Exp Brain Res (2008) 185:279–286
DOI 10.1007/s00221-007-1149-z

RESEARCH ARTICLE

Short and long duration transcranial direct current stimulation (tDCS) over the human hand motor area

Toshiaki Furubayashi · Yasuo Terao · Noritoshi Arai · Shingo Okabe · Hitoshi Mochizuki · Ritsuko Hanajima · Masashi Hamada · Akihiro Yugeta · Satomi Inomata-Terada · Yoshikazu Ugawa

Received: 7 July 2006 / Accepted: 19 September 2007 / Published online: 17 October 2007

Abstract The aim of the present paper is to study effects of short and long duration transcranial direct current stimulation (tDCS) on the human motor cortex. In eight normal volunteers, motor evoked potentials (MEPs) induced by transcranial magnetic stimulation (TMS) were recorded from the right first dorsal interosseous muscle, and tDCS was given with electrodes over the left primary motor cortex (M1) and the contralateral orbit. We performed two experiments: one for short duration tDCS (100 ms, 1, 3 or 5 mA) and the other for long duration tDCS (10 min, 1 mA). The stimulus onset asynchrony (SOA) between the onset of tDCS and TMS were 1–7 and 10–120 ms for the former experiment. In the latter experiment, TMS was given 0–20 min after the end of 10 min tDCS. We evaluated the effect of tDCS on the motor cortex by comparing MEPs conditioned by tDCS with control MEPs. Cathodal short duration tDCS significantly reduced the size of responses to motor cortical stimulation at SOAs of 1–7 ms when the intensity was equal to or greater than 3 mA. Anodal short duration tDCS significantly increased MEPs when the intensity was 3 mA, but the enhancement did not occur when using 5 mA conditioning stimulus. Moreover, both anodal and cathodal short duration tDCS decreased responses to TMS significantly at SOAs of 20–50 ms and enhanced them at an SOA of 90 ms. Long duration cathodal tDCS decreased MEPs at 0 and 5 min after the offset of tDCS and anodal long duration tDCS increased them at 1 and 15 min. We conclude that the effect at SOAs less than 10 ms is mainly caused by acute changes in resting membrane potential induced by tDCS. The effect at SOAs of 20–100 ms is considered to be a nonspecific effect of a startle-like response produced by activation of skin sensation at the scalp. The effect provoked by long duration tDCS may be short-term potentiation or depression like effects.

Keywords Transcranial direct current stimulation (tDCS) · Transcranial magnetic stimulation (TMS) · Motor cortex

T. Furubayashi (✉) · Y. Terao · N. Arai · S. Okabe · H. Mochizuki · R. Hanajima · M. Hamada · A. Yugeta · S. Inomata-Terada · Y. Ugawa
Department of Neurology, Division of Neuroscience, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: Furubayashi-tky@umin.ac.jp

H. Mochizuki
Department of Neurology, Higashi-Saitama National Hospital, Saitama, Japan

Y. Ugawa
Department of Neurology, School of Medicine, Fukushima Medical University, Fukushima, Japan

Introduction

Many studies using tDCS have been performed since Priori et al. (1998) reported the functional effects of very weak transcranial direct current stimulation (tDCS) on the human motor cortex. Nitsche et al. have extensively studied lasting excitability changes induced by transcranial direct current stimulation (tDCS) over several areas of the cerebral cortex, such as the motor cortex, somatosensory cortex, prefrontal cortex and the occipital cortex (Nitsche and Paulus 2000; Baudewig et al. 2001; Antal et al. 2001; Kincses et al. 2003; Lang et al. 2004; Matsunaga et al. 2004; Fregni et al. 2005; Dieckhöfer et al. 2006). In general, a weak anodal tDCS of long duration induces long lasting facilitatory effects whereas cathodal tDCS causes inhibitory effects. The outcome also depends on the intensity and duration of tDCS (Nitsche and Paulus 2000, 2001; Nitsche

et al. 2003b). The idea of these experiments was based on results in animal studies (Bindman et al. 1964, 1979; Purpura and McMurtry 1965), normal human subjects (Lippold and Redfearn 1964; Sheffield and Mowbray 1968; Hall et al. 1970) and psychiatric patients with depression or schizophrenia (Lifshitz and Harper 1968; Costain et al. 1964; Carney 1969; Arfai et al. 1970; Lolas 1977). These historical backgrounds have been summarized and described by Priori (2003) in a recent review.

Several basic mechanisms for the effects have been proposed. Anodal stimulation depolarizes the resting membrane potential of neurons while a cathodal stimulus elicits hyperpolarization. Both will alter spontaneous discharge rates and hence may change synaptic strength (Bindman et al. 1964; Purpura and McMurtry 1965). Liebetanz et al. (2002) suggested that lasting excitability changes, whether facilitatory or inhibitory, were produced by NMDA receptor activation. They also concluded that an alteration of the resting membrane potential plays a crucial role in triggering the DC-induced after effects (Liebetanz et al. 2002). On the other hand, Ardolino et al. (2005) claimed that these effects, at least for cathodal stimulation, have a non-synaptic mechanism of action based upon changes in neural membrane function. As mentioned above, the mechanisms remain to be elucidated. Most previous studies have investigated the relatively long lasting after-effects. Here we have studied some acute effects after short duration tDCS, and have compared them with the effects after long duration tDCS.

Subjects

Subjects were eight normal Japanese volunteers (7 male, 1 female), aged 29–50 years. They had no previous history of any neurological or psychiatric disorders. None of the subjects received acute or chronic medications during the experiments. They all participated in the main part of experiment 1. Three of them participated also in the brainstem electrical stimulation (BES) and occipital stimulation (Occ) experiments. Six subjects participated in the main part of experiment 2, and four of them in Occ experiment. All of them had given their written informed consent to take part in the experiments. The procedures described here were approved by the Ethics Committee of the University of Tokyo.

Methods

Transcranial magnetic stimulation (TMS)

In order to evaluate motor cortical excitability changes induced by tDCS, transcranial magnetic stimulation (TMS) was given with a figure of eight shaped coil connected with a Magstim 200 magnetic stimulator (Magstim Company). Anteromedially directed currents were induced in the brain to activate the left hand motor area. Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle (FDI) with a pair of surface cup electrodes with a belly-tendon montage. Signals were amplified with filters set at 100 and 3 kHz and recorded by a computer (signal processor DP-1200, GE Marquette Medical Systems) for the later off-line analysis. The intensity was adjusted to elicit a response of 0.2–0.4 mV in the relaxed FDI in each subject. This intensity corresponded to 1.2–1.3 times the resting motor threshold. Although the intensity might be smaller than that used by other investigators, we have shown that the strength was optimal to induce many suppression effects and did not mask inhibition, such as cerebellar suppression (Ugawa et al. 1995), short interval intracortical inhibition (Hanajima et al. 1998), transcallosal inhibition (Hanajima et al. 2001) or loud sound cortical suppression (Furubayashi et al. 2000). We fixed these intensities throughout the experiments.

Brainstem electrical stimulation (BES)

Three subjects participated in this experiment. BES was performed with a high voltage electric stimulator (Digitimer D180A stimulator). Cup electrodes were fixed at the posterior edges of the bilateral mastoid processes. The anode was on the left side and the cathode on the right. Subjects maintained the right FDI at rest. The stimulus intensity was carefully adjusted to elicit a control response with similar amplitude to that of cortical control responses in each subject.

Transcranial direct current stimulation (tDCS)

Transcranial direct current stimulation (tDCS) was applied to the left hand motor area at a current strength of 1, 3 and 5 mA with large electrodes similar to those used in the previous studies (Nitsche and Paulus 2000). Direct current was given with a pair of rectangular metallic electrodes soaked with gel (50 × 30 mm) connected to a constant direct current stimulator (electric stimulator 3F46, NEC San-Ei Instruments, Ltd) approved for using in humans by the Ministry of Health, Labor and Welfare of Japan. To exclude some distortion of an electrical field elicited by TMS when using a large metal electrode, we compared the thresholds of TMS over M1 between conditions with and without metal electrodes using a paired Student's *t*-test. Their means were 41.5% (±12.3) of the maximum stimulator output in the condition with electrodes and 39.8% (±11.1) without electrodes, and they did not differ significantly. For cathodal tDCS, the cathode was placed over the left hand motor area for FDI and the anode over the orbit,

and for anodal tDCS, they were reversed. For all kinds of experiments, we studied effects of anodal tDCS and cathodal tDCS. In addition, to exclude some nonspecific effects, tDCS (3 mA in experiment 1 and 1 mA in experiment 2) was given over the occipital area (10 cm posterior from Cz) and the right orbit in a part of experiments.

All subjects felt a slight tingling sensation during tDCS, but neither pain nor discomfort was evoked except a short duration pain at the onset and offset of strong tDCS.

Experimental procedures

We did two experiments. The first one was to study the acute effect of short duration tDCS. The second experiment was performed to confirm the previously reported lasting effects after tDCS for 10 min. Before the main experiments, we identified the left hand motor area for FDI using single pulse TMS in each subject. Subjects comfortably sat on a reclining chair with their arms supported. During the experiments subjects maintained the target muscle at rest using an audiovisual feedback of EMG discharges. A session in which any EMGs due to unintentional contraction were recorded was discarded in the analysis.

Experiment 1

In this experiment, we studied acute effects of 100 ms tDCS on the motor cortex. We used a randomized conditioning-test paradigm. The conditioning stimulus was 100 ms tDCS, and the test stimulus was TMS or BES. The interval between the conditioning and test stimuli was defined as a stimulus onset asynchrony (SOA). The SOA means the interval between the onsets of tDCS and TMS or BES. Conditioned trials in which the conditioning stimulus was given prior to the test stimulus at different SOAs were randomly intermixed with control trials in which the test stimulus was given alone. SOAs for TMS were 1, 2, 3, 5 and 7 ms, and 10–120 ms in steps of 10 ms. One session consisted of seven conditions including a control condition. For one mode of tDCS (same intensity and polarity), we performed at least three sessions to make a whole time course of the effect. Each condition consisted of ten trials. Intertrial intervals were randomly set at 10 ± 2 s by the computer. The effects at SOAs of 1–7 ms were studied using 100 ms tDCS at an intensity of 1, 3 and 5 mA. Effects at SOAs of 10 to 120 ms were studied by using 100 ms tDCS at an intensity of 3 mA. In the former effects, BES was also applied to see whether MEP size changes by tDCS reflected the cortical excitability changes. Because the deepest suppression was evoked at an SOA of 3 ms (see "Results"), we tried to evaluate spinal excitability using BES at that timing. SOAs between tDCS and BES were set at 6, 7, 8, 9 and 10 ms using 100 ms anodal tDCS at an intensity of 3 mA and cathodal tDCS at 5 mA. Those SOAs were used to compare their effects with that on cortical MEPs at a SOA of 3 ms to compensate the latency difference between MEPs to motor cortical stimulation and BES. Ugawa et al. (1991, 1994) reported that the latency difference between responses to TMS and BES in active FDI was about 3–4 ms, and about 5 ms in relaxed condition. Therefore, it was considered that the SOA of 8 ms in BES corresponded to the SOA of 3 ms in TMS experiments. The anodal and cathodal stimulation were also intermixed randomly using a special device to reverse the polarity of tDCS. We measured the sizes of individual responses in all subjects. The time course of the effect was plotted with SOAs on the abscissa and size ratios of the average conditioned response to that of the control response on the ordinate.

To exclude nonspecific effects, we also studied effects of tDCS over the occipital cortex (Occ) on MEPs. For this tDCS, one electrode was placed over an occipital area (10 cm posterior to CZ), and the other over the right orbit. In this experiment, the intensity was fixed at 3 mA and SOAs were the same as the latter part (SOAs of 10–120 ms with 100 ms tDCS) because we got a significant effect on MEP by tDCS over M1 with this intensity (see "Results"). In this experiment, anodal and cathodal stimulations were intermixed randomly in the same session.

Experiment 2

In this experiment, we compared lasting effects elicited by anodal and cathodal tDCSs for 10 min. The electrodes were placed over the same positions as in experiment 1 and currents were given for 10 min continuously. The intensity was fixed at 1 mA. In the present experiment, we could not induce the same long lasting effect as those in previous papers (see "Reults"). To elicit a long-term effect seen in previous papers, we may have used higher intensity stimulation as was used in the experiment 1 because such intensity stimulation may elicit a long lasing effect. However, because no reports have used 3 mA or higher intensity stimulation for 10 min, the ethical committee did not approve us to give higher intensity stimuli in long duration tDCS. MEPs were recorded 0, 1, 3, 5, 10, 15 and 20 min after the offset of tDCS. MEPs at each interval were compared with control MEPs which were recorded before tDCS. At each interval after tDCS, five MEPs were recorded, and twenty MEPs before tDCS. The order of two polarities of tDCS was counterbalanced among the subjects. To exclude some non-specific effects, we also studied lasting effects of tDCS over the occipital cortex. For this tDCS, procedures were the same as those described above except that electrodes were placed over occipital area and the right orbit.

In all experiments, they were separated from the preceding experiment by more than one week in the same subject.

Data analysis

We performed statistical comparisons using the pooled data from all subjects. The amplitude of MEPs was expressed with a ratio of the conditioned response size to the control response size (size ratio) for each individual. For all data, the normality and homogeneity were evaluated by the Kolmogorov–Smirnov test and Levene's test. Because they proved the normality and homogeneity of our data, we used the following processes.

In the former part of the first experiment (SOA = 1–7 ms), three factorial repeated measurements ANOVA was used to assess the effect of the polarity, intensity of tDCS and the SOA on the size of MEPs. Then post hoc comparisons were performed with Fisher's PLSD. For the latter part of the first experiment and the second experiment, two factorial repeated measurements ANOVA (SOA and mode of tDCS) was used for comparisons. Fisher's PLSD was used in post hoc analysis. In order to confirm that the same sized control responses were used in all experiments, control MEPs were compared among different experimental conditions with the one factor ANOVA.

In all statistical analyses, the statistical significant level was set at $P < 0.05$.

Results

No side effects of stimulation were noted in any individuals.

In all experimental conditions, control MEPs were carefully controlled. They were not significantly different in size among different experimental conditions [Experiment 1 (intervals shorter than 10 ms); 1 mA(+) = 0.32 ± 0.12 mV , 1 mA(−) = 0.32 ± 0.11 mV, 3 mA(+) = 0.30 ± 0.12 mV, 3 mA(−) = 0.35 ± 0.09 mV, 5 mA(+) = 0.34 ± 0.13 mV , 5 mA(−) = 0.41 ± 0.15 mV, Experiment 1 (SOA of 10 to 120 ms to M1); (+) = 0.37 ± 0.21 mV, (−) = 0.36 ± 0.14 mV, (SOA of 10to 120 ms for Occ); (+) = 0.36 ± 0.07 (−) = 0.42 ± 0.04, Experiment 2 (M1); (+) = 0.3 ± 0.11 mV, (−) = 0.4 ± 0.18 mV, (Occ); (+) = 0.27 ± 0.04, (−) = 0.26 ± 0.07, $F = 0.733$, $P = 0.725$]. It indicates that we investigated effects on similar sized MEPs in all experiments.

Experiment 1

Effects at intervals shorter than 10 ms

Figure 1 shows mean (±SD) size ratios at SOAs of 1–7 ms. The abscissa indicates the SOA, and the ordinate the size ratio. Three factors (polarity, intensity and SOA) ANOVA revealed that the polarity had a significant effect ($F = 9.445$, $P < 0.01$) on the size ratio whereas neither the intensity nor SOA had a significant effect (intensity;

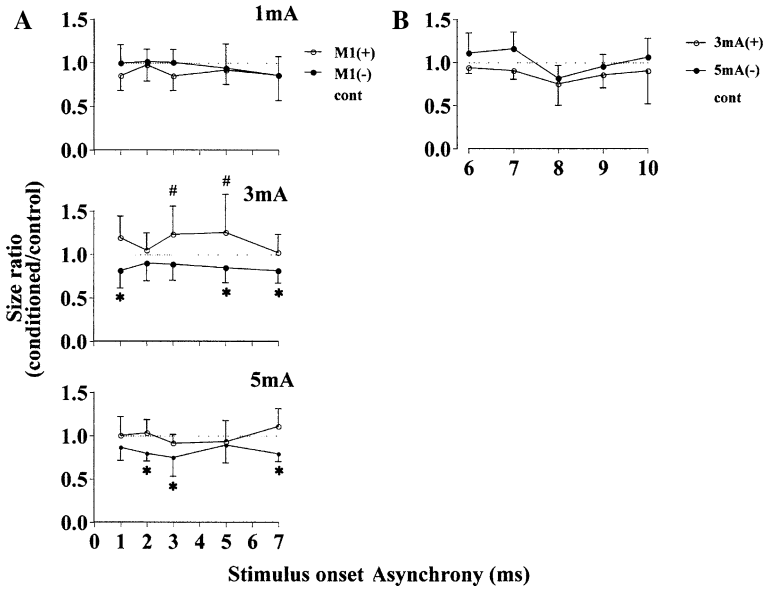

Fig. 1 Mean (±SD) time courses of effects of 100 ms tDCS on MEPs to TMS at short intervals (**a**). Cathodal stimulation on the motor cortex [M1 (−)] at an intensity of 3 or 5 mA decreased MEPs whereas M 1(−) stimuli at 1 mA had no influence on MEPs. Anodal stimulation [M1 (+)] at an intensity of 3 mA increased MEP at stimulus onset asynchronies (SOAs) of 3 and 5 ms whereas other intensities had no significant effects on MEPs. *Doted lines* show control response size. ($P < 0.05$, #: anodal stimulation, *: cathodal stimulation). The time courses of effects of the tDCS on MEPs to BES (**b**). MEP did not change at any SOAs when anodal stimulation at 3 mA or cathodal at 5 mA was given

$F = 1.768$, $P = 0.183$, SOA; $F = 1.507$, $P = 0.189$). A significant interaction was seen between polarity and intensity ($F = 6.335$, $P < 0.001$) even though no interaction was seen between other pairs of any factors (SOA and intensity; $F = 1.647$, $P = 0.095$, SOA and polarity; $F = 1.451$, $P = 0.207$). The three factors interaction was significant ($F = 2.379$, $P < 0.05$). Post hoc analysis showed that the MEP size ratios during cathodal stimulation were significantly smaller than those during anodal stimulation if the intensity was equal to or exceeded 3 mA (3 mA; $P < 0.01$, 5 mA; $P < 0.05$). In the case of cathodal tDCS at 3 mA, MEPs were significantly smaller than the control at SOAs of 1, 5 and 7 ms, and in the case of cathodal tDCS at 5 mA they were smaller than the control at SOAs of 2, 3, and 7 ms ($P < 0.05$). On the other hand, in the case of anodal tDCS at 3 mA, MEPs were significantly larger than the control at SOA of 3 and 5 ms. However, the anodal tDCS at 5 mA did not affect MEPs at any SOAs.

Figure 1b shows mean (±SD) size ratios of MEPs to BES at SOA of 6–10 ms. Two factors (polarity and SOA) ANOVA revealed that neither the polarity nor SOA had a significant effect (polarity; $F = 0.921$, $P = 0.438$, SOA; $F = 3.039$, $P = 0.063$). A significant interaction was not seen between these factors ($F = 0.571$, $P = 0.722$). In either case of anodal tDCS at 3 mA or cathodal at 5 mA, MEPs did not significantly differ from the control MEP at any SOAs.

Effects at SOAs 10–120 ms

Figure 2a shows MEP size ratios at SOAs of 10–120 ms when 3 mA, 100 ms tDCS was given. MEPs were similarly modulated through the whole SOAs in anodal and cathodal stimulations. The two factors ANOVA (polarity and SOA) revealed a significant effect of SOA ($F = 7.89$, $P < 0.001$) on the size ratio, but polarity had no significant effect on it ($F = 0.296$, $P = 0.599$). There was no significant interaction between the SOA and polarity ($F = 1.32$, $P = 0.216$). MEPs were significantly smaller than the control at SOAs of 20, 40 and 50 ms during anodal stimulation and at SOAs of 30 and 40 ms during cathodal tDCS ($P < 0.05$). MEPs tended to be larger at SOAs of 80–100 ms during tDCS of both polarities.

Figure 2b shows mean time courses during and after tDCS over the Occ. Two factors ANOVA revealed a significant effect of the SOA ($F = 3.84$, $P < 0.001$), but polarity had no effects ($F = 3.35$, $P = 0.104$). There was no significant interaction between the SOA and polarity ($F = 0.873$, $P = 0.576$). MEP size ratios were significantly smaller than the control at SOAs of 40–60 ms during anodal stimulation and at SOAs of 20, 40, 50, 60, 70 and 80 ms during cathodal tDCS ($P < 0.05$).

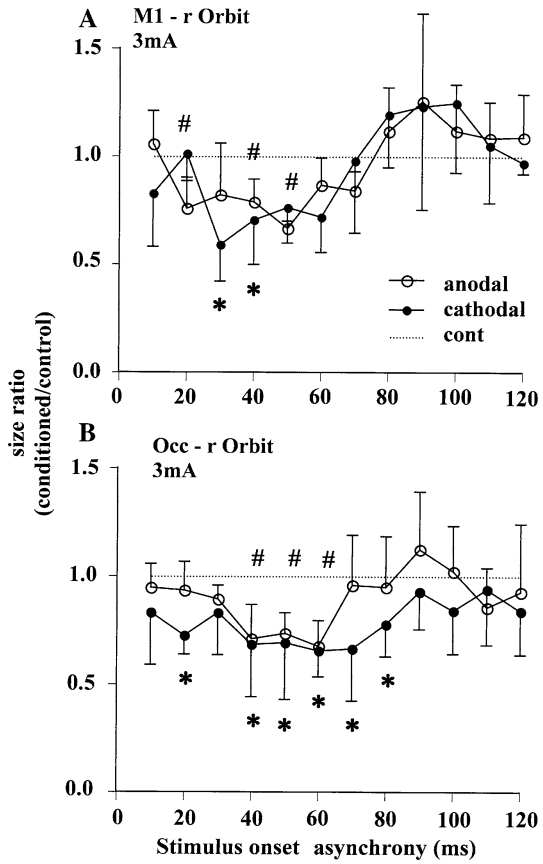

Fig. 2 Mean (±SD) time courses of effects on MEPs at stimulus onset asynchronies (SOAs) of 10–120 ms during 100 ms tDCS at 3 mA. (**a**) tDC stimulation was given with electrodes over the left M1 and right orbit. MEPs were similarly affected by tDCSs of both polarities. MEPs were suppressed at SOAs of 20–50 ms. (**b**) tDC stimulation between the occipital area (Occ) and right orbit. Cathodal and anodal tDCS over the OCC suppressed MEPs at SOA of 20–80 ms. ($P < 0.05$, #: anodal stimulation, *: cathodal stimulation)

Experiment 2

Figure 3 shows mean (±SD) size ratios after 10 min cathodal and anodal tDCS over the hand motor area. Two factors ANOVA (polarity and SOA) revealed a significant effect of polarity ($F = 8.54$, $P < 0.05$) but no effects of SOA ($F = 1.03$, $P = 0.264$). There was significant interaction between the SOA and polarity ($F = 2.29$, $P < 0.05$). Post hoc analysis showed that the MEP size ratios after cathodal stimulation were significantly smaller than those after anodal stimulation ($P < 0.05$). Cathodal tDCS decreased MEPs just after to 5 min after tDCS (0 min $P < 0.01$, 5 min $P < 0.05$). In contrast, anodal tDCS increased MEPs at 1 and 15 min (1 min $P < 0.05$, 15 min $P < 0.01$) after tDCS.

Figure 4 shows mean (±SD) size ratios after 10 min cathodal and anodal tDCS over the Occ. Two factors ANOVA revealed that neither polarity ($F = 2.34$, $P = 0.22$) nor SOA ($F = 1.11$, $P = 0.39$) had a significant effect on the

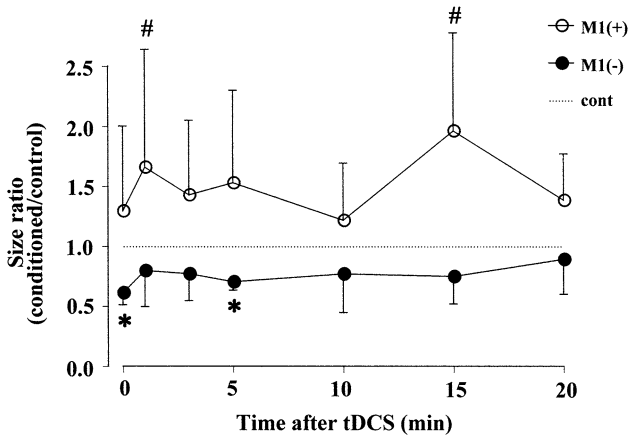

Fig. 3 Mean (±SD) time courses of after effects of 1 mA tDCS over the hand motor area for 10 min. Zero minute indicates the offset of tDCS. *Circles* and *dots* show anodal and cathodal tDCS, respectively. MEPs tended to be suppressed by cathodal tDCS and facilitated by anodal tDCS. Those were significantly inhibited at 0 and 5 min and facilitated at 1 and 15 min after the end of tDCS ($P < 0.05$, #: anodal stimulation, *: cathodal stimulation)

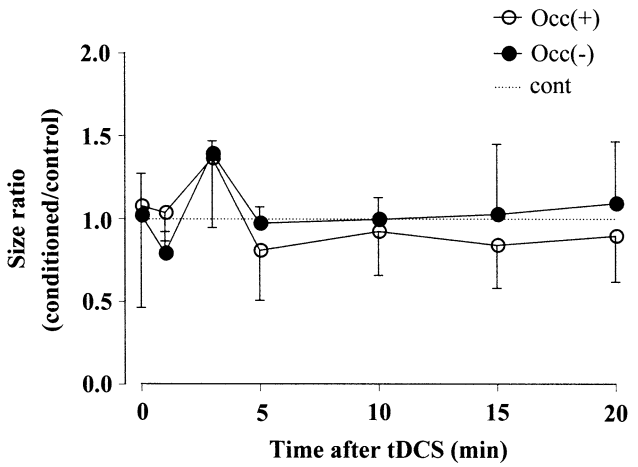

Fig. 4 Mean (±SD) time courses of effects of 1 mA tDCS over the occipital area for 10 min. *Circles* and *dots* show anodal and cathodal tDCS, respectively. No significant changes were seen

size ratio. There was no significant interaction between the SOA and polarity ($F = 1.02$, $P < 0.44$).

Discussion

Our present results can be summarized as follows. Just after the onset of tDCS (SOAs from 1–7 ms), MEPs to TMS were suppressed by cathodal stimulation if the intensity was equal to or exceeded 3 mA, and enhanced by anodal stimulation when the intensity was 3 mA. However, tDCS did not change MEPs to BES at SOAs from 6 to 10 ms. MEPs were suppressed at SOAs of 20–50 ms, and facilitated at SOAs of 80–100 ms for both anodal and cathodal tDCSs if the intensity was high. Also in a similar manner to previous studies of Nitsche et al. (Nitsche and Paulus 2000, 2001; Nitsche et al. 2003b), MEPs were suppressed after long-duration cathodal tDCS (10 min) and facilitated after long-duration anodal stimulation.

Hereafter we discuss the mechanism for the effect at each of three groups of the intervals after tDCS separately.

Effects at intervals shorter than 10 ms

The fact that the immediate effect was elicited on MEPs to TMS but not elicited on MEPs to BES indicates that it is a cortical event. Similar effect during tDCS has been reported by Nitsche and Paulus (2000). They showed motor cortical excitability changes during tDCS for 4 s.

Two possible mechanisms have been proposed to explain the after-effects of DC stimulation on the brain; one is a change in resting membrane potential (hyperpolarization or depolarization), the other is a change in synaptic efficacy. In the former hypothesis, cathodal stimulation is thought to cause hyperpolarization of the resting membrane potential, while anodal stimulation leads to depolarization (Bindman et al. 1964; Purpura and McMurtry 1965; Nitsche and Paulus 2001; Nitsche et al. 2003a, b). In addition, Ardolino et al. (2005) recently demonstrated that cathodal tDCS for 10 min over the motor cortex decreased both MEPs to TMS at rest and MEPs to transcranial electrical stimulation (TES) during slight voluntary contraction, in association with increased motor threshold. They concluded that tDCS did not affect synaptic mechanisms but had an influence through a resting neural membrane function. On the other hand, the after–effects following long-lasting tDCS may involve at least partially synaptic mechanisms as suggested by the pharmacological study (Nitsche et al. 2003a). We have no firm conclusion about the mechanisms for the long-term effect after tDCS.

In contrast, the mechanisms for the immediate or initial effects of tDCS may be explained by the membrane potential changes. Our current results of anodal and cathodal stimulation are consistent with this idea. The rapidness of the effect is also against some mechanism mediated by synaptic efficacy changes because they should not occur at such short intervals as 10 ms or shorter. The intensity needed to evoke this immediate effect was 3 mA or more, which was stronger than that required for the long-term effect (1 mA, see below). This may be compatible with the idea of changes in the resting membrane potentials.

Which neurons or their membrane potentials are affected by tDCS? One is superficial facilitatory or inhibitory interneurons in the cerebral cortex. Another is neuron at the

deeper cortical layers. The deeper layer cells may be affected by higher intensity (5 mA) stimulation as compared with weaker stimulation in long-term effects. This difference in threshold between neurons at different layers may explain some parts of our results. However, we have no good explanation for the finding that the facilitatory effects were gone when using anodal stimulation at 5 mA. One possible explanation may be based on the idea that deeper structures must be activated by higher intensity stimulation. The stimulation at 5 mA may activate some deeper cells as well as superficial internerons. This additional activation of deeper neurons may cancel out the facilitatory effects.

Effects at longer intervals in 100 ms tDCS

After the early effects, both anodal and cathodal stimulation evoked the same pattern of MEP modulation; MEP suppression at 20–50 ms and enhancement at 80–100 ms. These time courses are very similar to those provoked by a loud acoustic stimulus (100 ms duration, 110 dB intensity) (Furubayashi et al. 2000). Similar patterns of modulation have also been induced by non-specific stimuli such as pain (Valeriani et al. 1999). In our study, low stimulus intensity (1 mA) did not evoke any suppression (not shown). At an intensity of 3 mA in the present study, the subjects felt pain or saw phosphenes, while 1 mA tDCS did not produce such sensations. These suggest that the suppression was not caused by the stimulus itself but some perception of pain or phosphene. In addition, we performed an experiment of tDCS on the occipital cortex instead of the motor cortex, in order to investigate whether the effect of interest is non-specific or not. A similar time course of modulation was evoked even by the occipital stimulation. The observation that the present effect did not depend on the polarity of the conditioning stimulus and provoked by occipital stimulation also supports a non-specific effect. Such stimuli suppress the human motor cortex at 30–50 ms intervals and enhance the spinal cord at 80–100 ms. Thus, we suggest that the modulation of MEPs at SOAs of 20–100 ms is due to non-specific effects on the motor cortex and spinal cord produced by a novel stimulus.

Effects of 10 min tDCS

Previous studies have shown that the long lasting effects depend on the polarity of tDCS. The anodal tDCS elicited LTP-like effects, and the cathodal tDCS LTD-like effects. Our present results are consistent with these previous findings. The finding that no lasting effects were evoked by occipital stimulation also supports the notion that the lasting effect is not a nonspecific effect. One inconsistent point of the present result among these studies is the duration of the effects. In previous results, the effects lasted longer than 30 min after 9 min tDCS (Nitsche and Paulus 2001; Nitsche et al. 2003b). However, in our experiments, motor cortical suppression lasted only for a few minutes after the end of tDCS at an intensity of 1 mA. This short duration of suppression is very similar to the effect induced by weak tDCS of short duration (for 4 or 5 min at 1 mA) in other papers (Nitsche and Paulus 2000). One possible explanation for this discrepancy is that we studied the long duration tDCS in a small number of subjects. Another possibility is the anatomical difference between Japanese and European subjects. The threshold for MEPs to TMS may be higher in Japanese than in European subjects probably because the electric resistance of skull is higher in Japanese than Europeans (Terao et al. 2000). This difference may explain the fact that the time course of our results mimicked that of the effects elicited by weaker, shorter tDCS in Europeans. Cathodal tDCS also lasted shorter in our experiments than the other reports.

The trigger for the long-term effect is considered to be a membrane potential change that leads to NMDA receptor activation or calcium influx into neurons (Liebetanz et al. 2002; Nitsche et al. 2003a). One mA tDCS may be sufficient to trigger these steps in the chain. However, an intensity of 3 mA or stronger may be required to evoke the resting membrane potential changes detected by a single pulse TMS (see above). Either synaptic or non-synaptic (membrane potential changes) mechanisms, or both of them may explain the long-term tDCS effects (Liebetanz et al. 2002; Nitsche et al. 2003a; Ardolino et al. 2005). To explore the mechanisms for the long-term effect is beyond the scope of our present study. Nevertheless it is conspicuous that we have shown the threshold difference between the short interval effects and long lasting effects.

We have drawn two main conclusions from the present experiments. The short duration tDCS elicited the immediate cortical facilitation or inhibition depending on the current direction of tDCS. These effects may be explained by the resting membrane potential changes induced by tDCS. The long lasting effects after 10 min tDCS was shorter than those of previous European studies in Japanese. This is probably due to an anatomical difference between Japanese and Europeans (thicker skull in Japanese).

Acknowledgments Part of this work was supported by Research Project Grant-in-aid for Scientific Research No. 16500194, 18500236 from the Ministry of Education, Science, Sports and Culture of Japan, The Research Grant (15B-2) for Nervous and Mental Disorders from the Ministry of Health, Labor and Welfare of Japan, a grant from the Committee of the Study of Human Exposure to EMF, Ministry of Public Management, Home Affairs, Post and Telecommunications, grants from Life Science foundation of Japan and the association of radio-industry and business (ARIB). We are also grateful to Professor Rothwell (Institute of Neurology) for his helpful comments and suggestions

to the experiments and English revision. His visit to our laboratory and our visit to his laboratory were supported by Daiwa Foundation Awards.

References

Antal A, Nitsche MA, Paulus W (2001) External modulation of visual perception in humans. Neuroreport 12:3553–3555

Ardolino G, Bossi B, Barbieri S, Priori A (2005) Non-sysnaptic mechanisms underlie the after-effects o cathodal transcranial direct current stimulation of the human brain. J Physiol 568:653–663

Arfai E, Theano G, Montagu JD, Robin AA (1970) A controlled study of polarization in depression. Br J Psychiatry 116:433–434

Baudewig A, Nitsche MA, Paulus W, Frahm J (2001) Regional modulation of BOLD MRI responses to human sensorimotor activation by trascranial direct current stimulation. Magn Reson Med 45:196–201

Bindman LJ, Lippold OCJ, Redfearn JWT (1964) The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-klasting after-effects. J Physiol 172:369–382

Bindman LJ, Lippold OCJ, Milne R (1979) Prplonged changes in excitability of pyramidal tract neurones in the cat: a post-synaptic mechanism. J Physiol 286:457–477

Carney MW (1969) Negative polarisation of the brain in the treatment of manic states. Irish J Med Sci 2:133–135

Costain R, Redfearn JWT, Lippold OCJ (1964) A controlled trial of the therapeutic effects of polarization of the brain in depressive illness. Br J Psychiatry 110:786-799

Dieckhöfer A, Waberski TD, Nitsche M, Paulus W, Buchner H, Gobbelé R (2006) Transcranial direct current stimulation applied over the somatosensory cortex—differential effect on low and high frequency SEPs. Clin Neurophysiol 117:2221–2227

Fregni F, Boggio PS, Nitsche M, Bermpohl F, Antal A, Feredoes E, Marcolin MA, Rigonatti SP, Silva MTA, Paulus W, Pascual-Leone A (2005) Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. Exp Brain Res 166:23–30

Furubayashi T, Ugawa Y, Tearo Y, Hanajima R, Sakai K, Machii K, Mochizuki H, Shiio Y, Uesugi H, Enomoto H, Kanazawa I (2000) The human hand motor area is transiently suppressed by an unexpected auditory stimulus. Clin Neurophysiol 111:178–183

Hall KM, Hicks RA, Hopkins K (1970) The effects of low level DC scalp positive and negative current on the performance of various tasks. Br J Psychiatry 117:689–691

Hanajima R, Ugawa Y, Terao Y, Sakai K, Furubayashi T, Machii K, Kanazawa I (1998) Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves. J Physiol 509:607–618

Hanajima R, Uagwa Y, Machii K, Mochizuki H, Terao Y, Enomoto H, Furubayashi T, Shiio Y, Uesugi H, Kanazawa I (2001) Interhemispheric facilitation of the hand motor area in humans. J Physiol 531:849–859

Kincses TZ, Antal A, Nitsche MA, Bártfai O, Paulus W (2003) Facilitation of probabilistic classification learning by transcranial direct current stimulation of the prefrontal cortex in the human. Neuropsychologia 42:113–117

Lang N, Nitsche MA, Paulus W, Rothwell JC, Lemon RN (2004) Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. Exp Brain Res 156:439–443

Liebetanz D, Nitsche MA, Tergau F, Paulus W (2002) Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain 125:2238–2247

Lifshitz K, Harper P (1968) A trial of transcranial polarization in chronic Schizophrenics. Br J Psychiatry 114:635–637

Lolas F (1977) Brain polarization: Behavioral and therapeutic effects. Biol Phychiatry 12:37–47

Matsunaga k, Nitsche MA, Tsuji S, Tothwell JC (2004) Effects of transcranial DC sensorimotor cortex stimulation on somatosensory evoked potential in humans. Clin Neurophysiol 115:456–460

Nitsche MA, Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 527:633–639

Nitsche MA, Paulus W (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 57:1899–1901

Nitsche MA, Fricke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N, Henning S, Tergau F, Paulus W (2003a) Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. J Physiol 533:293–301

Nitsche MA, Nitsche MS, Klein CC, Tergau F, Rothwell JC, Paulus W (2003b) Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. Clin Neurophysiol 114:600–604

Priori A (2003) Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability. Clin Neurophysiol 114:589–595

Priori A, Berardelli A, Rona S, Accornero N, Manfredi M (1998) Polarization of the human motor cortex through the scalp. Neuro Report 9:2257–2260

Purpura DP, McMurtry JG (1965) Intracellular activities and evoked potential changes during polarization of motor cortex. J Neurophysiol 28:166–185

Sheffield LJ, Mowbray RM (1968) The effects of polarization on normal subjects. Br J Psychiatry 114:225–232

Terao Y, Ugawa Y, Hanajima R, Machii K, Furubayashi T, Mochizuki F, Enomoto H, Shiio Y, Uesugi H, Iwata NK, Kanazawa I (2000) Predominant activation of I1-waves from the leg motor area by transcranial magnetic stimulation. Brain Res 589:137–146

Ugawa Y, Rothwell JC, Day BL, Thompson PD, Marsden CD (1991) Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in man. Ann Neurol 29:418–427

Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I (1994) Magnetic stimulation of coticospinal pathways at the foramen magnum level in humans. Ann Neurol 36:618–624

Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I (1995) Magnetic stimulation over the cerebellum in humans. Ann Neurol 37:703–713

Valeriani M, Restuccia D, Di Lazzaro V, Oliviero A, Profice P, Le Pera D, Saturno E, Tonali P (1999) Inhibition of the human primary motor area by painful heat stimulation of the skin. Clin Neurophysiol 110:1475–1480