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Lasting effects of transcranial direct current stimulation on the inducibility of synaptic plasticity by paired-associative stimulation in humans

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Abstract

Background Transcranial direct current stimulation (tDCS) is capable of eliciting changes in cortical neuroplasticity. Increasing duration or repetition of tDCS during the after-efects of a frst stimulation has been hypothesized to enhance efficacy. Computational models suggest sequential stimulation patterns with changing polarities to further enhance effects. Lasting tDCS effects on neural plasticity are of great importance for clinical applications.

Objective The study systematically examined the infuence of diferent tDCS paradigms on long term potentiation (LTP)-like plasticity in humans, focusing on stimulation duration, repetition frequency and sequential combinations of changing polarities as the underlying characteristics.

Methods Amplitude changes of motor evoked potentials (MEP) were measured in response to paired associative stimulation (PAS) 6 h after application of diferent tDCS protocols. In total, 36 healthy participants completed the study, randomised into three groups with different stimulation protocols $(N=12 \text{ each})$.

Results tDCS was able to display lasting modulatory effects on the inducibility of LTP-like plasticity in the human motor cortex 6 h after stimulation. TDCS with the anode on primary motor cortex significantly increased MEP amplitudes following PAS induction. Further analyses highlighted single stimulation block duration to be of higher importance than repetitive protocols for efficacy of effects.

Conclusions tDCS is capable of inducing lasting changes in the brain's capability to interact with future stimuli. Especially, efects on the inducibility of LTP-like plasticity might only be detectable with specifc tests such as PAS and might otherwise be overlooked. Refned tDCS protocols should focus on higher current and duration of single stimulations instead of implementing complex repetitive schedules.

Keywords Brain stimulation, Transcranial magnetic stimulation, Cathodal, Anodal, Long-term potentiation

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Background

Transcranial application of weak currents to the human primary motor cortex (e.g. via transcranial direct current stimulation, tDCS) has been repeatedly shown to be capable of eliciting intracortical excitability changes [[1–](#page-10-0)[3\]](#page-10-1). Changes in cortical excitability might lead to a modulation of synaptic plasticity [[4](#page-10-2), [5](#page-10-3)] including associative long-term potentiation (LTP) and long-term depression (LTD). Long-term synaptic plasticity represents the basic mechanism for experience-dependent modifcation of synaptic transmission and has been described in virtually every brain region across species, including humans [[6\]](#page-10-4). While most studies on LTP/LTD are conducted in rodents, LTP-like plasticity in humans can be assessed via amplitude changes of motor evoked potentials (MEP) following paired associative and transcranial magnetic stimulation (PAS) [\[7](#page-10-5)]. To assess the interplay of changes in cortical excitability (i.e. excitation-inhibition balance) and associative long-term plasticity, we used tDCS paradigms that were intended to modulate excitation and PAS to read-out their effect on associative LTP $[8]$ $[8]$.

Changes evoked by tDCS evolve during stimulation but persist for prolonged timeframes after stimulation, depending on stimulation polarity, duration and intensity [[1,](#page-10-0) [3,](#page-10-1) [9,](#page-10-7) [10\]](#page-10-8). So far, most studies suggested that excitability modulations induced by tDCS do not exceed 120 min [[2,](#page-10-9) [11\]](#page-10-10). However, several fndings suggest signifcantly longer tDCS effects depending on differing stimulation protocols: In animal models, repetition of tDCS during the after-efects of a frst stimulation session has been shown to enhance efficacy $[12]$ $[12]$. In humans, repeated tDCS with an interstimulus interval (ISI) of 20 min could elicit prolonged enhancement in motor cortex excitability for approximately 6 h when the anode was placed on the primary motor cortex, whereas temporally contiguous stimulation or longer intervals between stimulation did not induce comparable effects $[13]$ $[13]$. Some studies even reported polarity-specifc EEG diferences following repeated tDCS with an interstimulus interval (ISI) of 20 min to last until the next day [\[14](#page-11-1)].

Based on recent computational models of neural network dynamics and synaptic plasticity, it has been hypothesized that tDCS triggers neural network remodeling and cell assembly formation [\[15](#page-11-2)]. In the same model, repetition of tDCS during after-efects of a prior stimulation (sequential stimulation) as well as reversing stimulation polarity during the ISI (biphasic/polyphasic stimulation) were proposed to enhance the duration and strength of stimulation efects [[15](#page-11-2)]. In animal models, tDCS has been shown to increase survival of synaptic spines and preferential formation of new spines after a combined peripheral stimulation and tDCS [[16\]](#page-11-3). Interestingly, these changes outlasted 24 h and depended on a secondary stimulation paradigm to fully materialize. While most known tDCS efects might rely on functional, short-term neural plasticity and/or a change in excitation-inhibition balance, it remains unclear, how lasting structural neuroplastic changes in humans are best induced and detected.

This study therefore systematically examined different tDCS paradigms regarding their infuence on inducibility of LTP-like plasticity in humans, focusing on repetition frequency and sequential combination of changing polarities as potential modifying characteristics. Based on recent fndings indicating an important functional and anatomical connection between parietal lobe and motor cortex for coordination of hand movements, we chose a non-typical return electrode positioning over the parietal cortex [[17](#page-11-4), [18](#page-11-5)]. We hypothesized that repeated tDCS signifcantly increases inducibility of LTP-like plasticity when the anode was placed on the primary motor cortex compared to sham stimulation and that reversing polarity of tDCS during the ISI of two (or more; biphasic/polyphasic) tDCS blocks further increases stimulation efects compared to an ISI without stimulation. Secondly, we hypothesized that increasing the number of anodal stimulation blocks signifcantly enhances LTP-like plasticity, even if the added stimulation duration remains constant. To detect these lasting (structural) changes neural plasticity we applied a paired associative stimulation paradigm several hours later.

Methods

Study population

In total, 36 right-handed, healthy participants fnished the study protocol (18 females, age range 21–33 years, mean age 24.3 ± 2.8 years). Participants were randomised into three groups with diferent stimulation protocols (12 participants each; 6 females each; no age diference between groups $[p=0.136]$). A narrow age range was chosen to reduce potential age related sources of variance. A thorough screening process including a structured interview [[19\]](#page-11-6) was implemented to rule out any relevant mental or somatic disorder or substance use (including smoking and excessive caffeine use $>$ 300 mg/d) as well as any CNS-active medication. Adhering to brain stimulation safety recommendations, subjects with metallic implants or ongoing pregnancy were excluded $[20]$ $[20]$. The screening process was complemented by self-report questionnaires that ruled out subjective experience of depressive symptoms (Beck Depression Inventory, BDI [\[21](#page-11-8)], total score 2.3 \pm 2.4; no group difference $[p=0.492]$ or excessive daytime sleepiness (Epworth Sleepiness Scale, ESS [\[22](#page-11-9)], total score 5.3 ± 3.0 ; no group difference $[p = 0.879]$).

Participants were recruited via the internet, official press communications and advertisements of the University Medical Center Freiburg, University of Freiburg, Germany.

Study design

All experiments were conducted at the Department of Psychiatry and Psychotherapy of the University Medical Hospital Freiburg, Germany.

Following a screening visit, all participants concluded three study visits with tDCS according to the respective experimental protocol, followed by motor evoked potentials (MEP) measurements and paired associative stimulation (PAS; see Fig. [1\)](#page-2-0). To avoid confounding metaplastic efects of repeated stimulation, a minimum break of 7 days between each study visit was implemented.

Each of the three study visits began with tDCS according to the scheduled protocol. Following tDCS, participants were instructed to spend the day avoiding any kind of activity involving physical activation (e.g. sportive activities) or daytime napping and to return to the study center in the afternoon. According to recent studies [\[13](#page-11-0)], we expected a window of peak efect size concerning inducibility of LTP-like plasticity starting 6 h after the end of tDCS. PAS was therefore induced and measured accordingly. MEPs were recorded prior to and 5, 30, and 60 min after the end of PAS in addition to prior to, and immediately after tDCS.

Transcranial direct current stimulation (tDCS)

tDCS was delivered by a battery-driven, micro-processor-controlled CE-certifed constant current stimulator (neuroConn GmbH, Illmenau, Germany) comprising target electrodes over the right motor cortex and parietal return electrodes $(5 \times 7$ cm each, sponges soaked with 10 ml saline solution) to allow for efficient stimulation of the motor cortex. Electrode placement for the target electrode was guided by using TMS to defne the motor cortical representational feld of the abductor pollicis brevis muscle (as proposed by Nitsche et al. [[1](#page-10-0)], while the return electrode was placed on an atypical location over the parietal cortex (P3 according to the 10–20 system), following experimental settings capable of inducing long lasting tDCS efects [[14\]](#page-11-1) in addition to recent fndings indicating an important functional and anatomical connection between parietal lobe and motor cortex for coordination of hand movements [\[17](#page-11-4), [18\]](#page-11-5). This electrode placement differs from classical electrode montages with the return electrode placed over the contralateral supraorbital area (e.g. $[23]$ $[23]$ $[23]$). For efficient flow of current and activation of neural structures under the electrodes a minimum distance of around six cm between both electrodes was chosen.

A constant current of 2 mA over each electrode was applied using a fade-in/fade-out design [30 s each] to decrease skin sensations during the beginning and end of the stimulation $[24]$ $[24]$ $[24]$. A standard sham protocol with 30 s fade-in followed by 30 s fade-out at the beginning and end of each block without active stimulation in between was applied for a duration corresponding to the respective anodal stimulation setting [\[25](#page-11-12)]. For each participant, one of the following predefned orders of experimental protocols was chosen in a pseudorandomized and balanced order based on study entry to prevent sequential effects: $1-2-3$, $3-1-2$ or $2-3-1$. Polarity setting 1 was designed to induce strong efects by placing the anode on the primary motor cortex; setting 2 was focused on optimizing efects of tDCS with the cathode on the primary motor cortex; while setting 3 represented the sham condition for each experiment (nomenclature according to [[26](#page-11-13)]).

For experiment B and C, more than one stimulation block was applied (biphasic/polyphasic). To stay within safety recommendations and to keep the conditions as comparable as possible, the total stimulation duration

Fig. 1 General schedule of one study visit. Following a screening visit, all participants concluded three study visits with tDCS according to the respective experimental protocol during the morning followed by a 6-h break. Afterwards, all participants received paired associative stimulation (PAS) on each visit. Immediately prior to (T_{DCS} 0) and after tDCS (T_{DCS} 1) as well as prior to (T_{PAS} 0) and 5, 30, and 60 min after PAS (T_{PAS}) 1–3), motor evoked potentials (MEP) were assessed. Study visits were separated by 1 week. Three difering tDCS protocols (1/2/3) were applied in a balanced, pseudorandomized order, once per visit, for each experiment (A/B/C)

was kept constant (e.g. 20 min of tDCS with the anode on the primary motor cortex).

Experiment A implemented one singular block of stimulation for a duration of 20 min to allow for the longest constant stimulation time at the chosen intensity with regards to standard safety criteria [[20\]](#page-11-7). Polarity settings 1–3 therefore comprised 20 min of tDCS with either the anode (A1) or cathode (A2) on the primary motor cortex, or sham tDCS (A3; see Fig. [2A](#page-3-0)).

Experiment B examined the effect of two sequential 10 min stimulation blocks with the anode on the primary motor cortex, with either a 20 min interstimulus interval (ISI; B1) or 20 min of tDCS with reversed polarity in between (B2). Two respective blocks of sham stimulation with an ISI of 20 min served as the sham condition (B3; see Fig. [2B](#page-3-0)).

Experiment C further prolonged the sequential stimulation pattern, with C1 including four blocks of 5 min of tDCS with the anode on the primary motor cortex, with three ten minutes ISI in between. For experimental sequence C2, two of the three ISI were replaced by 10 min of tDCS with reversed polarity each. To adhere to standard safety parameters, a total stimulation time of 40 min could not be exceeded. Therefore, only two of the three ten minutes ISIs were exchanged (see Fig. [2C](#page-3-0)).

Paired associative stimulation—transcranial magnetic stimulation (PAS)

TMS was applied by standard criteria using a fgure-ofeight coil with an outer diameter of 90 mm connected to a Magstim 200 stimulator (The Magstim Company, Whitland, UK). Optimal coil placement was defned as tangentially to the skull over the right primary motor cortex (M1) with the handle pointing in a posterior direction, at a lateral angle of 45° regarding the midline. To identify the optimal coil position for eliciting MEPs of maximal amplitude of the left abductor pollicis brevis (APB) muscle ('hotspot'), the coil was moved over M1 while administering 0.25 Hz stimuli (suprathreshold intensity). The identifed coil position was then recorded using a stereotaxic, optically tracked navigation system, consisting of a camera (Polaris Vicra P6, NDI, Waterloo, ON, Canada), custom-made software (Visor2, eemagine GmbH, Berlin, Germany), and passive sphere markers [[27\]](#page-11-14), and kept constant throughout measurements.

Resting motor threshold (RMT) was determined according to standard criteria [\[28](#page-11-15)], with stimulation intensity for MEP measurements adjusted to elicit MEPs with peak-to-peak amplitudes of on average 600– 1400 μV (SI 1 mV). At each measuring point, twenty TMS pulses were administered at a frequency of 0.1 Hz

Fig. 2 Overview of applied tDCS protocols. **A** Stimulation protocols for experiment A. Participants either received 20 min. of 2 mA tDCS with the anode on the primary motor cortex (A1), 20 min. of 2 mA tDCS with the cathode on the primary motor cortex (A2) or sham stimulation (A3). **B** Stimulation protocols for experiment B. Participants either received two blocks of 10 min. of 2 mA tDCS with the anode on the primary motor cortex (interstimulus interval [ISI] of 20 min.; B1), two blocks of 10 min. of 2 mA tDCS with the anode on the primary motor cortex, with 20 min. of 2 mA tDCS with reversed polarity in between (B2) or sham stimulation (B3). **C** Stimulation protocols for experiment C. Participants either received four blocks of 5 min. of 2 mA tDCS with the anode on the primary motor cortex (interstimulus interval [ISI] of 10 min.; C1), four blocks of 10 min. of 2 mA tDCS with the anode on the primary motor cortex with two blocks of 10 min. of 2 mA tDCS with reversed polarity in between (C2), or sham stimulation (C3)

and the corresponding peak-to-peak amplitudes of these pulses were averaged using Signal Software (CED, UK). For optimal MEP recordings, participants were instructed to relax the targeted left APB muscle during all measurements, which was monitored visually via a concurrent electromyogram (EMG). MEPs were recorded using silver/silver chloride electrodes (AMBU, Ballerup, Denmark) in a belly-tendon montage. Signals were band-pass fltered (20–2000 Hz), amplifed using an Ekida DC universal amplifer (EKIDA GmbH, Helmstadt, Germany), digitized at a 5 kHz sampling rate using a MICRO1401mkII data acquisition unit (CED), and stored on a computer for ofine analysis. MEPs with preceding muscle activity were excluded from analysis. MEPs were normalized by diving each post-PAS MEP with the corresponding pre-PAS MEP.

The chosen PAS protocol closely follows standard procedures [\[7](#page-10-5)]. In summary, the protocol comprised 200 pairs of peripheral and cortical stimuli, given at a frequency of 0.25 Hz (total duration \sim 13 min). The peripheral pulse was delivered to the median nerve of the left wrist at an intensity of 300% of the sensory perceptual threshold by a Digitimer DS7 electrical stimulator (Digitimer, Welwyn Garden City, Hertfortshire, UK) as constant current square wave pulses with a duration of 1000 μs.

The interstimulus interval (ISI) between the peripheral and cortical stimulation was set to 25 ms. Participants were instructed to direct their attention to the stimulated hand and count rarely occurring (4 stimuli in total), randomly intermittent, electrical stimuli to the thumb of the stimulated hand (200% perceptual threshold, cathode proximal, constant current square wave pulses, duration 200 μs), that were administered during the ISI to decrease infuences of difering attention levels [[29](#page-11-16)[–31](#page-11-17)]. These stimuli were administered through an additional electrode placed distal to the peripheral electrode on the thumb.

Statistical analyses

MEP mean amplitudes were considered as primary outcome parameters. To test for MEP amplitude diferences, repeated-measures analyses of variance (ANOVA) with the within-subject factors condition (tDCS protocols) and timepoint (T_{DCS} 0–1, T_{PAS} 0–3) were conducted for each experiment. Simple two-tailed t-tests were conducted post-hoc to test for diferences between specifc MEPs. In addition, one sample t-test comparing the sample mean against a hypothetical mean of 1 (due to normalization) were conducted (Fig. [7](#page-8-0)).

Descriptive values are given as means and standard deviations. For the estimation of efect sizes, partial ETA squared values were calculated (low:<0.06; medium: ≥ 0.06 and < 0.14; large: ≥ 0.14). The level of signifcance was set at *p*<*0.05* (two-tailed). In cases of violations of sphericity, the Greenhouse–Geisser adjustment was applied. For subgroup analysis PAS response was defned as reaching a normalized (to baseline) MEP amplitude greater than one at T_{PAS} 2 in the sham condition. All analyses were conducted with the statistical software IBM SPSS Statistics (Version 29).

Results

No short‑term and long‑term MEP amplitude diferences after tDCS

To control for immediate and delayed excitability increasing efects of the diferent tDCS protocols, we compared MEP amplitudes prior to and after tDCS $(T_{DCS}$ 0–1). MEP amplitudes did not differ in any of the experiments (experiment 1: timepoint: $F=0.7$, $p=0.792$, $pETA²=0.007$; condition: $F=0.6$, $p=0.576$, $pETA^2 = 0.115$; interaction timepoint x condition: $F = 0.6$, $p=0.576$, $pETA^2=0.115$; experiment 2: timepoint: F=1.1, $p=0.308$, $pETA^2=0.094$; condition: F=0.07, $p=0.932$, $pETA^2=0.014$; interaction timepoint \times condition: $F = 0.07$, $p = 0.932$, $pETA^2 = 0.014$; experiment 3: timepoint: $F=1.2$, $p=0.306$, $pETA^2=0.095$; condition: F=1.3, $p=0.312$, $pETA^2=0.208$; interaction timepoint \times condition: $F = 1.3$, $p = 0.312$, $pETA^2 = 0.208$).

Moreover, MEP amplitudes post tDCS and pre PAS did not difer in any condition (experiment 1: timepoint: F=1.186, *p*=*0.2840*; condition: F=2.927, *p*=0.0676; interaction timepoint \times condition: $F = 1.238$, $p = 0.3030$; experiment 2: timepoint: $F=3.180$, $p=0.6102$; condition: $F=2.229$, $p=0.1236$; interaction timepoint \times condition: F=0.5015, $p=0.6102$; experiment 3: timepoint: F=0.007446, *p*=*0.9318*; condition: F=0.09192, $p=0.9124$; interaction timepoint \times condition: $F = 0.5729$, $p = 0.5694$).

Induction of long‑term potentiation‑like plasticity by paired associative stimulation

Efects of PAS on MEP amplitude were measured at three timepoints at 5, 30 and 60 min following PAS induction $(T_{PAS} 1-3)$ and compared to a baseline measurement immediately prior to PAS (T_{PAS} 0; see Fig. [3\)](#page-5-0). To assert the general feasibility of the chosen PAS paradigm, averaged data across all sham conditions $(A3, B3, C3; N=36)$ was analyzed. Focusing on the timepoint displaying the largest efect on LTP-like plasticity, which was expected around 30 min after conclusion of PAS (T_{PAS} 2), MEP amplitudes signifcantly difered from baseline with PAS inducing an increase of 0.18 μ V (F=4.4, *p*=0.042, $pETA^2 = 0.112$.

Fig. 3 Induction of long-term potentiation by PAS. Efects of paired associative stimulation (PAS) on motor evoked potentials (MEP) amplitudes were measured at three timepoints following PAS induction (T_{PAS} 1–3) and compared to a baseline measurement immediately prior to PAS (T_{PAS} 0). T_{PAS} 2 displayed significantly increased MEP amplitudes compared to T_{PAS} 0. Normalized MEP amplitudes, averaged across all experiments. Means±SEM

Lasting modulatory efects of single block tDCS (experiment A)

One singular block of tDCS for a duration of 20 min led to a polarity-specifc modulation of inducibility of LTP-like plasticity by PAS 6 h later (for stimulation protocol see Fig. [2A](#page-3-0)). Specifcally, a signifcant interaction between timepoint of MEP measurement and condition of stimulation protocol could be detected $(F=2.9, p=0.013, pETA²=0.211; timepoint: F=1.5,$ $p=0.233$, $pETA^2=0.120$; condition: $F=0.9$, $p=0.436$, $pETA^2 = 0.073$.

Post-hoc testing revealed that tDCS with the anode on the primary motor cortex was the major factor of the significant interaction, with T_{PAS} 3 displaying a significantly higher MEP amplitude compared to T_{PAS} 0 (A1, F=5.7, $p=0.036$, $pETA^2=0.341$). In addition, tDCS with the anode on the primary motor cortex led to significantly higher MEP amplitudes at T_{PAS} 3 compared to sham (F=6.1, $p = 0.032$, $pETA^2 = 0.355$). Interestingly, while the infuence of prior tDCS with the anode on the primary motor cortex led to a later rise in MEP amplitudes following PAS than expected and detected after sham stimulation, participants displayed no increase in MEP amplitude following tDCS when the cathode was placed on the primary motor cortex (A2), which suggests an induced suppression of the expected PAS efects (see Fig. [4](#page-6-0)). For individual data please refer to Fig. S1 (supplements).

No lasting modulatory efects of sequential tDCS (experiment B)

Sequential blocks of tDCS did not lead to a signifcant protocol-specifc modulation of inducibility of LTP-like plasticity by PAS 6 h later (for stimulation protocol see Fig. [2](#page-3-0)B; timepoint: $F = 2.8$, $p = 0.054$, $pETA^2 = 0.204$; condition: $F = 2.8$ $p = 0.085$, $pETA^2 = 0.201$; interaction timepoint x condition, $F=1.7$, $p=0.197$, $pETA^2=0.133$).

Exploratory post-hoc testing indicated that the most prominent differences were between T_{PAS} 2 and T_{PAS} 3 where all conditions displayed an increase in MEP amplitudes (F=5.3, $p=0.042$, pETA²=0.324). In addition, biphasic sequential tDCS displayed signifcantly lower MEP amplitudes at T_{PAS} 2 compared to T_{PAS} 0 (B2, F=5.7, $p=0.037$, $pETA²=0.340$) and compared

Fig. 4 Lasting modulatory efects of single block transcranial direct current stimulation (tDCS). Main efects of tDCS on inducibility of LTP-like plasticity. Efects of paired associative stimulation (PAS) on motor evoked potential (MEP) amplitudes were measured at three timepoints following PAS induction (T_{PA5} 1–3) and compared to a baseline measurement immediately prior to PAS (T_{PA5} 0). A significant interaction between timepoint of MEP measurement and stimulation protocol could be detected. Post-hoc testing revealed T_{PAS} 3 to display a significantly higher MEP amplitude compared to T_{PAS} 0 following tDCS with the anode on the primary motor cortex (experiment A1, indicated by *). In addition, at T_{PAS} 3, anodal tDCS led to significantly higher MEP amplitudes compared to sham (not marked). Means \pm SEM

to monophasic sequential tDCS at T_{PAS} 2 (B1, F=6.9, $p = 0.023$, pETA²=0.387; see Fig. [5](#page-7-0)). For individual data please refer to Fig. S1 (supplements).

No lasting modulatory efects of sequential tDCS at higher frequencies (experiment C)

Sequential blocks of tDCS with higher frequency but reduced duration of singular stimulation blocks (see Fig. [2C](#page-3-0)) failed to induce a signifcant modulation of inducibility of LTP-like plasticity by PAS 6 h later (timepoint: $F = 0.8$, $p = 0.488$, $pETA^2 = 0.070$; condition: $F = 0.3$, $p=0.745$, pETA²=0.026; interaction timepoint \times condition: F=0.4, $p=0.879$, $pETA²=0.035$; see Fig. [6](#page-8-1)). For individual data please refer to Fig. S1 (supplements).

Discussion

General duration and characterization of tDCS efects in humans

A frst major conclusion is that tDCS was able to produce lasting modulatory efects on the inducibility of LTPlike plasticity in the human motor cortex. Direct efects

of tDCS on excitability were probably prevented by the atypical electrode positioning. Even in the absence of immediate efects on the MEP amplitudes, the chosen tDCS protocols did show polarity- and frequency-specifc efects on the inducibility of neuroplastic changes by PAS 6 h later. These results indicate that maintained tDCS efects other than direct excitability changes modulate associative long-term plasticity induction, even after 6 h. Tis could be due to complex metaplastic changes (i.e. changes of excitation-inhibition balance), which might be of decisive importance for the underlying longterm plasticity processes frequently reported efective in motor rehabilitation [[32,](#page-11-18) [33](#page-11-19)].

These findings add to the data on long-term tDCS efects and demonstrate, for the frst time, clear changes in the response to plasticity-modulating interventions as late as 6 h after tDCS.

Specifcally, 20 min of sustained tDCS with the anode on the primary motor cortex (experiment A1) led to a signifcant long-term boost of inducibility of LTP-like plasticity by PAS. While PAS regularly led to an increase

Fig. 5 No lasting modulatory effects of sequential transcranial direct current stimulation (tDCS). Main effects of tDCS on inducibility of LTP-like plasticity. Efects of paired associative stimulation (PAS) on motor evoked potential (MEP) amplitudes were measured at three timepoints following PAS induction (T_{PAS} 1–3) and compared to a baseline measurement immediately prior to PAS (T_{PAS} 0). No main effects were found. Exploratory post-hoc testing indicated biphasic sequential tDCS with direction changes of the electrical feld (experiment B2) to induce signifcantly lower MEP amplitudes at T_{PAS} 2 compared to T_{PAS} 0 (indicated by *). In addition, at T_{PAS} 2, biphasic sequential tDCS led to significantly lower MEP amplitudes compared to monophasic tDCS (not marked). Means ± SEM

in MEP amplitudes with a maximum after around 30 min following sham tDCS, sustained 20 min tDCS with the anode on the primary motor cortex led to a slower increase in MEP amplitudes reaching a maximum at around 60 min past PAS. At this timepoint, MEP amplitudes were signifcantly higher compared to sham stimulation and signifcantly higher than baseline values. As no further MEPs were measured, it remains unclear how long the induced MEP increase would have been sustained.

Repetitive tDCS blocks are not superior to sufficiently **powered single stimulations**

Despite our initial hypothesis and modelling data hinting at positive efects [\[15](#page-11-2)], repetitive tDCS protocols did not increase tDCS efficacy in this study (experiments $B1/C1$). It appears that the duration of singular stimulation blocks is highly important for the longevity of efects and multiple shorter stimulation blocks do not add up to reach the same efect size. Instead, shorter stimulation blocks

as typically applied in sequential settings appear underpowered to induce lasting effects. Comparing normalized amplitudes at T_{PAS} 3 (60 min after stimulation) across settings, only 20 min and 2×10 min monophasic tDCS with the anode on the primary motor cortex showed increased amplitudes, with only 20 min tDCS with the anode on the primary motor cortex displaying changes superior to averaged sham response across all conditions (see Fig. [7](#page-8-0)). A diferent interpretation could be, that tDCS protocols with more than one stimulation block inhibit later inducibility of LTP-like plasticity (e.g. by inducing stronger short-term efects with inhibited afterphases). However, we did not detect stronger short-term efects for those protocol in our (limited) MEP data.

The findings are in line with animal model data hinting at higher stimulation intensities needed to affect neural circuits than historically expected $[34]$. The authors recommend 4–6 mA instead of the state-of-the-art usage of $1-2$ mA (as applied in this study) [\[34\]](#page-11-20). However, experimental data in humans did not demonstrate

Fig. 6 No lasting modulatory efects of sequential transcranial direct current stimulation (tDCS) at higher frequencies. Main efects of tDCS on inducibility of LTP-like plasticity. Efects of paired associative stimulation (PAS) on motor evoked potential (MEP) amplitudes were measured at three timepoints following PAS induction (T_{PAS} 1–3) and compared to a baseline measurement immediately prior to PAS (T_{PAS} 0). No significant modulation of inducibility of LTP-like plasticity could be detected. Means±SEM

Fig. 7 Overview of normalized motor evoked potentials (MEP) amplitudes at T_{PAS} 3. Effects of paired associative stimulation (PAS) on motor evoked potential (MEP) amplitudes 60 min. after induction (T_{PAS} 3) indicated a clear increase only following transcranial direct current stimulation with the anode on the primary motor cortex (tDCS; experiment A1), with all other tDCS protocols showing no diference to sham (averaged across all experiments for this visualization). One sample t-test comparing the sample mean against a hypothetical mean of 1 (due to normalization): A1: p=0.0362*, A2: p=0.2432, B1: p=0.7951, B2: p=0.4900, C1: p=0.6004, C2: p=0.6099, Sham: p=0.2698. Means±SEM

a clear correlation between increasing current intensity and effect sizes $[2, 35]$ $[2, 35]$ $[2, 35]$ $[2, 35]$. For cathodal tDCS, the relationship between stimulation intensity, repetition frequency, and efect size and direction appears more complex with some intensities leading to diminished efects, others to anodal-tDCS-like enhancement of neuroplasticity [[2,](#page-10-9) [15](#page-11-2)]. To date, general recommendations for optimal stimulation range between 1 and 3 mA [\[35,](#page-11-21) [36](#page-11-22)].

Diferential efects of tDCS with direction changes of the electrical feld (anodal/cathodal)

In experiment A2, PAS was not able to induce LTP-like efects after 20 min of tDCS with the cathode on the primary motor cortex earlier in the day. Instead, in experiment B2, the biphasic setting of two-times 10 min of tDCS with the anode on the primary motor cortex with 20 min of reversed polarity tDCS during the ISI showed a significant reduction of MEP amplitudes at T_{PAS} 2 compared to T_{PAS} 0, and compared to two-times monophasic 10 min tDCS without stimulation during the ISI. TDCS with the cathode on the primary motor cortex appears to have a diminishing efect on inducibility of LTP-like plasticity that might be enhanced by carefully combining phases of changing polarity. In contrast to our initial hypothesis, the polarity of the longest single tDCS block might determine the direction of the efect more than the order of stimulations. We initially designed the protocol to increase the efect size of two tDCS blocks with the anode on the primary motor cortex by adding tDCS in reverse polarity to the ISI. However, the resulting paradigm can be better interpreted as a tDCS of 20 min duration with the cathode on the primary motor cortex, with 10 min of reversed polarity tDCS beforehand acting as a preconditioning efect (priming; [\[37](#page-11-23)]). It is to note, though, that all interpretations of tDCS efects with the cathode on the primary motor cortex in this dataset rely on exploratory data and lack robust statistical support.

Limitations

The study was conceptualized to further understand ongoing tDCS efects that might infuence the brains capability to interact with stimuli long after conclusion of stimulation (e.g. $[14]$ $[14]$). The electrode placement, mainly the placement of the 'return' electrode on the parietal cortex was chosen based on recent fndings indicating the importance of the parietal cortex specifcally for hand movements [\[17](#page-11-4), [18\]](#page-11-5). In addition, this electrode placement reduced the direct efects of tDCS on excitability and MEP amplitudes, thereby improving the measurability of long-term plasticity induced by PAS. However, the resulting electric feld is diferent compared to the classical electrode placement (over M1 and the contralateral supraorbital area) and may not necessarily be comparable to studies using the standard montage.

In addition, the chosen electrode placement might have reduced the expected efficacy of the stimulation by resulting in a distance between electrodes slightly smaller compared to the standard electrode montage [[38](#page-11-24)]. In general, choosing high defnition tDCS approaches might be useful to further clarify the results [[39](#page-11-25)].

As can be seen in Figs. [4](#page-6-0) and [5](#page-7-0), MEP changes following sham stimulation depicted a slightly diferent trajectory across experiments and only clearly displayed the expected changes when combined across experiments $(N=36, Fig. 3)$ $(N=36, Fig. 3)$ $(N=36, Fig. 3)$. To our understanding, these differences are most likely due to random variations in stimulation response and pronounced by a limited sample size $[40]$ $[40]$. This might add to overall variance and diminish the explanatory power of the results. However, exploratory analysis of subgroups with a clear PAS response following sham supported the main analysis (lower signifcance levels due to smaller sample size of $N=7-8$, supplements Fig. S2).

The current study aimed at understanding long-term efects of tDCS on neuroplasticity given the heterogeneity of results in previous clinical studies.

As the study was conducted in healthy participants, results might not translate to populations with potentially disturbed levels of neuroplasticity as in major depressive disorder (MDD). In addition, the study examined neuroplastic efects in the motor cortex, while from a clinical perspective other areas, e.g. prefrontal brain regions, might be of higher importance. However, there is growing support on the transferability of neurostimulation efects between brain regions [\[41](#page-11-27)].

Conclusions

The current study shows tDCS to be capable of inducing long term plasticity-inducing effects several hours after stimulation. This effect duration might be frequently overlooked in regular study designs because detection relies on specifc interventions to discriminate underlying efects on inducibility of neural plasticity [[5](#page-10-3)]. PAS has the potential to provide a tool for detecting these long-term, possibly structural, tDCS efects. In addition, the study adds to the understanding of the interaction between repetition patterns and stimulation intensity in designing optimal tDCS protocols [[15\]](#page-11-2).

Besides promising results in initial clinical trials [\[42](#page-11-28), [43\]](#page-11-29), tDCS recently failed to demonstrate efficacy in augmenting standard treatment for MDD patients [\[44](#page-11-30), [45\]](#page-11-31). However, plasticity processes are not immediate, and antidepressant treatment response requires longlasting restorative effects $[46]$ $[46]$ $[46]$. The underlying expectation of therapeutic efects of time-limited interventions

by tDCS implies the existence of longer lasting efects than commonly conceptualized. In addition, the lacking clinical efect appears to be inconsistent with a growing body of data on plasticity-modulating tDCS efects (e.g. $[5]$ $[5]$ $[5]$.

As a conclusion, there is a pressing need for refned tDCS protocols [[47\]](#page-11-33). In analogy to optimized rTMS protocols using pulsed protocols [[48\]](#page-11-34) and predictions from modelling studies [[15](#page-11-2)], it has been expected that spaced, sequential tDCS would be more efective. Regarding short-term efects on MEP amplitudes and long-term efects on synaptic plasticity, we could not confirm this assumption. Therefore, further research on optimized tDCS protocols is necessary [\[47](#page-11-33), [49](#page-11-35)].

Abbreviations

- tDCS Transcranial direct current stimulation
- ESS Epworth Sleepiness Scale
- BDI Beck Depression Inventory
- PAS Paired associative stimulation
- MEP Motor evoked potentials
- TMS Transcranial magnetic stimulation
- LTP Long-term potentiation
- LTD Long-term depression
ISI Interstimulus interval
- ISI Interstimulus interval
APB Abductor pollicis brev
- Abductor pollicis brevis
- RMT Resting motor threshold
CNS Central nervous system
- Central nervous system MDD Major depressive disorder

Supplementary Information

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Supplementary Material 1: Fig. S1: Systematic presentation of all experimental series including individual data. Means±SEM

Supplementary Material 2: Fig. S2: Differential effects of paired associative stimulationon motor evoked potentialamplitudes in a subgroup of defned PAS responders depending on prior tDCS. PAS responders were defined by reaching a normalizedMEP amplitude greater than one at T_{PAS} 2 in the sham condition.. Means±SEM.

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Author contributions

Conceptualization, Supervision, Resources: EW, KD, CN, LF. Investigation: EW, JD, CHZ, SI, MK. Validation, and Methodology: SV, EW, BF, SF, CN, LF. Writing original draft, Visualization: SV, SF, CN, LF. Writing—review and editing: EW, JD, KD, BF, CH, SI, MK. All authors provided fnal approval and agreed to be accountable for all aspects of the work.

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Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to German data protection laws but are available in accordance with these from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University Medical Center Freiburg (477/18). All participants signed written informed consent prior to the study and received fnancial compensation for their participation.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- 1. Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology. 2001;57(10):1899–901.
- 2. Jamil A, Batsikadze G, Kuo H-I, Labruna L, Hasan A, Paulus W, et al. Systematic evaluation of the impact of stimulation intensity on neuroplastic after-efects induced by transcranial direct current stimulation. J Physiol (Lond). 2017;595(4):1273–88.
- 3. Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol (Lond). 2000;527(Pt 3):633–9.
- 4. Nitsche MA, Roth A, Kuo M-F, Fischer AK, Liebetanz D, Lang N, et al. Timing-dependent modulation of associative plasticity by general network excitability in the human motor cortex. J Neurosci. 2007;27(14):3807–12.
- 5. Frase L, Mertens L, Krahl A, Bhatia K, Feige B, Heinrich SP, et al. Transcranial direct current stimulation induces long-term potentiation-like plasticity in the human visual cortex. Transl Psychiatry. 2021;11(1):17.
- 6. Cooke SF. Plasticity in the human central nervous system. Brain. 2006;129(7):1659–73.
- 7. Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain. 2000;123(3):572–84.
- 8. Morya E, Monte-Silva K, Bikson M, Esmaeilpour Z, Biazoli CE, Fonseca A, et al. Beyond the target area: an integrative view of tDCS-induced motor cortex modulation in patients and athletes. J Neuroeng Rehabil. 2019;16(1):141.
- 9. Nitsche MA, Fricke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N, et al. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. J Physiol (Lond). 2003;553(Pt 1):293–301.
- 10. Salehinejad MA, Ghanavati E, Reinders J, Hengstler JG, Kuo M-F, Nitsche MA. Sleep-dependent upscaled excitability, saturated neuroplasticity, and modulated cognition in the human brain. Elife. 2022;11:e69308.
- 11. Huang Y-Z, Lu M-K, Antal A, Classen J, Nitsche M, Ziemann U, et al. Plasticity induced by non-invasive transcranial brain stimulation: a position paper. Clin Neurophysiol. 2017;128(11):2318–29.
- 12. Bindman LJ, Lippold OCJ, Redfearn JWT. The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and

(2) in the production of long-lasting after-efects. J Physiol (Lond). 1964;172(3):369–82.

- 13. Monte-Silva K, Kuo M-F, Hessenthaler S, Fresnoza S, Liebetanz D, Paulus W, et al. Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. Brain Stimul. 2013;6(3):424–32.
- 14. Frase L, Piosczyk H, Zittel S, Jahn F, Selhausen P, Krone L, et al. Modulation of total sleep time by transcranial direct current stimulation (tDCS). Neuropsychopharmacology. 2016;41(10):2577–86.
- 15. Lu H, Gallinaro JV, Rotter S. Network remodeling induced by transcranial brain stimulation: a computational model of tDCS-triggered cell assembly formation. Netw Neurosci. 2019;3(4):924–43.
- 16. Gellner A-K, Reis J, Holtick C, Schubert C, Fritsch B. Direct current stimulation-induced synaptic plasticity in the sensorimotor cortex: structure follows function. Brain Stimul. 2020;13(1):80–8.
- 17. Reibelt A, Quandt F, Schulz R. Posterior parietal cortical areas and recovery after motor stroke: a scoping review. Brain Commun. 2023;5(5):fcad250.
- 18. Goldenkoff ER, Logue RN, Brown SH, Vesia M. Reduced facilitation of parietal-motor functional connections in older adults. Front Aging Neurosci. 2021;13: 595288.
- 19. Sheehan DV, Lecrubier Y, Harnett-Sheehan K, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59(Suppl 20):22–33 quiz 34–57.
- 20. Bikson M, Grossman P, Thomas C, Zannou AL, Jiang J, Adnan T, et al. Safety of transcranial direct current stimulation: evidence based update 2016. Brain Stimul. 2016;9(5):641–61.
- 21. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4:561–71.
- 22. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep. 1991;14(6):540–5.
- 23. Ghasemian-Shirvan E, Mosayebi-Samani M, Farnad L, Kuo M-F, Meesen RLJ, Nitsche MA. Age-dependent non-linear neuroplastic efects of cathodal tDCS in the elderly population: a titration study. Brain Stimul. 2022;15(2):296–305.
- 24. Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: state of the art 2008. Brain Stimul. 2008;1(3):206–23.
- 25. Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clin Neurophysiol. 2006;117(4):845–50.
- 26. Bikson M, Esmaeilpour Z, Adair D, Kronberg G, Tyler WJ, Antal A, et al. Transcranial electrical stimulation nomenclature. Brain Stimul. 2019;12(6):1349–66.
- 27. Jung NH, Delvendahl I, Kuhnke NG, Hauschke D, Stolle S, Mall V. Navigated transcranial magnetic stimulation does not decrease the variability of motor-evoked potentials. Brain Stimul. 2010;3(2):87–94.
- 28. Awiszus F. Chapter 2: TMS and threshold hunting. Suppl Clin Neurophysiol. 2003;56:13–23.
- 29. Stefan K, Wycislo M, Classen J. Modulation of associative human motor cortical plasticity by attention. J Neurophysiol. 2004;92(1):66–72.
- 30. Kuhn M, Wolf E, Maier JG, Mainberger F, Feige B, Schmid H, et al. Sleep recalibrates homeostatic and associative synaptic plasticity in the human cortex. Nat Commun. 2016;7:12455.
- 31. Maier JG, Kuhn M, Mainberger F, Nachtsheim K, Guo S, Bucsenez U, et al. Sleep orchestrates indices of local plasticity and global network stability in the human cortex. Sleep 2019; 42(4).
- 32. Liao W-W, Chiang W-C, Lin K-C, Wu C-Y, Liu C-T, Hsieh Y-W, et al. Timingdependent efects of transcranial direct current stimulation with mirror therapy on daily function and motor control in chronic stroke: a randomized controlled pilot study. J Neuroeng Rehabil. 2020;17(1):101.
- 33. Elsner B, Kugler J, Mehrholz J. Transcranial direct current stimulation (tDCS) for upper limb rehabilitation after stroke: future directions. J Neu‑ roeng Rehabil. 2018;15(1):106.
- 34. Vöröslakos M, Takeuchi Y, Brinyiczki K, Zombori T, Oliva A, Fernández-Ruiz A, et al. Direct effects of transcranial electric stimulation on brain circuits in rats and humans. Nat Commun. 2018;9(1):483.
- 35. Agboada D, Mosayebi-Samani M, Kuo M-F, Nitsche MA. Induction of long-term potentiation-like plasticity in the primary motor cortex with repeated anodal transcranial direct current stimulation—better efects with intensifed protocols? Brain Stimul. 2020;13(4):987–97.
- 36. Mosayebi Samani M, Agboada D, Kuo M-F, Nitsche MA. Probing the relevance of repeated cathodal transcranial direct current stimulation over the primary motor cortex for prolongation of after-efects. J Physiol (Lond). 2020;598(4):805–16.
- 37. Fujiyama H, Hinder MR, Barzideh A, van de Vijver C, Badache AC, Manrique-C MN, et al. Preconditioning tDCS facilitates subsequent tDCS efect on skill acquisition in older adults. Neurobiol Aging. 2017;51:31–42.
- 38. Caulfeld KA, George MS. Optimized APPS-tDCS electrode position, size, and distance doubles the on-target stimulation magnitude in 3000 electric feld models. Sci Rep. 2022;12(1):20116.
- 39. Kuo H-I, Bikson M, Datta A, Minhas P, Paulus W, Kuo M-F, et al. Comparing cortical plasticity induced by conventional and high-definition 4×1 ring tDCS: a neurophysiological study. Brain Stimul. 2013;6(4):644–8.
- 40. Lahr J, Paßmann S, List J, Vach W, Flöel A, Klöppel S. Efects of diferent analysis strategies on paired associative stimulation. A pooled data analysis from three research labs. PLoS ONE. 2016;11(5):e0154880.
- 41. Klöppel S, Lauer E, Peter J, Minkova L, Nissen C, Normann C, et al. LTP-like plasticity in the visual system and in the motor system appear related in young and healthy subjects. Front Hum Neurosci. 2015;9:506.
- 42. Brunoni AR, Moffa AH, Sampaio-Junior B, Borrione L, Moreno ML, Fernandes RA, et al. Trial of electrical direct-current therapy versus escitalopram for depression. N Engl J Med. 2017;376(26):2523–33.
- 43. Brunoni AR, Valiengo L, Baccaro A, Zanão TA, Oliveirade JF, Goulart A, et al. The sertraline vs. electrical current therapy for treating depression clinical study: results from a factorial, randomized, controlled trial. JAMA Psychiat. 2013;70(4):383–91.
- 44. Aust S, Brakemeier E-L, Spies J, Herrera-Melendez AL, Kaiser T, Fallgatter A, et al. Efficacy of augmentation of cognitive behavioral therapy with transcranial direct current stimulation for depression: a randomized clinical trial. JAMA Psychiat. 2022;79(6):528–37.
- 45. Burkhardt G, Kumpf U, Crispin A, Goerigk S, Andre E, Plewnia C, et al. Transcranial direct current stimulation as an additional treatment to selective serotonin reuptake inhibitors in adults with major depressive disorder in Germany (DepressionDC): a triple-blind, randomised, sham-controlled, multicentre trial. The Lancet. 2023;402(10401):545–54.
- 46. Castrén E. Neuronal network plasticity and recovery from depression. JAMA Psychiat. 2013;70(9):983–9.
- 47. Voineskos D, Blumberger DM. Transcranial direct current stimulation as a treatment for major depressive disorder. Lancet. 2023;402(10401):506–7.
- 48. Blumberger DM, Mulsant BH, Thorpe KE, McClintock SM, Konstantinou GN, Lee HH, et al. Efectiveness of standard sequential bilateral repetitive transcranial magnetic stimulation vs bilateral theta burst stimulation in older adults with depression: the FOUR-D randomized noninferiority clinical trial. JAMA Psychiat. 2022;79(11):1065–73.
- 49. Kang W, Lee J, Kim YR, Chung WR, Na DL, Shon Y-M, et al. Analyzing the advantages of subcutaneous over transcutaneous electrical stimulation for activating brainwaves. Sci Rep. 2020;10(1):7360.

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