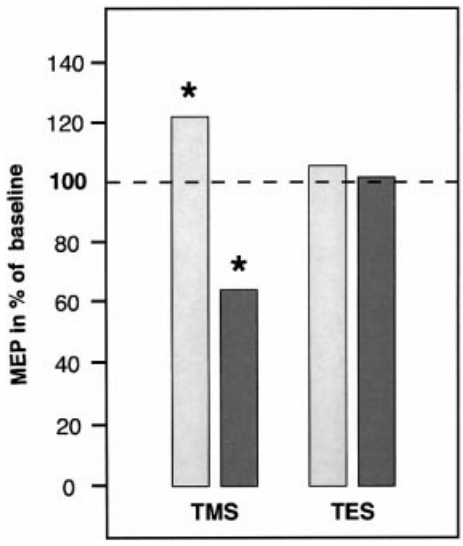

Fig. 4 Comparison of MEP amplitudes evoked by TMS and TES before and after 5 min of tDCS. Columns indicate baseline-standardized MEP amplitudes produced by a random sequence of 20 pulses of each TES and TMS after 5 min of either cathodal (open columns) or anodal tDCS (filled columns) in three subjects. Asterisks indicate significant changes of the evoked potentials after tDCS as compared with the baseline values that are displayed as 100% (Student's *t*-test, $P < 0.05$).

polarity of DC stimulation. These effects are most likely produced at the NMDA receptor, which is blocked by DMO in a non-competitive manner (Wong *et al*., 1988; Tortella *et al*., 1989; Franklin and Murray, 1992). However, DMO in addition causes a partial inhibition of voltage-dependent Ca^{2+} and Na^{+} channels at higher doses (Netzer *et al*., 1993). If DMO were to contribute with a relevant inhibition of Ca^{2+} and Na^{+} channels to our results, the drug should, like CBZ, increase the MEP threshold and the MEP size by itself. However, according to our results and that of a previous study (Ziemann *et al*., 1998*a*), DMO in an equal dose left the MEP thresholds and MEP sizes unchanged, in contrast to CBZ. Thus, it is unlikely that these additional actions of DMO contributed to the effect on the tDCS after-effects.

NMDA receptors are able to function in two ways: first, in signal coding (excitatory synaptic transmission), and secondly in induction of synaptic plasticity (leading to a modification of synaptic strength) (D'Angelo and Rossi, 1998). In this regard, our results on the DMO-related suppression of both cathodal and anodal after-effects suggest that the induction of synaptic plasticity rather than a pure glutamatergic, i.e. excitatory, signal transmission may represent the relevant contribution of NMDA receptors to the after-effects of tDCS.

The duration of the after-effects in our current experiments with 5 min of tDCS has been restricted to several minutes. Thus, in the current experiments we have no evidence for LTD or LTP. However, by applying 13 min of anodal tDCS, an increase in cortical excitability could be achieved for up to 90 min in a parallel study (Nitsche and Paulus, 2001). In addition, in the human LTD- and LTP-like effects could be produced by certain types of associated stimulation, i.e. repetitive TMS in combination with deafferentation, short-

term sensory stimulation or paired somatosensory and TMS (Hamdy *et al*., 1998; Ziemann *et al*., 1998*b*; Stefan *et al*., 2000). With respect to the time course, the achieved excitability changes of a few minutes would be compatible with post-exercise central inhibition for the case of cathodal stimulation and short-term potentiation, or post-tetanic potentiation for anodal effects (Samii *et al*., 1996). In contrast to post-tetanic potentiation, short-term potentiation and depression are NMDA receptor-dependent (Malenka, 1991; Xie *et al*., 1996), and therefore seem to represent the more likely mechanisms.

How can DC-induced shifts in the membrane potential cause lasting excitability changes promoted by the NMDA receptor? An important feature of the NMDA receptor is coupled to the voltage sensitivity of this receptor. Above the resting membrane potential, the inward current induced by a particular concentration of glutamate increases with depolarization of the membrane potential. Equally, the capability of these receptors to mediate an increase in synaptic strength is voltage dependent (MacDonald and Wojtowicz, 1982; Artola *et al*., 1990).

Combining the action of the DC stimulation at neuronal membranes, leading to increased firing rates in the case of anodal stimulation with the voltage-dependent activation of NMDA receptors in the initiation of long-term effects, we suggest that in the case of anodal tDCS, tDCS-driven post-synaptic membrane depolarization, accompanied by enhanced pre-synaptic input due to increased firing rates, leads to an NMDA receptor-mediated augmentation of synaptic strength, presumably via the increase in intracellular Ca^{2+} levels.

A possible mechanism for the cathodal after-effects could be based on a DC-induced hyperpolarization of the membrane potential, which leads to a depression of synaptic strength.

The combination of post-synaptic hyperpolarizing pulses with low frequency pre-synaptic stimulation induces a prolonged excitability depression *in vitro* (Frégnac *et al*., 1990). In addition, we know from *in vivo* animal experiments that cathodal stimulation produces a hyperpolarization of the neuronal membrane (Bindman *et al*., 1964; Purpura and McMurry, 1965). The prolongation of these effects, beyond the period of the DC stimulation that in animals could be achieved for up to several hours (Bindman *et al*., 1964), is possibly NMDA receptor-mediated. In line with this hypothesis, the suppression of cathodal after-effects by DMO in our experiments strongly suggests that they, analogous to STD and LTD, are mediated by NMDA receptors.

On the polarity-specific action of CBZ and the interference of DMO with both cathodal and anodal after-effects, we assume that a combination of glutamatergic and membrane mechanisms is necessary to induce after-effects of weak tDCS. These results further suggest that polarity-driven alterations of resting membrane potentials represent the crucial mechanism of the DC-induced after-effects that consequently lead to both a change in spontaneous discharge rates and to an NMDA receptor-mediated alteration of synaptic strength.

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