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Probing the relevance of repeated cathodal transcranial direct current stimulation over the primary motor cortex for prolongation of after-effects

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Key points

- To explore the capability of cathodal transcranial direct current stimulation (tDCS) to induce late-phase long-term depression (LTD) via repeated stimulation.
- Conventional (1 mA for 15 min) and intensified (3 mA for 20 min) protocols with short (20 min) and long (24 h) intervals were tested.
- Late-phase plasticity was not induced by a single repetition of stimulation.
- Repetition reduced the efficacy of stimulation protocols with higher intensities.

Abstract Transcranial direct current stimulation (tDCS) has shown promising results in pilot studies as a therapeutic intervention in disorders of the central nervous system, but more sustained effects are required for clinical application. To address this issue, one possible solution is the use of repeated stimulation protocols. Previous studies indicated the possibility of extending the after-effects of single intervention cathodal tDCS by repeating the tDCS, with relatively short intervals between repetitions being most effective. In this study, we thus investigated the effects of repeated stimulation protocols at short and long intervals, for a conventional tDCS protocol (1 mA for 15 min) and a newly developed optimized protocol (3 mA for 20 min). In 16 healthy participants, we compared single interventions of conventional and optimized protocols, repeated application of these protocols at intervals of 20 min and 24 h, and a sham tDCS session. tDCS-induced neuroplastic after-effects were then monitored with transcranial magnetic stimulation (TMS)-induced motor evoked potentials (MEPs) until the following evening after stimulation. The results revealed that the duration of the after-effects of repeated conventional and optimized protocols with short intervals remained nearly unchanged compared to the respective single intervention protocols. For the long-interval (24 h) protocol, stimulation with

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the conventional protocol did not significantly alter respective after-effects, while it reduced the efficacy of the optimized protocol, compared with respective single interventions. Thus late-phase plasticity could not be induced by a single repetition of stimulation in this study, but repetition reduced the efficacy of stimulation protocols with higher intensities. This study provides further insights into the dependency of tDCS-induced neuroplasticity on stimulation parameters, and therefore delivers crucial information for future tDCS applications.

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Introduction

Application of a weak direct current via electrodes placed over the scalp (transcranial direct current stimulation, tDCS) can bidirectionally induce neuroplasticity in the targeted area. The direction, magnitude and duration of respective effects depend on stimulation parameters such as polarity and intensity/duration. Anodal tDCS, which refers to surface inward current over the target area, enhances cortical excitability, while cathodal tDCS, which refers to outward current over the target area, results in excitability reduction with standard protocols at the macroscopic level (Nitsche & Paulus, 2000, 2001; Nitsche *et al.* 2003*b*). These effects alter symptoms of neurological and psychiatric disorders accompanied by pathological alterations of cortical excitability, such as in stroke (Allman *et al.* 2016), Parkinson's disease (Schoellmann *et al.* 2019), depression (Brunoni *et al.* 2017) and schizophrenia (Andrade, 2013).

However, the overall efficacy of the technique is currently limited, most probably caused by sub-optimal stimulation protocols (Lefaucheur *et al.* 2017). Earlier studies indicated that 1 mA tDCS for 4 s over the primary motor cortex alters cortical excitability during stimulation (Nitsche & Paulus, 2000). Increasing the duration of stimulation to some minutes induces long-term potentiation (LTP)- and long-term depression (LTD)-like neuroplastic after-effects depends on the stimulation polarity (Nitsche & Paulus, 2001; Nitsche *et al.* 2003*b*). These results show that stronger and/or longer stimulation extends the neuromodulatory after-effects of tDCS within specific windows of stimulation intensity and duration. However, recent studies revealed a non-linear dosage dependency of tDCS-induced neuroplasticity, when stimulation duration and intensity exceed the limits of these 'classic' protocols. While 1 mA cathodal tDCS for 15 min significantly reduced cortical excitability, no significant effects were observed with 2 mA for the same duration (Jamil *et al.* 2017), and prolongation of 2 mA cathodal tDCS to 20 min resulted in an excitability enhancement (Batsikadze *et al.* 2013; Mosayebi Samani *et al.* 2019). A dosage-dependent non-linearity of tDCS after-effects has also been revealed by other studies (Bastani & Jaberzadeh, 2013; Kidgell *et al.* 2013; Ho *et al.* 2016), which can partially be explained by the dependency of the direction of plasticity on the amount of neuronal calcium influx (Lisman, 1989, 2001). Thus, for enhancing the efficacy of tDCS, increasing stimulation intensity, and duration, might have its limitations.

Animal studies revealed the possibility of extending neurophysiological after-effects of plasticity-inducing stimulation from early- to late-phase plasticity by means of repeated stimulation protocols with short time intervals (Vickers *et al.* 2005; Reymann & Frey, 2007; Ahmed *et al.* 2015) In accordance, studies in humans have also shown that single intervention cathodal tDCS-induced excitability-reducing after-effects can be extended by repeated tDCS protocols with certain inter-stimulation intervals. In a former study, the excitability-diminishing after-effects of a single intervention of cathodal tDCS with 1 mA for 9 min over the primary motor cortex, which induces after-effects of about 1 h duration, were enhanced by repeating the same protocol with short intervals (3 or 20 min), but abolished when the interval was extended to 3 or 24 h (Monte-Silva *et al.* 2010). In another study, however, the excitability-diminishing after-effects of a single intervention of 1 mA cathodal tDCS for 5 min, which induces short-term depression-like effects, were reversed or unchanged by repeated tDCS protocols with 3 min and 30 min intervals, respectively (Fricke *et al.* 2011). These results suggest that cathodal tDCS can effectively reduce cortical excitability if stimulation is repeated within specific intervals, but that, in addition to the interval between interventions, the stimulation protocol itself is also of critical relevance. It is, however, also important to mention that, in contrast to results in animal models, so far repeated stimulation in humans has gradually enhanced the efficacy of various cathodal stimulation protocols, but has not resulted in late-phase effects, which should last for more than 3 h.

In a recent study, we systematically titrated cathodal tDCS parameters for the human motor cortex model with different intensities (1, 2 and 3 mA) and durations (15, 20 and 30 min), to explore systematically the impact of these parameters on after-effects, and identify protocols that result in stronger and/or longer lasting after-effects. The results revealed that stimulation with 1 mA for

15 min, and 1 mA for 30 min induced a significant MEP amplitude diminution, while stimulation with 2 mA for 20 min resulted in a significant corticospinal excitability enhancement. Protocols with higher stimulation intensity (specifically stimulation with 3 mA for 20 min) induced again a significant excitability diminution lasting for about one and half hours after stimulation, and thus induced longer lasting excitability-diminishing after-effects than the other protocols (Mosayebi Samani *et al.* 2019). Since a former cathodal tDCS repetition study with a standard stimulation protocol with 1 mA did not lead to after-effects lasting for more than a few hours, we were interested to explore if repetition of a protocol, whose single application had shown superior effects, would also induce improved effects with a repeated protocol. Accordingly, in the present study, tDCS with the cathode positioned over M1 was applied with 1 mA for 15 min (conventional protocol), and 3 mA for 20 min (optimized protocol). To explore if repeated tDCS protocols with different intervals prolong the after-effects, we compared the impact of single interventions of conventional and optimized cathodal tDCS with the effects of repeated application with intervals of 20 min and 24 h on motor cortex excitability. These intervals were selected since in a previous study the 20 min interval prolonged the neuroplastic after-effects compared to a single intervention protocol, and an interval of 24 h is often used for repeated tDCS, but did reduce the neurophysiological effects of tDCS in that study (Monte-Silva *et al.* 2010). In accordance with previous studies, we hypothesized that the cathodal tDCS-induced excitability diminution would be enhanced by repeated application of conventional and optimized protocols with the short interval, and these effects would be reduced with the long interval. Furthermore, we hypothesized that the high intensity protocol should improve tDCS-induced neuroplastic after-effects more than the low intensity (1 mA) condition for repeated stimulation. This study aimed to provide further information about the dependency of tDCS-induced neuroplasticity on the respective stimulation parameters, and thereby to deliver crucial information for future applications of cathodal tDCS.

Methods

Ethical approval

The study conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database and was approved by the local ethics committee of the Leibniz Research Center for Working Environment and Human Factors (IfADo; approval reference no. GCBS 01EE1501). All participants gave written informed consent before starting the study, and were financially compensated for participation.

Participants

Sixteen healthy, non-smoking participants (7 males, mean age 25.56 ± 4.96 years standard deviation (SD)) were recruited. All participants were right-handed according to the Edinburgh handedness inventory (Oldfield, 1971). None of the participants had a history of neurological or psychiatric disease, current or previous drug abuse, alcohol abuse, present pregnancy or metallic head implants, and all fulfilled the exclusion criteria for non-invasive electrical or magnetic brain stimulation (Rossi *et al.* 2009; Bikson *et al.* 2016).

Transcranial direct current stimulation over the motor cortex

tDCS was applied with a battery-powered constant current stimulator (neuroCare, Ilmenau, Germany), through a pair of saline-soaked surface sponge electrodes (7 × 5 cm, 35 cm^2) placed on the scalp. The target electrode was fixed over the motor cortex representational area of the right abductor digiti minimi muscle (ADM) as identified by TMS, and the return electrode was placed contralaterally over the right orbit (Nitsche & Paulus, 2000; Nitsche *et al.* 2003*b*). The participants received two single interventions of cathodal tDCS of conventional (1 mA for 15 min), and optimized (3 mA for 20 min) protocols and two additional repeated cathodal tDCS protocols with 20 min and 24 h intervals, for each single protocol. Taking into account all single and repeated protocols, including sham stimulation, this resulted in 7 sessions per participant (Fig. 1). For sham stimulation, 1.0 mA stimulation was delivered for 15 s followed by 15 min with 0.0 mA stimulation. All protocols were conducted with a 10 s ramp-up and down at the start, and end of stimulation.

Motor cortical excitability assessment

Single pulse TMS was delivered by a PowerMAG stimulator (Mag&More, Munich, Germany) to measure excitability changes of the representational motor cortical area of the right ADM, indexed as the amplitude of motor evoked potentials (MEPs). The TMS pulses were delivered via a figure-of-eight-shaped coil (diameter of one winding 70 mm; peak magnetic field 2 T) at a frequency of 0.25 Hz with 10% jitter. The coil was held tangentially to the scalp at an angle of 45° to the sagittal plane with the coil handle pointing laterally and posterior. Surface EMG was recorded from the right ADM in a belly-tendon montage. The signals were amplified, and filtered (1000; 3 Hz–3 kHz) using D440-2 (Digitimer, Welwyn Garden City, UK), and were digitized (sampling rate, 5 kHz) with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK), controlled by Signal Software (Cambridge Electronic Design, v. 2.13).

Experimental procedures

The study was performed in a cross-over, single-blinded, randomized design. At the beginning of each session, participants were seated in a comfortable chair with head- and arm-rests. Then single-pulse TMS was conducted at a frequency of 0.25 Hz over the left motor cortex for identification of the representational area of the right ADM, in which the largest MEPs were produced by a given TMS intensity (hot spot determination). The TMS intensity (SI1mV) was then adjusted to elicit MEPs with on average 1 mV peak-to-peak amplitudes. Finally, baseline cortical excitability was determined by recording 25 MEPs with that TMS intensity from the right ADM. Prior to intervention, a topical anesthetic cream (EMLA, 2.5% lidocaine + 2.5% prilocaine) was applied to the stimulation site, in order to decrease somatosensory sensations and sufficiently blind the participants (McFadden *et al.* 2011; Guleyupoglu *et al.* 2014). Afterwards, tDCS electrodes were mounted onto the head, and tDCS was applied. After finishing the intervention, tDCS electrodes were removed and corticospinal excitability was monitored by 25 MEPs obtained by TMS with baseline intensity every 5 min for up to 30 min, and then at 60 min, 90 min, 120 min, then the same evening, the next morning, the next noon, and the next evening after tDCS (Fig. 1). A waterproof pen was used to mark the position of the TMS coil on the scalp, as well as EMG electrodes on the hand. Different tDCS protocols were applied in separate sessions and in randomized order with a minimum 1 week interval between each session to avoid carry-over effects (Nitsche *et al.* 2008).

Calculations and statistics

MEP amplitudes were first visually inspected to exclude trials in which background electromyographic activity was present. Then, the individual means of MEP amplitudes recorded at each time point were calculated for all subjects and all conditions separately. The post-intervention mean MEP amplitudes were then normalized to the respective individual mean baseline MEP amplitude (quotient of post-intervention *versus* pre-intervention MEP amplitudes).

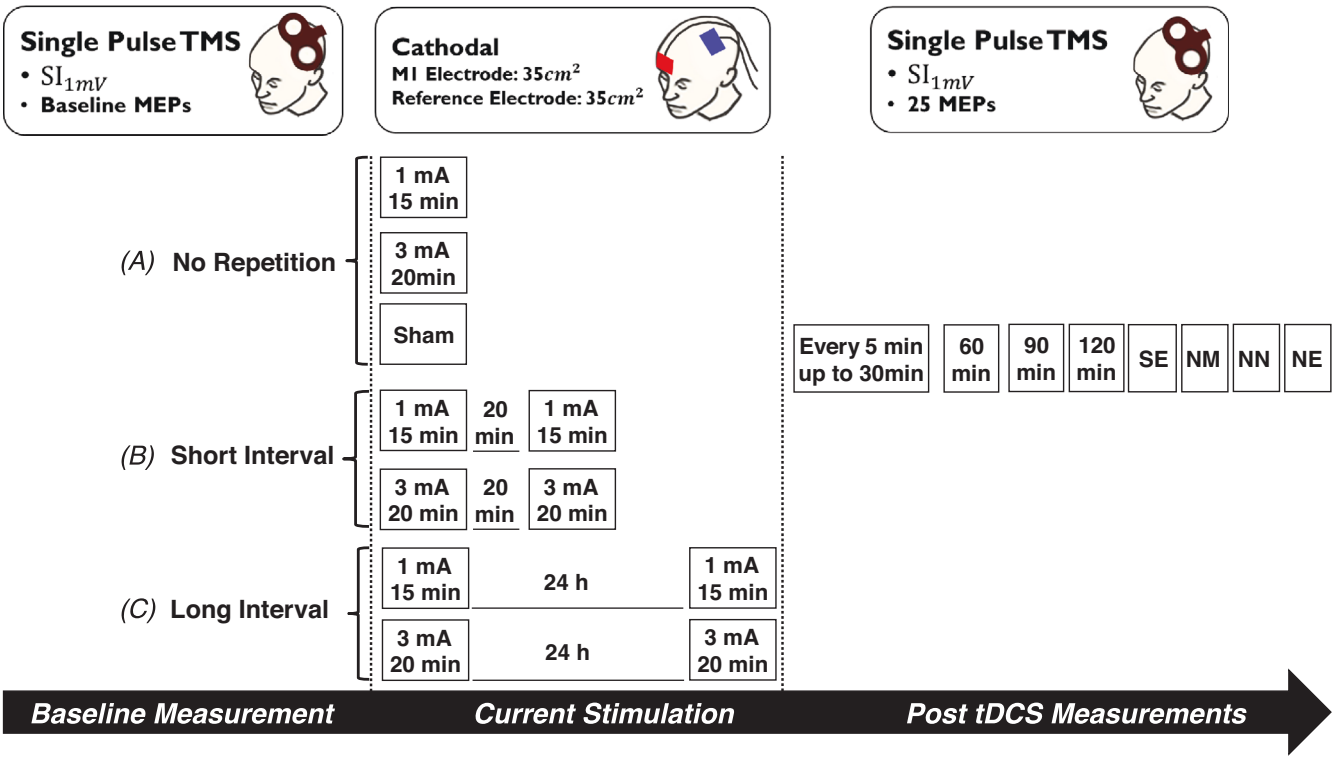

Figure 1. Course of the study
To obtain baseline motor cortex excitability, twenty-five single-pulse TMS-generated MEPs were recorded from the right ADM. Afterwards, cathodal tDCS was applied as a single intervention with the conventional, optimized, or sham protocols (no repetition) (*A*), or the same stimulation protocols were repeated with a 20 min (short) (*B*) or a 24 h (long) (*C*) interval. The after-effects were monitored with TMS-induced MEPs of baseline intensity every 5 min for up to 30 min and at the following time points: 60 min, 90 min, 120 min, the same evening (SE, ∼7 h after tDCS), the next morning (NM, ∼24 h after tDCS), noon the next day (NN, ∼4–5 h after NM) and the next evening (NE, ∼4–5 h after NN). [Colour figure can be viewed at wileyonlinelibrary.com]

The effects of baseline measures 'SI1mV' and 'baseline MEP' on tDCS after-effects

To investigate if baseline measures differed between sessions, two separate one-way repeated measures ANOVAs were performed with 'condition' (7 levels) as within-subject factor and 'SI_{1mV}' or 'baseline MEP' as dependent variables.

Overall effects of tDCS protocols and impact of the covariates 'age' and 'gender'

To determine if the respective active stimulation conditions altered cortical excitability relative to sham, and if the effects of the real stimulation protocols differed from each other, and also to investigate a possible impact of the covariates 'age' and 'gender' on tDCS-induced MEPs, the normalized MEP amplitudes of all time points (14 time points) were grand-averaged and pooled into three epochs (this compensates for variability between single time points): 0–30 min after stimulation (early after-effects), 60–120 min (late after-effects) and same-day evening to next-day evening (very late after-effects). For these parameters, a repeated measures ANCOVA was calculated with normalized MEPs as dependent variable, 'condition' (7 levels) and 'epoch' (4 levels) as within-subject factors, and 'age' and 'gender' as covariates.

Qualitative assessment of tDCS protocols

After finishing each session, participants were asked to fill in a questionnaire which contained: (1) guessed intensity of applied direct current (0, 1 and 3 mA), (2) rating scales for the presence and amount of visual phenomena, itching, tingling and pain during stimulation, and (3) rating scales for the presence and amount of skin redness, headache, fatigue, concentration difficulties, nervousness and sleep problems within 24 h after stimulation. The side-effects were rated on a numerical scale from zero to five, zero representing no and five extremely strong sensations.

Mauchly's test of sphericity was conducted, and the Greenhouse-Geisser correction was applied when necessary, for the ANCOVA. The critical significance level was set at $P < =0.05$. *Post hoc* t tests were Bonferroni-corrected for multiple comparisons. Statistical analysis was performed with SPSS (IBM Corp. Version 25.0).

Results

All participants completed the entire study.

No difference of SI1mV and baseline MEPs between conditions

Baseline MEP and $SI1_{mV}$ are listed in Table 1. The respective one-way ANOVAs showed no significant differences of baseline MEP and $SI1_{mV}$ across conditions (baseline MEP: d.f. $= 6$, $F = 0.592$, $P = 0.736$; $SI1_{mV}$: d.f. $= 3.112$, $F = 0.989$, $P = 0.404$).

Table 1. MEP baseline measurements and TMS stimulation intensities

Experimental session	SI_{1mv} (%)	Baseline MEP (mV)
Sham	57.18 ± 15.11	1.01 ± 0.08
Conventional protocol	57.40 ± 16.00	0.98 ± 0.15
Conventional protocol with 20 min interval	57.68 ± 16.50	1.01 ± 0.09
Conventional with 24 h interval	58.68 ± 16.82	1.04 ± 0.14
Optimized protocol	57.28 ± 15.30	1.01 ± 0.08
Optimized protocol with 20 min interval	58.71 ± 16.62	1.04 ± 0.10
Optimized protocol with 24 h interval	56.50 ± 15.91	1.01 ± 0.01

Data are presented as means ± SD; SI_{1mV} refers to the maximal stimulator output (%MSO) which was required for generating ~1 mV MEP. The ANOVAs showed no significant differences between baseline MEP and SI_{1mV} across sessions.

Overall effects of tDCS protocols and impact of the covariates 'age' and 'gender'

The two-factorial ANCOVA ('condition' 7 levels, 'epoch' 4 levels) revealed significant main effects of condition (d.f. $= 6$, $F = 4.608$, $P < 0.001$), epoch (d.f. $= 3$, $F = 15.286$, $P < 0.001$) and the respective interaction (d.f. $= 6.882$, $F = 2.205$, $P = 0.042$) (Fig. 2; Table 2). *Post hoc* tests comparing the respective active tDCS protocols with baseline cortical excitability measures for the first 30 min after stimulation (early epoch) revealed a significant reduction of MEP amplitudes after all active protocols, except for the optimized protocol with a 24 h interval. For the late epoch (60–120 min after stimulation) only the optimized single intervention tDCS protocol resulted in a significant reduction of MEP amplitudes relative to baseline values. For the very late epoch, no significant differences *versus* baseline were revealed. In addition, *post hoc* comparisons between the active and sham protocols showed a significant excitability diminution of all active protocols for the early epoch, except for optimized protocol with the 24 h interval. For the late epoch, only the optimized single intervention and optimized tDCS protocols with a 20 min interval induced significant motor cortical excitability diminutions. No significant effects were found for the very late epoch (same evening to next-day evening). Furthermore, *post hoc* tests comparing MEPs between active stimulation protocols indicated that neuroplastic after-effects of the single intervention optimized protocols (for early and late epochs) were

significantly larger than those obtained with the repeated optimized protocol with a 24 h interval. In addition, no effect on tDCS-induced MEP alterations was observed for either age or gender, as shown by the respective ANCOVA results (Table 2).

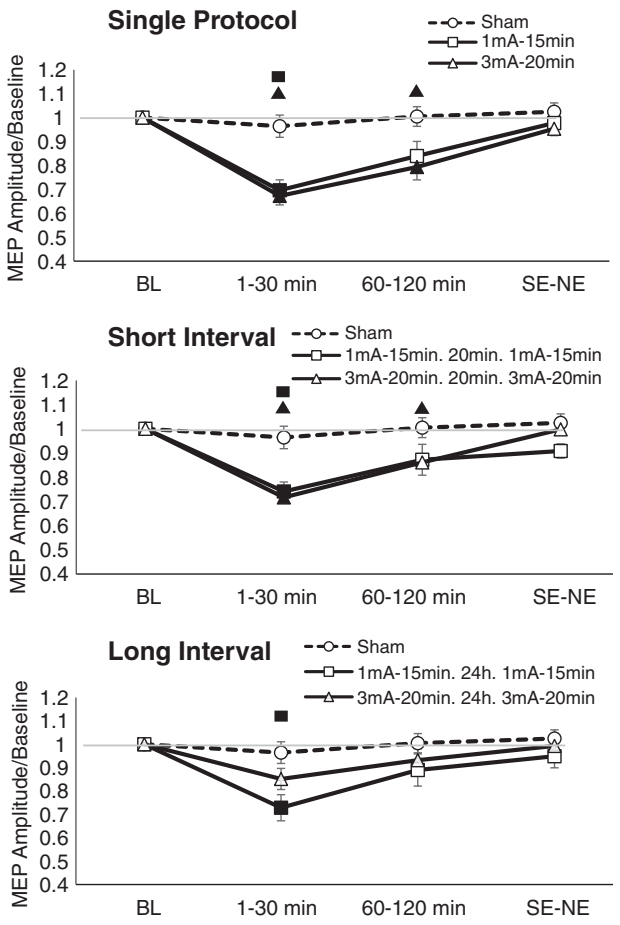

Figure 2. MEP amplitudes grand-averaged for early, late and very late tDCS post-stimulation effects
MEP were grand-averaged and pooled into three epochs of early (0–30 min), late (60–120 min), and very late (same evening (SE) or next evening (NE)) excitability changes. The single intervention protocol includes conventional and optimized tDCS protocols. The short interval protocol includes repeated conventional and optimized tDCS with a 20 min interval. The long interval protocol includes repeated conventional and optimized tDCS with a 24 h interval. Statistical data indicate that, in comparison with sham tDCS, all protocols significantly reduced MEP amplitudes in the early epoch (0–30 min after stimulation), except for the optimized protocol with a 24 h interval. For the late epoch (60–120 min after stimulation), only the optimized single intervention and the optimized tDCS with a 20 min interval induced significant motor cortical excitability diminutions. No significant effects were found for the very late epoch. Error bars represent standard error of means. Filled symbols indicate a significant difference between cortical excitability and the respective baseline values. Floating symbols in each sub-figure indicate a significant difference between the respective active condition and the sham stimulation condition. SE, same evening; NE, next evening.

Qualitative assessment of tDCS protocols

Participants' guesses of received stimulation intensity are shown in Table 3. Frequencies of correct and wrong guesses were relatively similar in all intervention conditions. Ratings of the presence and intensity of side-effects, including visual phenomena, itching, tingling and pain during stimulation and skin redness, headache, fatigue, concentration difficulties, nervousness and sleep problems within 24 h after stimulation, are documented in Table 4. Side-effects were minor, or not present in all conditions.

Discussion

In this study, we explored if repetitive stimulation with short or long intervals extends the neuroplastic after-effects of low and high dosages of single interventions of cathodal tDCS. In a sham-controlled repeated measures design, single intervention cathodal tDCS protocols with 1 mA for 15 min and 3 mA for 20 min and two repeated cathodal tDCS protocols with short (20 min) and long (24 h) intervals, for both single intervention protocol parameter combinations, were tested.

In general, the results of the study show that all cathodal tDCS protocols significantly reduced cortical excitability. The respective excitability alterations reflect, however, early-phase LTD-like neuroplasticity, since the duration of the after-effects was shorter than 3 h (Huang *et al.* 2004; Reymann & Frey, 2007). For repeated stimulation with short intervals, the after-effects of stimulation with conventional and optimized protocols remained nearly unchanged, compared to the respective single intervention protocols. For the long interval (24 h) protocols, stimulation with the conventional protocol did not significantly alter respective after-effects, while stimulation with the optimized protocol reduced after-effects, compared with the respective single interventions.

These results are in general accordance with previous findings from other studies conducted in healthy humans, in which late-phase LTD-like plasticity could not be induced by repeated cathodal stimulation with short intervals, and after-effects were reduced with an intervention interval of 24 h (Monte-Silva *et al.* 2010). Similarly, repeated continuous theta burst stimulation (cTBS), another non-invasive brain stimulation tool suited to induce LTD-like plasticity, did not induce late-phase LTD, when repeated with short intervals, in one study (Gamboa *et al.* 2011). However, other studies using comparable cTBS protocols showed gradual enhancements of LTD-like plasticity with spaced protocols, which were nevertheless still in the range of early-phase plasticity (Goldsworthy *et al.* 2012, 2013). Interestingly, in the latter study, intensified stimulation – similar to the results of the present study – reduced the excitability reduction in the case of repeated stimulation. In contrast, prolonged effects in the range of late-phase plasticity were elicited at the

Table 2. Results of the ANCOVA conducted for tDCS-induced MEP alterations and the impact of the covariates 'age' and 'gender' on tDCS-induced after-effects

	Factor	d.f.	*F* value	*P* value
Overall effects of tDCS on MEP amplitudes	Condition	6	4.608	<0.001*
	Epoch	*3*	15.286	<0.001*
	Condition × epoch	6.882	2.205	0.042*
	Age	1	0.997	0.336
	Condition × age	6	2.402	0.072
	Epoch × age	3	0.440	0.726
	Condition × epoch × age	6.882	1.112	0.363
	Gender	1	0.024	0.880
	Condition × gender	6	1.067	0.389
	Epoch × gender	3	0.41	0.989
	Condition × epoch × gender	6.882	1.689	0.121

The two-factorial repeated-measures ANCOVA conducted for grand-averaged pooled MEPs to discern active *vs*. sham stimulation protocols revealed significant main effects of stimulation condition, and epoch, and a respective significant interaction. In addition, results show no significant effects of either age or gender on the tDCS-generated neuromodulatory after-effects. Asterisks indicate significant results. d.f., degrees of freedom.

Table 3. Frequency table of participants' guesses of received stimulation intensity

	Sham	1 mA for 15 min	1 mA for 15 min with a 20 min interval	1 mA for 15 min with a 24 h interval	3 mA for 20 min	3 mA for 20 min with a 20 min interval	3 mA for 20 min with a 24 h interval
Wrongly guessed	9	8	9	10	10	9	9
Correctly guessed	7	8	7	6	6	7	*7*
Total	16	16	16	16	16	16	16

behavioural level via spaced theta burst stimulation over the frontal eye field in healthy humans, and the parietal cortex in patients affected by visual neglect, especially when more than two interventions were combined (Nyffeler *et al.* 2006, 2009; Cazzoli *et al.* 2012). Studies in animal models to induce late-phase LTD are comparatively rare, but showed a respective potential of slice preparations with repeated pharmacological (Shinoda *et al.* 2005) and also electrical stimulation interventions (Ahmed *et al.* 2015). Interestingly in these studies, spacing between interventions differed between minutes, and 24 h, and the repetition rate was usually larger than one.

The question emerges of why repeated plasticity-inducing cathodal tDCS did not generate late-phase LTD-like plasticity in healthy humans in the present and some previous studies, or in studies in humans in which related non-invasive brain stimulation protocols were applied. One explanation might be the challenge of translating results from animal *in vitro* and/or *in vivo* studies to human studies, which require different stimulation parameters, such as intensity, duration and inter-stimulation intervals, and present differences in spontaneous activity, neuro-transmitter and neuromodulator concentration, among others (Brunoni *et al.* 2011; Nitsche *et al.* 2012). Standard animal *in vivo* stimulation protocols affect relatively small populations of neurons in the target area, while tDCS protocols in humans stimulate hundreds of thousands of neurons of diverse origin, including excitatory and inhibitory neurons, concurrently. In addition, magnitude and direction of plasticity have been shown to be critically affected by the number of repetition blocks and the inter-stimulation interval. Three stimulation blocks with 10 min intervals were applied to induce late-phase LTD in an animal slice model (Ahmed *et al.* 2015), while in most studies in humans, including the present one, only one repetition was applied. Interestingly, however, larger repetition frequencies might induce larger effects in humans, and should therefore be tested in future studies (Nyffeler *et al.* 2006, 2009; Cazzoli *et al.* 2012).

With respect to the mechanistic foundation of these effects, it has been shown in animal models, that NMDA receptors, trafficking of AMPA receptors and calcium channel activities are involved in the early and late phases of long-term plasticity (Malenka & Bear, 2004), and modification of gene expression and protein synthesis are required for the maintenance of LTD (Manahan-Vaughan *et al.* 2000; Sajikumar & Frey, 2003; Pelletier & Cicchetti,

Table 4. Participant ratings of the presence and intensity of side-effects

	Side-effects	Sham	1 mA for 15 min	1 mA for 15 min with a 20 min interval	1 mA for 15 min with a 24 h interval	3 mA for 20 min	3 mA for 20 min with a 20 min interval	3 mA for 20 min with a 24 h interval
During stimulation	Visual phenomenon	0.00, 0.25	0.50, 1.00	0.00, 1.00	0.00, 0.00	0.00, 1.00	0.00, 0.125	1.00, 2.00
	Itching	0.00, 1.00	0.00, 1.00	0.00, 1.00	0.00, 0.00	0.00, 1.00	0.00, 1.00	1.00, 1.25
	Tingling	0.00, 1.00	0.50, 1.00	0.00, 0.25	1.00, 1.25	1.00, 1.00	1.00, 2.00	0.00, 2.25
	Pain	0.00, 0.25	0.00, 0.25	0.00, 0.25	0.00, 1.25	0.00, 0.00	0.00, 1.00	0.50, 2.00
24 h after stimulation	Redness	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.00, 0.25	0.00, 1.00	0.00, 0.00	0.00, 2.00
	Headache	0.00, 0.25	0.00, 0.25	0.00, 1.00	0.00, 1.25	0.00, 1.00	0.00, 1.00	0.00, 1.00
	Fatigue	0.50, 1.00	0.00, 1.00	0.00, 1.00	0.00, 1.25	0.50, 1.00	0.00, 1.00	0.00, 1.00
	Concentration difficulties	0.00, 0.00	0.00, 0.25	0.00, 0.00	0.00, 1.00	0.00, 0.00	0.00, 0.00	0.00, 1.00
	Nervousness	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.00, 1.00	0.00, 0.00	0.00, 0.00	0.00, 0.00
	Sleep problem	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.00, 1.00	0.00, 0.00	0.00, 0.00	0.00, 0.00

Side-effects were visual phenomena, itching, tingling and pain during stimulation and skin redness, headache, fatigue, concentration difficulties, nervousness and sleep problems within 24 h of stimulation. The presence and intensity of the side-effects were rated on a numerical scale from zero to five, zero representing no effect and five extremely strong sensations. Data are presented as medians, and interquartile ranges (IQR).

2014). Similar mechanisms have been described for tDCS-induced cortical excitability alterations in humans (Stagg *et al.* 2009; Nitsche *et al.* 2012; Monai *et al.* 2016). Basic mechanisms of action of tDCS in humans are thus similar to mechanisms revealed in animal models. Animal studies have, however, also described forms of LTD triggered by metabotropic glutamate receptors (mGluRs) (Collingridge *et al.* 2010; Lisman, 2017), including late-phase LTD (Shinoda *et al.* 2005). While these different forms of LTD share similarities, they might have a discernable impact on the direction and rate of LTD plasticity induction. Whereas the contribution of NMDA receptors to tDCS-induced cortical excitability alterations is well studied (Nitsche *et al.* 2003*a*; Stagg *et al.* 2009; Monai *et al.* 2016), a potential impact of metabotropic glutamate receptors, which might be relevant for late-phase LTD induction, has not been revealed so far for cathodal tDCS in humans. A potential missing effect of tDCS on these receptors might contribute to the limited efficacy of tDCS to induce late-phase LTD. At present, these explanations are, however, speculative, and should be explored directly in future studies.

Another important feature of the results is that the 24 h interval of interventions resulted in diminished LTD-like effects of tDCS. Importantly, this diminution was present only for the intensified stimulation protocol and only when the second tDCS intervention was applied at a time far beyond the time point when the single intervention resulted in MEP alterations. Homeostatic regulatory mechanisms, which control for the amount of neuroplastic alterations to avoid neuronal network destabilization, might help to explain these results. Here, prior synaptic activity influences the magnitude and direction of subsequently induced plasticity (Wexler & Stanton, 1993). According to the Bienenstock-Cooper-Munro rule, a prolonged decrease of postsynaptic activity will shift the synaptic modification threshold, reducing the amount of LTD induced by a respective intervention (Bienenstock *et al.* 1982). Non-invasive brain stimulation (NIBS) studies in humans have shown similar mechanisms (Siebner *et al.* 2004; Monte-Silva *et al.* 2010; Fricke *et al.* 2011), and also showed that synergistic or homeostatic effects of repeated stimulation critically depend on the respective intervals. With respect to the latter, the pattern of results obtained in the present study fits nicely to those of a previous one, suggesting that intervals longer than a few minutes are required for the induction of homeostatic effects in case of LTD-inducing protocols (Monte-Silva *et al.* 2010). They furthermore suggest that homeostatic counter-regulation is more easily induced by intensified stimulation protocols, which might lead to stronger saturation of the system, and that the mechanism driving these effects is beyond overt excitability alterations observable by MEP alterations.

The failure to induce late-phase LTD in the present study does not, however, imply that it is in principle not possible to induce such kind of plasticity in the human brain by tDCS. On the one hand, as outlined above, increasing the number of repetitions could enhance the effects of intervention, similarly to other NIBS protocols such as TBS. On the other hand, it has been shown that enhancement of global dopaminergic activity in combination with cathodal tDCS induces late-phase LTD-like plasticity (Kuo *et al.* 2008), and that this effect is at least partially driven by D2 receptors (Fresnoza *et al.* 2014). The mechanisms of this synergistic effect have not

been explored in detail so far, but one possibility might be that NMDA receptor activity diminutions induced by D2 receptor activation reduce spontaneous activity of the stimulated neuronal networks, which might support the effects of cathodal tDCS.

For blinding purposes, we used local anaesthetic cream to decrease tDCS-induced somatosensory sensations, as reported in previous studies for 2 mA tDCS (McFadden *et al.* 2011). Our results show that the frequency of correct guesses did not differ between conditions (Table 3). However, the participants' slightly higher rating of the presence and intensity of side-effects during stimulation under the high stimulation intensity (Table 4) implies that the topical anaesthetic cream might have limited efficacy at this intensity (here, 3 mA), which might potentially reduce the quality of blinding.

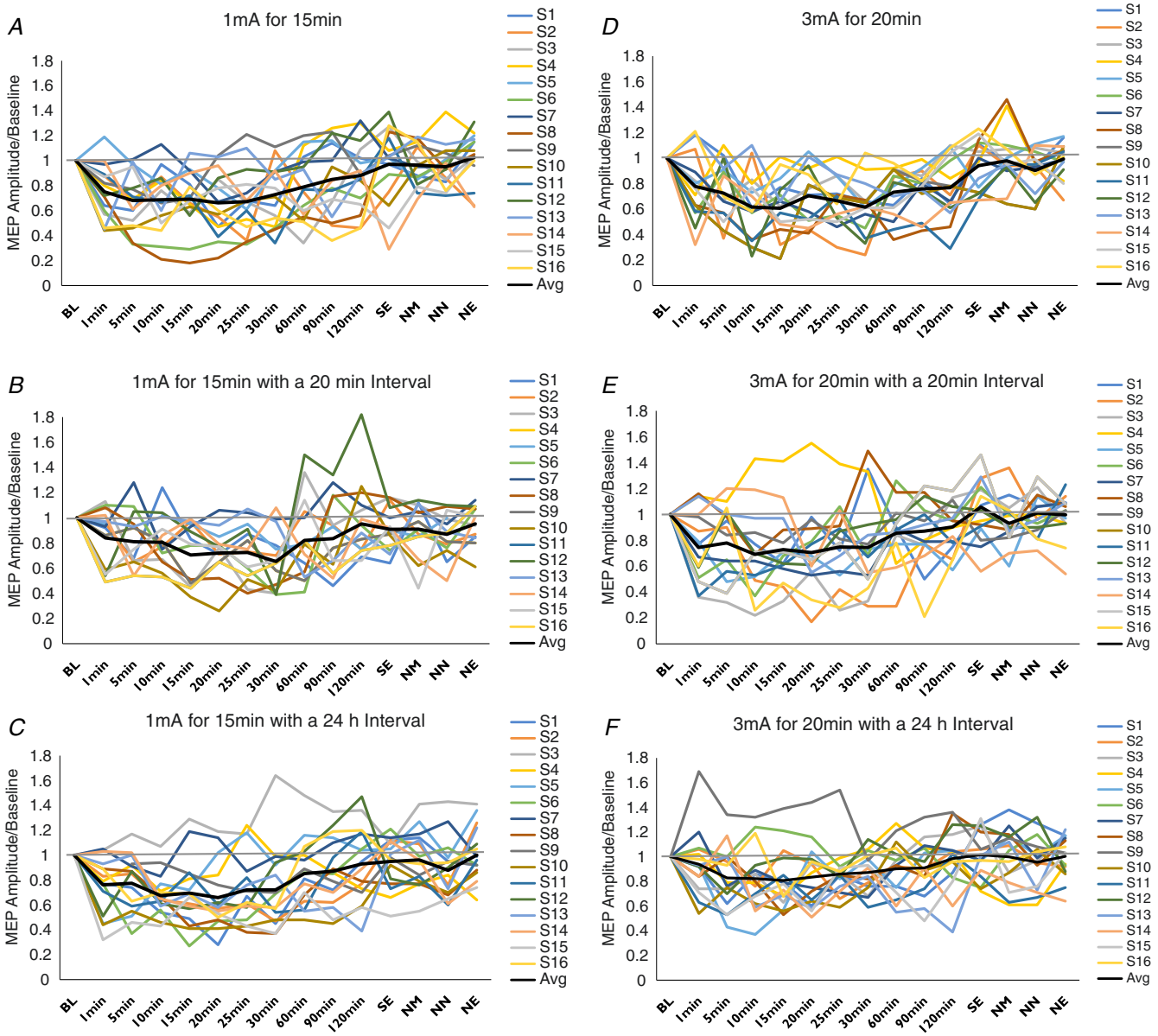

Figure 3. Intra-individual motor cortical excitability changes after single and repeated sessions of cathodal transcranial direct current stimulation (tDCS) over the primary motor cortex
The panels show individual excitability alterations after single intervention with the conventional protocol (1 mA for 15 min) (*A*), repeated intervention with the conventional protocol with a 20 min interval (*B*), repeated intervention with the conventional protocol with a 24 h interval (*C*), single intervention with the optimized protocol (3 mA for 20 min) (*D*), repeated stimulation with the optimized protocol with a 20 min interval (*E*), repeated intervention with the optimized protocol with a 24 h interval (*F*). Each coloured line in each graph represents MEP values of one participant (S1–S16). MEP amplitudes are normalized to baseline values individually. SE, same evening; NM, next morning; NN, next noon; NE, next evening. [Colour figure can be viewed at wileyonlinelibrary.com]

Limitations and future directions

In the present study, we probed the neurophysiological effects of tDCS at the group level, but individual characteristics affect the outcomes of tDCS and other NIBS protocols (Ridding & Ziemann, 2010; Wiethoff *et al.* 2014). Accordingly, the data obtained in the present experiment show some variability (Fig. 3). Potential contributing factors are anatomical and biophysical differences of individual brains, including genetics, time of day, and brain state (Kuo *et al.* 2006; Li *et al.* 2015; Moliadze *et al.* 2018). Thus, to improve stimulation efficacy at the level of the individual, an important next step would now be to understand/control for individual factors affecting the physiological and behavioural outcome of tDCS (Huang *et al.* 2017).

Moreover, the targeted population in this study comprised healthy young humans. One-to-one transferability of our results from motor to other cortical areas, as well as transferability to different age populations and patient groups, should not be taken for granted because the effects of transcranial electrical stimulation (tES) depend on brain state, anatomical differences, and differences of neuromodulator activities and cortical excitability between healthy humans and respective patients, and should be explored in future studies. In general, and in contrast to the inducibility of late-phase LTP-like effects, it seems to be more difficult to induce late-phase LTD-like effects in humans, independent from the specific plasticity induction tool. In this respect, non-linearities of the effects of cathodal tDCS, which are dependent largely on stimulation intensity, but possibly also on other parameters, play a prominent role (Jamil *et al.* 2017; Mosayebi Samani *et al.* 2019). The respective mechanisms are largely unexplored but might be important for the informed development of optimized stimulation protocols. Furthermore, based on results of animal and some human studies, a promising way forward might be spaced stimulation with a frequency larger than one, which should be explored systematically in future studies, or a combination of stimulation and pharmacological interventions. In this connection, it needs also to be stressed that the results of the present study do not allow us to derive assumptions about the physiological effects of multi-session once-daily stimulation protocols, which are often used in clinical trials. Finally, a further limitation of this study is that we included only one sham condition, because inclusion of a higher number of sham conditions would have reduced the feasibility of this already relatively laborious study.

Conclusion

The main results of this study show that late-phase plasticity was not induced by a single repetition of cathodal tDCS with short and/or long intervals using conventional and intensified stimulation protocols. We investigated the effects of repeated stimulation protocols with short (20 min), and long intervals (24 h) for a conventional protocol (1 mA for 15 min) and a newly developed optimized tDCS protocol (3 mA for 20 min). Our results revealed that, compared to the single intervention protocols, the duration of after-effects of repeated conventional and optimized protocols remained largely unchanged, or was reduced. The results of the present study are thus not in accordance with the induction of late-phase LTD by a single repetition of cathodal tDCS, but hint at a partially non-linear, probably homeostatic, counter-regulation. Since, in other studies, more frequent repetition of interventions induced cumulative effects and combinations of cathodal tDCS with pharmacological interventions induced late-phase effects, these might be promising approaches for future studies.

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Additional information

Competing interests

M. A. Nitsche is member of Advisory Board of Neuroelectrics. None of the remaining authors have potential conflicts of interest to be disclosed.

Author contributions

The study was performed at the neuromodulation laboratory of the Department of Psychology and Neuroscience, Leibniz Research Center for Working Environment and Human Factors (IfADo). M.M.S.: conceptualization, data curation, formal analysis, investigation, writing – original draft, visualization. D.A.: data curation, formal analysis, writing – review and editing. M.K: conceptualization, project administration, supervision, formal analysis, methodology, validation, writing – review and editing. M.A.N.: conceptualization, funding acquisition, supervision, writing – review and editing. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the study. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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LTD, MEP, neuroplasticity, repeated tDCS, TMS