Shaping the Optimal Repetition Interval for Cathodal Transcranial Direct Current Stimulation (tDCS)

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Monte-Silva K, Kuo M-F, Liebetanz D, Paulus W, Nitsche MA. Shaping the optimal repetition interval for cathodal transcranial direct current stimulation (tDCS). J Neurophysiol 103: 1735–1740, 2010. First published January 27, 2010; doi:10.1152/jn.00924.2009. Transcranial DC stimulation (tDCS) is a plasticity-inducing noninvasive brain stimulation tool with various potential therapeutic applications in neurological and psychiatric diseases. Currently, the duration of the aftereffects of stimulation is restricted. For future clinical applications, stimulation protocols are required that produce aftereffects lasting for days or weeks. Options to prolong the effects of tDCS are further prolongation or repetition of tDCS. Nothing is known thus far about optimal protocols in this behalf, although repetitive stimulation is already performed in clinical applications. Thus we explored the effects of different break durations on cathodal tDCS-induced cortical excitability alterations. In 12 subjects, two identical periods of cathodal tDCS (9-min duration; 1 mA) with an interstimulation interval of 0 (no break), 3, or 20 min or 3 or 24 h were performed. The results indicate that doubling stimulation duration without a break prolongs the aftereffects from 60 to 90 min after tDCS. When the second stimulation was performed during the aftereffects of the first, a prolongation and enhancement of tDCS-induced effects for ≤120 min after stimulation was observed. In contrast, when the second stimulation followed the first one after 3 or 24 h, the aftereffects were initially attenuated, or abolished, but afterwards re-established for up to 120 min after tDCS in the 24-h condition. These results suggest that, for prolonging the aftereffects of cathodal tDCS, stimulation interval might be important.

INTRODUCTION

Transcranial DC stimulation (tDCS), a noninvasive, neuroplasticity-generating brain stimulation tool, is increasingly used for therapeutic purposes in neurological and psychiatric diseases accompanied by pathological alterations of cortical excitability and activity, such as epilepsy (Fregni et al. 2006c; Liebetanz et al. 2006), migraine (Antal et al. 2007), stroke (Boggio et al. 2007; Hummel et al. 2005; Schlaug et al. 2008), and depression (Boggio et al. 2008; Fregni et al. 2006a). By application of a weak DC, tDCS elicits cortical excitability changes in the human brain in a painless and reversible way (Nitsche and Paulus 2000). The induced excitability alterations depend on the duration, current density, and direction of the current flow. Anodal stimulation enhances excitability, whereas cathodal stimulation reduces it (Nitsche and Paulus 2000, 2001; Nitsche et al. 2003b). In the currently available protocols, tDCS has been shown to modify cortical excitability for up to ~1 h after stimulation (Nitsche and Paulus 2001; Nitsche et al. 2003b). For clinical purposes, however, longer-lasting effects are needed. In principle, these can be achieved by prolonging stimulation duration or enhancing stimulation strength, and both have been done in clinical applications (Ferrucci et al. 2009; Fregni et al. 2006b; Ohn et al. 2008). Although these protocols are well within safety limits (Liebetanz et al. 2009), it is a general goal to keep current exposure as low as possible. Moreover high-intensity stimulation might be painful (Furubayashi et al. 2008) and affect different neuronal populations, compared with weaker stimulation, because of increased stimulation depth, which might not be intended in each case. Another option to prolong the aftereffects of tDCS might be the repetition of tDCS sessions. Functionally, repetitive tDCS has been shown to induce cumulative effects when applied once daily in humans (Boggio et al. 2007, 2008). It is, however, unclear whether this repetition rate (stimulation once daily) is optimal suited to stabilize tDCS-induced plasticity. In animal experiments, repetition of DC stimulation during the aftereffects of a first stimulation session has been shown to enhance its efficacy (Bindman et al. 1964; Gartside 1968). However, repeated plasticity induction might also result in homeostatically driven antagonistic effects (Siebner et al. 2004). Here we aimed to explore repetitive tDCS protocols, which could lead to longer duration and stabilization of the stimulation aftereffects. We compared single sessions of cathodal tDCS with the effects of repetitive stimulation during or after the aftereffects of the first stimulation.

METHODS

Subjects

Twelve different healthy volunteers took part in each stimulation protocol: tDCS applied constantly for 9 min (9-0-0 protocol), five men, seven women, 24.31 ± 6.1 (SD) yr of age; for 18 min (9-0-9 protocol), four men, eight women, 26.83 ± 6.2 yr of age; tDCS applied with interstimulation intervals of 3 min (9-3-9 protocol), six men, six women, 25.73 ± 5.52 yr of age; with interstimulation intervals of 20 min (9-20-9 protocol), five men, seven women; 25.96 ± 4.18 yr of age; interstimulation intervals of 3 h (9-3h-9 protocol), six men, six women; 26.23 ± 7.18 yr of age; and interstimulation intervals of 24 h (9-24h-9 protocol), six men, six women; 27.58 ± 6.13 yr of age. Age (t-test, P > 0.05) and sex (χ², P > 0.05) did not differ significantly between the groups of participants. The subjects were between 18 and 45 yr old and had no history of chronic or acute neurological, psychiatric, or medical disease, no family history of epilepsy, no pregnancy, no cardiac pacemaker, and no previous surgery involving implants to the head (cochlear implants, aneurysm clips, brain electrodes). Subjects were recruited from the campus of the Georg-August-University. Before the sessions, written informed consent was obtained. The experiments were approved by...
Direct current stimulation of the motor cortex

Continuous direct currents were transferred by saline-soaked surface sponge electrodes (35 cm²) and delivered by a specially developed, battery-driven constant current stimulator (Schneider Electronic, Gleichen, Germany) with a maximum output of 2 mA. The motor cortical electrode was positioned over the motor cortex representational area of the right abductor digitii minimi muscle (ADM), as identified by transcranial magnetic stimulation (TMS), and the other electrode above the right orbit. This electrode arrangement is known to result in significant excitability changes in the human motor cortex (Nitsche and Paulus 2000). tDCS was applied once or twice (see METHODS) with a current intensity of 1 mA for 9 min (cathodal tDCS), which induces cortical excitability diminutions lasting for ~1 h after the end of stimulation (Nitsche et al. 2003b).

Monitoring of motor cortical excitability

TMS-elicited motor-evoked potentials (MEPs) were recorded to monitor excitability changes of the representational motor cortical area of the right ADM. Single-pulse TMS was conducted by a Magstim 200 magnetic stimulator (Magstim, Whiteland, Dyfed, UK) with a figure-of-eight magnetic coil (diameter of 1 winding = 70 mm, peak magnetic field = 2.2 T). The coil was held tangentially to the skull, with the handle pointing backward and laterally at an angle of 45° from midline. The optimal position was defined as the site where stimulation resulted consistently in the largest MEPs. Surface EMG was recorded from the right ADM with Ag-AgCl electrodes in a belly-tendon montage. The signals were amplified and filtered with a time constant of 80 ms and a low-pass filter of 2.0 kHz and then digitized at an A/D rate of 5 kHz and further relayed into a laboratory computer using the Signal software and CED 1401 hardware (Cambridge Electronic Design, Cambridge, UK). The intensity was adjusted to elicit, on average, baseline MEPs of 1 mV peak-to-peak amplitude and was kept constant for the poststimulation assessment.

Experimental procedures

The experiments took place in an air-conditioned laboratory with a constant temperature of 20°C to guarantee constant skin temperature throughout the experimental sessions. The volunteers were seated in a comfortable chair with head and arm rests. The left motor-cortical representational field of the right ADM was identified using TMS (the coil position that produced to the largest MEPs in the resting ADM–optimal site). Identification of the hot spot took ~20–30 min in each subject. For all stimulation protocols, the intensity of the magnetic cortical stimulus was adjusted to elicit MEPs with a peak-to-peak amplitude of an average 1 mV. Determination of baseline TMS intensity took 5–10 min for each subject. Twenty-five MEPs were recorded after stable MEP amplitudes were obtained, at a frequency of 0.25 Hz for baseline determination. The motor cortical tDCS electrode was fixed on the ADM hot-spot, and the other was fixed at the contralateral forehead, just above the orbit. Afterward, motor cortical tDCS was performed as follows.

Experiment A

This experiment was designed to explore the effect of single cathodal tDCS applied constantly for 9 (9-0-0 protocol) or 18 min (9-0-9 protocol) on motor cortex excitability.
ability diminution for \( \leq 120 \) min after stimulation was observed. Compared with the 9-0-0, the 9-20-9 and 9-3-9 protocols prolonged the inhibitory effects generated by cathodal tDCS from 60 to 120 min after stimulation. Furthermore, the aftereffects were significantly enlarged in the 9-20-9 condition for \( 15\%-20\% \) compared with the 9-0-0 condition (Fig. 2B). When the second cathodal stimulation was applied 3 (9-3h-9) or 24 h (9-24h-9) after the first stimulation, the cathodal tDCS-induced inhibitory aftereffects were attenuated or abolished during the first 30 min after stimulation. The 9-3h-9 protocol abolished the inhibitory effect of cathodal tDCS until 60 min after stimulation; a relevant inhibitory effect appeared only 60 min after the second tDCS session. For the 9-24h-9 protocol, the excitability-diminishing effects of cathodal tDCS were more relevantly delayed and prolonged until 120 min after the end of stimulation (Fig. 2C). Comparison of the 9-0-0 with the other stimulation conditions shows that 18-min continuous stimulation (9-0-9 condition) prolongs the aftereffects for \( \leq 120 \) min after stimulation. The same result emerges for the 9-3-9 and the 9-20-9 conditions. For the 9-20-9 condition, the tDCS-induced inhibition is additionally significantly larger than the inhibition accomplished by 9-0-0 stimulation. With regard to the long breaks, inhibition was significantly reduced, if the second stimulation followed the first after a 3-h break for 25 min after tDCS, and a trend for such an effect was seen for the 24-h break condition compared with the 9-0-0 stimulation protocol (for results of the single t-test, see Supplemental Table S2). Comparison of the 9-0-9 condition with the 9-20-9 condition to explore which protocol was optimally suited to enhance the aftereffects of cathodal tDCS shows that a 20-min break between stimulation conditions results in a significantly increased magnitude of the excitability diminutions between 20 and 30 min after tDCS, whereas the duration of the aftereffects only differed trendwise between these conditions, with some advantage for the 20-min break session (Supplemental Table S2). Sex did not affect the inhibitory impact of cathodal tDCS on motor cortex excitability (for the results of the ANOVA, see Supplemental Table S1).

**FIG. 1.** Experimental course. First, transcranial magnetic stimulation (TMS) was applied over the left motor cortical representational area of the right ADM with an intensity to elicit motor evoked potentials (MEPs) with a peak-to-peak amplitude of on average 1 mV (baseline determination). Afterward, motor cortical cathodal transcranial direct current stimulation (tDCS; 1 mA; 9 min) was performed as follows: 1) **experiment A** (no intervals), a) single tDCS for 9 min (9-0-0 protocol) and b) single tDCS for 18 min (9-0-9 protocol); 2) **experiment B** (short intervals), a) repeated tDCS with inter-tDCS pause of 3 min (9-3-9 protocol) and b) repeated tDCS with inter-tDCS pause of 20 min (9-20-9 protocol); and 3) **experiment C** (long intervals), a) repeated tDCS with inter-tDCS pause of 3 h (9-3h-9 protocol) and b) repeated tDCS with inter-tDCS pause of 24 h (9-24h-9 protocol). For experiments 2 and 3, the aftereffects of tDCS were evaluated by TMS up to next evening after intervention. For experiment 1, TMS recordings were performed until 120 min and until the same evening after the stimulation for 9-0-0 and 9-0-9 paradigms, respectively. ne, next evening; nm, next morning; na, next afternoon; se, same evening.
DISCUSSION

This is the first study showing that 1) prolongation of cathodal tDCS duration is able to prolong the aftereffects relevantly beyond 1-h duration and that 2) for fractionated application of tDCS, the interstimulation break is of critical importance. Prolonging stimulation duration from 9 to 18 min relevantly prolonged the after-effects of cathodal tDCS from 60 to 90 min. When the second tDCS session was performed during the aftereffects of the first, i.e., with short interstimulation intervals (9-3-9 and 9-20-9 protocols), a prolongation of tDCS-induced effects for \( \geq 120 \) min after stimulation was observed. For the 20-min interval, the magnitude of inhibition was furthermore enhanced. C: when the 2nd stimulation followed the 1st after 3 or 24 h (9-3h-9 and 9-24h-9 protocols), the cathodal tDCS-induced inhibitory aftereffects were attenuated. Filled symbols indicate significant deviations of the post-tDCS MEP amplitudes from baseline values; # marks significant deviations of each tDCS protocol from the 9-0-0 protocol (t-test, independent samples, \( P < 0.05 \)). Error bars indicate SE. ne, next evening; nm, next morning; na, next afternoon; se, same evening. *Significant difference between the baseline-standardized MEPs marked by brackets.

Repetitive tDCS: the importance of timing

Concordant with previous data, a single session of 9-min cathodal tDCS produced inhibitory aftereffects on cortical excitability lasting for an hour after the end of stimulation (Nitsche and Paulus 2001; Nitsche et al. 2003b). Prolonging continuous stimulation duration without a break for 18 min increased the duration of the aftereffects; however, it was less exaggerated than the duration increase between 5-, 7-, and 9-min stimulation shown in a previous study (Nitsche et al. 2003b). This result confirms principally those of previous studies that suggested that the duration of tDCS-induced plasticity is related to stimulation duration (Bindman et al. 1964; Nitsche and Paulus 2000); however, as shown here, a saturation effect might apply. The main finding of our study is that repeating cathodal tDCS during the aftereffects of the first session prolongs the duration of the aftereffects for 1 h and increases their amplitude. Interestingly, the magnitude of cathodal tDCS-induced inhibition was only enhanced when the second followed the 1st after 3 h and 24 h (9-3h-9 and 9-24h-9 protocols), the cathodal tDCS-induced inhibitory aftereffects were attenuated.
second stimulation session. Taken together, our results are indicative for a stimulation timing–dependent plasticity regulation in the human motor cortex. Specifically, a second stimulation session performed during the aftereffects of the first is suited to prolong and enhance the magnitude of tDCS-induced excitability diminution. This result is consistent with early animal experimentation in DC stimulation, which furthermore suggests that these effects are protein synthesis dependent (Bindman et al. 1964; Gartside 1968). A similar effect is also described for spinal plasticity (Jung et al. 2009).

Proposed mechanisms of action of prolongation of the aftereffects of tDCS by repetitive cathodal tDCS

Interestingly, repetition of tDCS had a different impact on inhibitory plasticity depending on the break between sessions. Specifically a second stimulation performed during the aftereffects of the first one resulted in enhanced amplitude and duration of the aftereffects of cathodal tDCS, which was slightly larger than the efficacy enhancement of stimulation accomplished by pure prolongation of stimulation without a break. Such cumulative effects of repetitive plasticity-inducing stimulation have been also shown for long-term depression (LTD) protocols in slice preparations (Kamikubo et al. 2006; Shinoda et al. 2005). Here, repetitive LTD induction resulted in long-lasting excitability diminutions, which were dependent on protein phosphatase and metabotropic glutamate receptors and resulted in a reduction in the number of functional AMPA receptors in the postsynaptic density. Although in the reported studies the aftereffects explored were much longer lasting than those in this one, the principle mechanism of action might be similar. Beyond slice experiments, systematic physiological studies exploring cumulative effects of repeated plasticity induction are rare. However, simulation and cognitive studies exploring the optimum repetition interval for learning tasks and stabilization of plasticity propose that there might be a restricted time window to improve functions (Hotermans et al. 2006; Langemann et al. 2008; Matsumoto and Mizunami 2002; Pavlik and Anderson 2008). Specifically, an early “viscosity” phase was suggested, which encompasses alterations of N-methyl-d-aspartate (NMDA) and CaMKII activity, and only turns into stable plasticity if repeated excitation causes the subsequently temporally altered concentrations of AMPA subunits in the postsynaptic membrane to be stabilized (Langemann et al. 2008). Because it is known that tDCS effects depend on activation of NMDA receptors (Liebetanz et al. 2002; Nitsche et al. 2003a), such a mechanism, which, however, has to be substantiated further experimentally, could explain the prolonging effect of a second tDCS session during the aftereffect of the first, and also the absence of such a cumulative action of the second stimulation, if it is applied after the aftereffects of the first stimulation have vanished. The reduced effect of repeated tDCS on excitability, which is accomplished when the second stimulation is applied hours after the first one, is not completely explained by this mechanism. Thus far, tDCS aftereffects have mainly be quantified by single-pulse TMS; however, this effect shows that the impact of tDCS lasts longer than the overt effects on cortico-cortical excitability and is a first hint toward separate presumably intracortical plasticity mechanisms. It might be argued that some stabilization of plasticity takes place during that prolonged period, which makes it more difficult for further interventions to induce plastic alterations. In that way, this effect is similar to homeostatic mechanisms of neuroplasticity regulation, which are postulated to limit neuroplastic alterations to avoid neuronal network destabilization and have been shown to work within similar time frames in animals (Abbott and Nelson 2000; Abraham and Tate 1997; Davis 2006; Turrigiano and Nelson 2004). For humans, homeostatic mechanisms have also been shown but only within a much shorter time course (Bliem et al. 2008; Iyer et al. 2003; Lang et al. 2004; Müller et al. 2007; Nitsche et al. 2007; Siebner et al. 2004). The reason for missing homeostatic effects with regard to short-term breaks between stimulation, and their restriction to long-term intervals in this experiment, might depend on the specific plasticity induction protocols used in the different studies. Whereas we applied tDCS in the present study, repetitive transcranial magnetic stimulation, or paired associative stimulation, in some studies in combination with tDCS, were administered in the preceding-mentioned studies. These protocols differ in focality of effects, timing dependency, supra- versus subthreshold stimulation, and duration and magnitude of the aftereffects. Differences in these factors might be responsible for the seemingly opposing effects and should be explored systematically in future experiments. Interestingly, even not in all of the above-mentioned studies, in which other plasticity induction procedures than tDCS were applied, homeostatic plasticity was induced.

General remarks

The results of this study deliver the first neurophysiological evidence that 1) fractionation of cathodal tDCS is suited to prolong its inhibitory neuroplastic effects and 2) specific timing of repetition intervals is important for optimizing cumulative effects. tDCS is increasingly used for therapeutic purposes, with some promising initial results in pain treatment, motor rehabilitation after stroke, and epilepsy therapy, among others (Fregni et al. 2005, 2006a,b,c; Hummel et al. 2005). Here neuroplastic excitability alterations are needed that last for days or weeks. In exploring fractionated tDCS protocols, which are optimally suited to induce long-lasting neuroplastic cortical excitability alterations, the results of this study might help to develop tDCS protocols optimized for clinical application.

Interestingly, MEP variations tended to increase, both intraindividually, with larger time lags between the experiments (Supplemental Fig. S1). Although this might at least in part be explained by methodological aspects, e.g., slight differences of electrode or coil positions between MEP measures, it may also be caused by physiological alterations. Long intervals between plasticity induction procedures might be more prone to spontaneous changes of cortical activity and excitability, possibly related to diurnal or other variations, which could affect the efficacy of plasticity induction procedures. Moreover, these changes, which might differ between individuals, could have an impact on the interaction of the consecutive stimulation protocols.

Beyond tDCS, fractionation might also be important for other therapeutically applied neuroplasticity induction procedures, such as repetitive transcranial magnetic stimulation (rTMS). Currently in most clinical studies, rTMS is applied once daily. Our results indicate that the initial excitability decrease might be suboptimal within the first 30 min when the repeated stimulation is applied.
after 24 h. This should be taken into account in the development of treatment protocols with a technique that is expected to have effective therapeutic purposes. It also shows that MEPs should be studied with a delay in 24-h repetitive stimulation protocols when used as a surrogate marker. Otherwise, the main effect may be missed. Systematic studies are needed to explore whether these protocols are really optimally suited for achieving the intended functional effects.

REFERENCES


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