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Effects of multifocal transcranial direct current stimulation targeting the motor network during prolonged experimental pain

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Short title: Motor network tDCS modulates endogenous pain mechanisms

What's known? Prolonged tonic pain reduces corticomotor excitability and conditioning pain modulation (CPM)

What's new? Multifocal tDCS over the resting state motor network may counteract corticomotor inhibition and CPM reduction

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ABSTRACT

Background: Antinociceptive effects of transcranial direct current stimulation (tDCS) over the primary motor cortex (M1) have been extensively studied in the past years. However, M1 does not work in isolation, but it rather interacts within a network, the so-called resting-state motor network.

Objective: To explore the anti-nociceptive effects of a new multifocal tDCS approach administered to regions linked to the resting state motor network (network-tDCS) compared to sham tDCS.

Methods: Healthy individuals were included in this randomized, parallel and double-blinded study comprising two consecutive interventions with 24-h interval of either active (n=19) or sham (n=19) network-tDCS. Prolonged pain was induced by application of topical capsaicin on the dorsum of the hand during a 24-h period. Assessments of corticomotor excitability (transcranial magnetic stimulation), pain ratings (numerical rating scale, NRS), skin pain sensitivity on the arm (heat and mechanical), temporal summation of pain (TSP) and conditioned pain modulation (CPM) were performed at baseline (Day1-baseline), after 25 min of capsaicin application and before the first tDCS session (Day1-post-cap), and after the second tDCS session (Day2).

Results: Comparing Day2 to Day1-baseline measures, there was reduced corticomotor excitability ($P<0.05$) and impaired CPM-effect ($p<0.05$) after sham but not after active network-tDCS. Pain NRS ratings increased at Day2 compared to Day1-post-cap ($P<0.01$) in both groups whereas no significant changes were found in pain sensitivity and TSP.

Conclusions: Present findings demonstrate that tDCS applied over regions linked to the resting state motor network reverts the inhibition of corticomotor excitability and CPM impairment both provoked by prolonged experimental pain for 24 hours.

Key words: Pain, transcranial direct current stimulation, motor network, conditioned pain modulation, corticomotor excitability.

INTRODUCTION

Transcranial direct current stimulation (tDCS) is a non-invasive technique used to modulate brain function and promote neuroplasticity through excitation and inhibition of brain circuits (Lefaucheur et al., 2017). Anodal tDCS of the primary motor cortex (M1) facilitates cortical excitability (Nitsche et al., 2005), theta-band functional connectivity (Notturno et al., 2014), metabolite concentration (Tremblay and Lafleur, 2016), and motor learning (Ammann et al., 2016; Fan et al., 2017; Nitsche et al., 2003).

Behavioural studies have shown that M1 tDCS modulates pain processing in healthy (Mylius et al., 2012) and clinical populations (Lefaucheur et al., 2017) and may improve descending pain inhibitory controls assessed by conditioned pain modulation (CPM) (Flood et al., 2016) in healthy individuals. Neuroimaging studies indicate that these changes may be due to the modulatory effects of anodal M1 tDCS on brain regions typically involved in anti-nociceptive processes, i.e. thalamus, cingulate cortex (Naegel et al., 2018), and periaqueductal grey (PAG) (Meeker et al., 2019). Using experimentally-induced mechanical hyperalgesia, anodal M1 tDCS normalizes BOLD responses within the descending pain modulatory network, including anterior cingulate cortex (ACC) and PAG, when compared to sham stimulation (Meeker et al., 2019). This normalization of BOLD responses to painful mechanical stimuli could suggest that increased corticomotor excitability induced by M1 tDCS might also improve anti-nociceptive mechanisms in longer-lasting pain conditions. However, there is no evidence of tDCS-modulation of descending pain control mechanisms during prolonged pain.

Implementation of multifocal tDCS protocols allows targeting multiple brain regions simultaneously. Analysis of neurophysiological effects of tDCS through transcranial magnetic stimulation (TMS) combined with electroencephalography showed that tDCS of left dorsolateral prefrontal cortex (DLPFC) and parietal regions reduced the N100 amplitude of TMS-evoked potentials relative to baseline (Hill et al., 2018), while single-site DLPFC tDCS produced no significant effects. Likewise, multifocal tDCS targeting M1 bilaterally (Maezawa et al., 2019) and tDCS applied simultaneously over left M1 and left DLPFC (Vaseghi et al., 2015a) increased corticomotor excitability compared to single-site anodal M1 tDCS. Fischer et al. (2017) developed a new multifocal tDCS approach based on the resting-state motor network. The authors observed that multifocal tDCS administered over the left M1 cortex and its associated resting state motor network regions (network-tDCS) increased the corticomotor excitability beyond traditional single-site protocols. Underlining the role of these multifocal paradigms on augmented corticomotor excitability, analysis of the

modulatory effects of traditional single-site anodal M1 tDCS at different intensities showed that higher current intensities do not facilitate corticomotor excitability further (Agboada et al., 2019). Overall, these findings suggest that targeting functionally associated areas can produce a more widespread modulation of the corticomotor excitability (Vaseghi et al., 2015b), and could affect central pain modulatory mechanisms.

Using a prolonged tonic pain model lasting 24-hours, this study analyzed the effects of multifocal tDCS over the resting state motor network on neurophysiological and psychophysical pain parameters. It was hypothesized that 1) prolonged tonic pain compared with baseline would reduce corticomotor excitability and CPM efficacy in the sham condition, and 2) active network-tDCS, compared with sham network-tDCS, would increase corticomotor excitability and improve CPM efficacy during prolonged tonic pain.

METHODS

Participants

Healthy, right-handed individuals (N=38, Table 1) participated in this parallel, randomized, and double-blinded study. Participants were randomly assigned into two sex-matched groups (www.random.org): Active network-tDCS (n=19) or sham network-tDCS (n=19). Prior to inclusion, all participants completed a tDCS (Thair et al., 2017) and TMS (Rossi et al., 2011) safety screen questionnaire. Participants had no previous chronic or current acute pain, allergy to chili (capsaicin), or neurologic disorders at the time of the experiment. All individuals received written and verbal description of the experimental procedures, provided written informed consent, and the experiment was performed in accordance with the Declaration of Helsinki. All procedures were approved by the local ethics committee (VN-20180092). This study presents parts of parameters included in the ClinicalTrials.gov registration (NCT04165980). Primary outcome was the corticomotor excitability and secondary outcomes were quantitative sensory testing measures.

Experimental protocol

The study comprised 3 experimental sessions on 2 consecutive days (Fig. 1) at the Center for Neuroplasticity and Pain (CNAP, Denmark) where the first and third sessions were at the same time of day for each participant. The first session at Day1 started with familiarization to all experimental measures. All participants were exposed to the prolonged tonic pain model

based on topical application of capsaicin to the dorsal part of the right hand. A recording sequence of pain intensity provoked by topical capsaicin, corticomotor excitability, heat and mechanical skin sensitivity, cuff pressure algometry (pain sensitivity measures), temporal summation of pain (TSP), and CPM was performed at baseline on Day1 (Day1-baseline), after 25 minutes (Farina et al., 2001) of exposition to topical capsaicin on Day1 (Day1-post-cap), and at the end of Day2. TSP and CPM were recorded to assess for effects on central pain mechanisms. Finally, participants underwent two identical sessions of either active or sham network-tDCS after Day1-post-cap and before Day2 assessments. Participants completed pain-related questionnaires at Day1-baseline and Day2 (before other assessments): The Pittsburgh Sleep Quality Index for assessing sleep quality (Buysse et al., 1989), the Pain Catastrophizing Scale for pain-related catastrophizing thinking (Sullivan, 1995), and the Positive and Negative Affect Schedule (PANAS) for evaluating mood changes (Watson et al, 1988).

Capsaicin-induced pain

Prolonged tonic pain was induced in a period of 24 hours by application of a 4 x 4 cm topical capsaicin patch (8%, Qutenza, Grunenthal GmbH) on an adjacent position to the first dorsal interosseous (FDI) muscle on each day in all participants. On Day1, the capsaicin patch was positioned on the distal part of the first metacarpus (Farina et al., 2001) and on Day2 on the center of the third metacarpus (Fig. 1). Both topical capsaicin patches were kept in the same position until the experiment was finished on Day2 in order to produce sustained pain. Participants were asked to rate the current perceived intensity of pain on a 0 – 10 numerical rating scale (NRS) every 20 minutes after the application of each capsaicin patch during each session. The verbal anchors of the NRS was defined as ‘no pain’ and ‘worst pain imaginable’, respectively. At the end of the Day1 session, a pain diary was provided to participants in order for them to record perceived pain every hour (except sleeping hours) until they return to the laboratory on the following day.

Corticomotor excitability

Electromyographic activity (EMG) was acquired using Ag–AgCl surface electrodes (Neuroline 720, Ambu® A/S, Denmark) positioned over the belly of the right FDI muscle. EMG signals were sampled at 4 kHz, pre-amplified (1000x gain) and bandpass filtered at 5Hz-1kHz. TMS (Magstim 200, Magstim Company, UK) was delivered using a focal figure-of-eight coil ($D70^2$, Magstim Company, UK). Data were digitized by a 16-bit data-acquisition

system (National Instruments, NI6122) and saved by custom-made LabVIEW software (Mr. Kick, Aalborg University).

Participants wore an EEG neoprene cap with 10-10 international configuration (NE056 Headcap R, Neuroelectrics, Spain) and were seated comfortably on a dental chair, with the head and neck supported by a head rest. Coil position was set in a postero-anterior orientation with a 45° angle from the sagittal plane and monitored throughout the experiment by the same examiner (LG). Optimal cortical representation (hotspot) of the FDI muscle was determined as the highest and most stable motor-evoked potential (MEP) in 3 trials to single-pulse TMS. The hotspot was documented using the X-Y coordinate system from C_Z point of the EEG cap. Rest motor threshold (RMT) was determined as the minimum output power that elicits 5 out of 10 MEPs of at least 50 μV over the hotspot of the FDI muscle at rest. MEPs were measured as the peak-to-peak amplitude. Corticomotor excitability was assessed as the average of 10 MEPs at 120% RMT (approx. 5-6 s as interstimulus interval). Assessment of corticomotor excitability using 10 MEPs on the FDI muscle has shown acceptable intersession (Intraclass Correlation Coefficient, ICC=0.93) and intrasession (ICC=0.99) relative reliability as well as intersession (Standard Error of the Measure; SEM=3.67) and intrasession (SEM=3.60) absolute reliability in young adults (Bastani and Jaberzadeh, 2012).

Heat skin sensitivity

Warm detection (WDT) and heat pain thresholds (HPT) were recorded (Pathway, Medoc Ltd, Israel). A 3x3 cm thermal stimulation probe was positioned on the distal part of the volar forearm. Ascending ramp stimuli of 1 °C/s were delivered from a starting temperature of 32 °C until subjects identified the relevant threshold by pressing a stop button. After each ascending ramp, the temperature returned to baseline.

Warm detection thresholds were based on series of 4 ramp stimuli delivered with a randomized interstimulus interval between 6 to 10 s. During each ramp, participants were instructed to press the stop button as soon as warmth or an increase in temperature was felt (Rolke, 2006) and the corresponding temperature value was extracted for further analysis and defined as WDT. The final WDT was expressed as the mean of 4 series of ramp stimuli.

Heat pain thresholds were recorded by 3 ramp stimuli delivered with a randomized interstimulus interval between 6 to 10 seconds. During each ramp, participants were instructed to press the stop button as soon as their sensation changed from warm to a pain (Rolke, 2006) and the corresponding temperature value was extracted and defined as HPT. The resulting HPT was expressed as the mean of 3 series of ramp stimuli.

Mechanical skin pain sensitivity

Mechanical pain thresholds (MPT) were assessed using seven pinprick stimulators (MRC Systems GmbH, Germany) exerting forces ranging from 8 mN to 512 mN over the distal part of the volar forearm. Starting with the lightest, each stimulator was applied in an ascending order until the participant reported a perception of sharpness or pain. If the pain threshold was not reached, the value of 1024 mN was registered (Lo Vecchio et al., 2018). The MPT was expressed as the geometric mean of five series of ascending/descending series of stimuli.

Cuff pressure pain sensitivity

A user-independent cuff algometer (NociTech, Aalborg, Denmark) comprising two 10-cm wide air-pressured cuffs (VBM Medizintechnik GmbH, Sulzam Neckar, Germany), an electronic visual analogue scale (eVAS; anchored at 0 cm [no pain] and 10 cm [worst pain imaginable]), and a stop button were used to assess cuff pressure pain detection thresholds (PDT) and pain tolerance thresholds (PTT). Each cuff was applied over the widest portion of each lower leg (upper cuff rim approx. 5 cm distal to the knee). The cuff pressure system inflated the cuffs with rates set at 1 kPa/s and a maximum cuff pressure at 100 kPa. During each pressure ramp, participants were instructed to begin sliding the dial of the eVAS as soon as the pressure became painful and to push the stop button when they could no longer tolerate pressure pain. The pressure value corresponding to 1 cm on the eVAS defined the PDT and PTT was defined as the pressure when the participant pushed the stop button (Graven-Nielsen et al., 2017). If the PTT was not reached, the PTT was registered as 100 kPa. PDTs and PTTs were recorded bilaterally with approx. 2 minutes interval between legs.

Temporal summation of pain

Cuff algometry was used to assess TSP by 10 cuff inflations (1 s duration, 1 s pause) on the right leg to a pressure equivalent to PTT. Participants scored the pain intensity evoked by the cuff stimulations using the eVAS. The stop button could be used if the pain intensity became intolerable. Participants were instructed to rate their pain on the first inflation and adjust the dial if their pain changed after each subsequent inflation. Maximum eVAS value for each inflation was extracted and as a result 10 values were used for further analysis. Normalization of each eVAS was expressed as the subtraction of the eVAS value from the first stimulation from each eVAS values from the subsequent cuff stimuli. The TSP-effect was expressed as the summation of the 10 normalized eVAS values in each assessment (Bement et al., 2020).

Conditioned pain modulation

The CPM protocol consisted of a constant cuff pressure stimulation applied to the left leg (conditioning stimulus) at 70% of the PTT (recorded on the left leg for assessment of the pain sensitivity in the same session) and simultaneously one ramped cuff stimulation (test stimulus) at 1 kPa/s applied to the right leg. Participants were instructed to rate their initial pain perception of the conditioning stimulus on a NRS anchored with 0 as 'no pain' and 10 as 'worst pain imaginable'. Immediately after this, the PDT and PTT were recorded for the right leg (identically to cuff pain sensitivity assessments). The CPM-effect (Graven-Nielsen et al., 2017) was calculated separately for PDT and PTT, as the conditioned values minus values recorded prior to the TSP assessment.

Network tDCS intervention

This study applied tDCS targeting the left M1 and its associated resting state brain regions linked to the motor system (i.e., bilateral M1, prefrontal and parietal cortices) obtained through a seed-based functional connectivity analysis of the left M1 (Fischer et al., 2017). The network-tDCS was administered using Starstim 32 tDCS system (Neuroelectrics, Spain) with 3.14 cm^2 Ag/AgCl gelled electrodes fitted into a neoprene cap (NE056 Headcap R, Neuroelectrics, Spain) corresponding to the international 10-10 international EEG system. Double-blinding to tDCS interventions was performed using the double-blinding mode of a commercial software (NIC2, Neuroelectrics, Spain). The examiner (LG) was both the operator and assessor, and blinding of this person was ensured since a third-party established a randomized code for the active and sham tDCS protocols as well as the concealment of the technical specifications (current intensity, total delivered charge and electrode configuration) in the mentioned software.

Every active or sham network-tDCS session lasted 20 minutes. Electrode configuration and amperage was based on the algorithm developed by Fischer et al.(Fischer et al., 2017): C1=872 μA, C2=888 μA, C3=1135 μA, C4=922 μA, FZ=-1843 μA, P3=-1121 μA, P4=-1036 μA and T8=183 μA. It resulted in a total injected current of 4 mA. For active network-tDCS, direct current was delivered continuously during each 20-minute session. For sham network-tDCS, the same current was only delivered during the first 30 seconds and during the last 30 seconds of the 20-minute session, in order to emulate the somatosensory perception (paresthesia) of active tDCS on the scalp. In each session, participants were instructed to relax and keep their eyes open.

At the end of Day 2, participants were asked to report the most common sensations or side effects they felt (tingling, itchy, pricking, burning, painful) during the tDCS sessions. Subsequently, participants were asked to guess the protocol they believed they received (active/sham).

Statistics

Data are presented as mean and standard deviation of the mean (SD) in text and tables and as mean and standard error of the mean (SEM) in figures. Statistical analysis were performed using Statistical Package for Social Sciences (SPSS, v25.0, IBM, USA). Statistical significance was set at $p<0.05$. Normal distribution of all parameters was assessed using visual inspection and Shapiro-Wilk's test. In case of data was not normally distributed, parameters were log transformed before analysis. A two-way mixed model analysis of variance (ANOVA) was used to assess within-subjects effects of *time* (Day1-baseline, Day1-post-cap, and Day2 – except for current pain NRS scores where only Day1-post-cap and Day2 were considered since participants were pain-free at Day1-baseline), and between-subjects effects of *group* (active network-tDCS and sham network-tDCS) for pain NRS scores, MEPs, WDT, HPT, MPT (including ceiling values), MPT (excluding ceiling values), TSP-effect, PDT CPM-effect, PTT CPM-effect (including saturated values) and PTT CPM-effect (excluding participants with saturated values). In order to measure changes in the pain ratings collected during lab hours and from the pain diaries, a two-way mixed model ANOVA was run with the two factors *time* (Day1-post-cap, off-lab hours, and Day2) and *group* (active network-tDCS and sham network-tDCS). Day1-post-cap included the average NRS pain ratings reported after the first capsaicin application at the lab; off-lab hours included the averaged NRS pain ratings reported in pain diaries between Day1 and Day2; and Day2 included the averaged NRS pain ratings reported at the lab on Day 2. A two-way mixed model ANOVA was applied to analyze within-subjects effect of *time* (Day1-baseline, Day2) and between-subjects effects of *group* (active network-tDCS and sham network-tDCS) for questionnaires data. If sphericity was violated, Greenhouse-Geisser approximation was applied. When ANOVA factors or interactions were significant, post-hoc analyses were performed using Bonferroni corrections for multiple comparison. A Chi^2 test was applied in order to measure between-subjects effect of *group* for assessing *tDCS* blinding (active, sham).

RESULTS

Capsaicin-induced pain

The current pain NRS scores were zero at baseline, 2.8±1.6 and 3.3±2.3 (Day1-post-cap) and 4.1±2.0 and 5.2±2.7 (Day2), in the active and sham network-tDCS groups, respectively. The two-way ANOVA on pain NRS scores revealed a main effect of *time* ($F_{(1,36)}$=11.57, p=0.002, η^2=0.243). No main effect of *group* ($F_{(1,36)}$=2.45, p=0.126, η^2=0.064), or interactions ($F_{(1,36)}$=0.326, p=0.571, η^2=0.009) were observed.

The averaged NRS pain ratings were 3.5±1.5 and 4.2±2.1 (during Day1-post-cap), 4.7±1.9 and 5.9±2.6 (during off-lab hours) and 4.3±1.8 and 5.5±2.3 (during Day2), in the active and sham network-tDCS groups, respectively. The ANOVA of averaged NRS pain ratings resulted in an effect of *time* ($F_{(2,72)}$=10.38, p<0.001, η^2=0.224). In both groups, pairwise comparisons showed that averaged NRS pain ratings were exacerbated during off-lab hours (p=0.001) and during Day2 (p=0.001) compared to Day1-post-cap. No significant effect of *group* was found ($F_{(1,36)}$=3.81, p=0.059, η^2=0.096) nor interactions ($F_{(2,72)}$=0.480, p<0.621, η^2=0.013).

Psychometric results

The ANOVA of Positive PANAS revealed a main effect of *time* (Table 2; $F_{(1,36)}$=21.3, p<0.001, η^2=0.372) indicating that the positive affect was reduced due to prolonged pain in both groups. Similarly, Negative PANAS revealed a main effect of *time* (Table 2; $F_{(1,36)}$=4.73, p<0.037, η^2=0.116) showing an increase in Negative Affect in both groups.

The ANOVA on Pittsburgh Sleep Quality Index scores revealed a main effect of *time* (Table 2; $F_{(1,36)}$=8.17, p=0.007, η^2=0.18) and interaction ($F_{(1,36)}$=14.29, p<0.002, η^2=0.28) although no main effect of *group* ($F_{(1,36)}$=, p=0.29, η^2=0.032). Post-hoc analysis showed an increase of Pittsburgh Sleep Quality Index scores from Day1-baseline to Day2 (p<0.001) in the sham network-tDCS group, indicating that sleep quality was aggravated in that group.

The ANOVA on Pain Catastrophizing Scale scores revealed a main effect of *time* (Table 2; $F_{(1,36)}$=8.17, p=0.007, η^2=0.185) and an interaction ($F_{(1,36)}$=4.44, p=0.042, η^2=0.110). There was no significant main effect of *group* ($F_{(1,36)}$=1.44, p=0.238, η^2=0.038). Post-hoc analysis showed an increase in pain catastrophizing scores at Day2 compared to Day1-baseline (p=0.001) in the sham network-tDCS group, reflecting that pain catastrophizing was worsened.

Corticomotor excitability

The ANOVA of RMT did not showed significant effects of *time* (Fig 2A; $F_{(2,72)}$=2.98, p=0.057, η^2=0.077), *group* ($F_{(2,72)}$=0.21, p=0.65, η^2=0.006), or interaction ($F_{(2,72)}$=0.192, p=0.826, η^2=0.005).

The ANOVA of MEP amplitudes demonstrated a significant main effect of *time* ($F_{(2,72)}$=5.54, p=0.006, η^2=0.133) and a significant interaction between *time* and *group* (Fig. 2B; $F_{(2,72)}$=3.61, p=0.032, η^2=0.091). Post-hoc analysis showed decreased MEP amplitudes at Day2 compared with Day1-baseline in the sham network-tDCS group (p=0.002) and increased MEP amplitudes in the active network-tDCS group compared with the sham network-tDCS group in Day2 assessment (Fig. 2B, p=0.018). There was no significant main effect of *group* ($F_{(2,72)}$=2.586, p=0.117, η^2=0.067).

Heat skin sensitivity

The ANOVA of WDTs indicated no significant effect of *time* (Table 3, $F_{(1.695,61.008)}$=0.54, p=0.557, η^2=0.015), *group* ($F_{(1,36)}$=0.62, p=0.437, η^2=0.017), or interaction ($F_{(1.695,61.008)}$=0.23, p=0.758, η^2=0.006). Likewise, the two-way ANOVA of HPTs showed no effect of *time* (Table 3; $F_{(1.223,44.026)}$=3.91, p=0.285, η^2=0.033), *group* ($F_{(1,36)}$=0.37, p=0.545, η^2=0.091), or interaction ($F_{(1.223,44.026)}$=0.26, p=0.661, η^2=0.007).

Mechanical skin pain sensitivity

The ANOVA of MPTs showed no main effect of *time* (Table 3; $F_{(1.357,48.867)}$=0.51, p=0.536, η^2=0.014), group ($F_{(1,36)}$=0.16, p=0.689, η^2=0.005), or interaction ($F_{(1.357,48.867)}$=0.07, p=0.862, η^2=0.002).

Analysis of individual data shows that 8 subjects in the active and 6 subjects in the sham group showed a ceiling value (1024 mN), at some point. Two of those 6 subjects in sham group exhibited a ceiling value in all 5 repetitions at Day1-baseline, and Day1-post-cap; and one of those two subjects also showed ceiling values in the 5 repetitions at Day2. The ANOVA of MPTs removing the ceiling values (1024 mN) revealed no main effect of time (Table 3; $F_{(1.230,\ 44.298)}$=0.38, p=0.582, η^2=0.011), group ($F_{(1,36)}$=0.003, p=0.960, η^2=0.001) nor interactions ($F_{(1.230,\ 44.298)}$=0.046, p=0.877, η^2=0.001).

Cuff pressure pain sensitivity

The ANOVA of PDTs on the right leg showed no effects of *time* (Table 3; $F_{(2,72)}$=0.19, p=0.831, η^2=0.005), *group* ($F_{(1,36)}$=0.001, p=0.972, η^2=0.000), or interaction ($F_{(2,72)}$=0.15,

p=0.857, η^2=0.004). Likewise, no effects were observed with the ANOVA of PTTs over *time* (Table 3; $F_{(2,72)}$=1.29; p=0.283, η^2=0.034), *group* ($F_{(1,36)}$=0.73, p=0.397, η^2=0.020), or interaction ($F_{(2,72)}$=0.20, p=0.818, η^2=0.006).

The ANOVA of PDTs on the left leg resulted in no main effects of *time* (Table 3; $F_{(2,72)}$=0.68, p=0.509, η^2=0.019), *group* ($F_{(1,36)}$=0.07, p=0.795, η^2=0.002), or interaction ($F_{(2,72)}$=0.33, p=0.720, η^2=0.009). Likewise, there were no main effects of *time* (Table 3; $F_{(2,72)}$=0.15, p=0.238, η^2=0.039), *group* ($F_{(1,36)}$=0.57, p=0.454, η^2=0.016), or interaction ($F_{(2,72)}$=2.48, p=0.091, η^2=0.064) for the PTTs on the left leg.

Conditioned pain modulation

The ANOVA of the PDT CPM-effect showed a main effect of *time* (Fig. 3A; $F_{(2,72)}$=7.33, p=0.001, η^2=0.169), and post-hoc test showed a CPM-effect reduction at Day1-post-cap compared with Day1-baseline (p<0.001). There was no main effect of *group* ($F_{(2,72)}$=3.40, p=0.073, η^2=0.086) or interaction ($F_{(2,72)}$=1.86, p=0.163, η^2=0.049).

The ANOVA of the PTT CPM-effect revealed no main effects of *time* ($F_{(2,72)}$=1.34, p=0.270, η^2=0.036) or *group* ($F_{(2,72)}$=0.14, p=0.714, η^2=0.004) but a significant interaction (Fig. 3B; $F_{(2,72)}$=4.51, p=0.014, η^2=0.111). Post-hoc analysis revealed that the PTT CPM-effect was decreased Day2 compared with Day1-baseline recordings in the sham network-tDCS group (p=0.013). Moreover, increased PTT CPM-effect was found in the active network-tDCS group compared with the sham network-tDCS group when recorded at Day2 (p=0.018). Three participants in the active network-tDCS group and 3 participants in the sham network-tDCS group showed saturated (PTT > 100 kPa) responses during the CPM paradigm. Two-way ANOVA of the PTT CPM-effect excluding these participants still showed a significant interaction ($F_{(2,60)}$=4.76, p=0.012, η^2=0.137) and neither main effects of *group* ($F_{(2,60)}$=0.40, p=0.530, η^2=0.013) nor *time* ($F_{(2,60)}$=1.22, p=0.302, η^2=0.039). Post hoc analysis indicated a significant reduction of PTT CPM-effect at Day2 compared with Day1-baseline (p=0.020) and a group difference only at Day2 (p=0.025).

Temporal summation of pain

The ANOVA of the TSP-effect showed no main effects of time (Table 3, $F_{(1.610,57.969)}$=0.31, p=0.688, η^2=0.009), or interaction ($F_{(1.610,57.969)}$=1.06, p=0.340, η^2=0.029) but there was a main effect of group ($F_{(1,36)}$=4.303, p=0.045, η^2=0.107) with higher TSP-effect in the sham network-tDCS group at all time points.

Side-effects and blinding assessments

Few mild side-effects were reported during tDCS sessions and those that occurred were well tolerated. No skin lesions at the site of tDCS electrodes were induced and the side-effects disappeared once the stimulation period was over. Qualitative descriptors of the scalp sensations during tDCS sessions included tingling (52.6% in active network-tDCS and 31.5% in sham network-tDCS), itchiness (26.3%, 21%), pricking (21%, 21%), burning (15.7%, 26.3%) and scalp pain (15.7%, 15.0%). Assessment of blinding revealed that 9 out of 19 subjects in the active network-tDCS group and 4 out of 19 subjects in the sham network-tDCS group correctly guessed the protocol they received. Chi^2 test of tDCS blinding revealed no significant effect of group (χ^2=2.09, p=0.170), suggesting that guessing success was due to chance and blinding was adequate in both groups.

DISCUSSION

The present study addressed the consequences of one day of tonic experimental pain on pain mechanisms as well as corticomotor excitability and whether it was possible to revert them after two sessions of resting state motor network tDCS. The results suggest that network-tDCS counteracted the reduction of corticomotor excitability and CPM-effect induced by prolonged tonic pain.

Counteraction of pain-related corticomotor inhibition after network-tDCS

The results of the current study expand the evidence of inhibited corticomotor excitability due to short-lasting pain from 5-30 minutes (Cheong et al., 2003; Farina et al., 2003; Larsen et al., 2018; Le Pera et al., 2001) to prolonged pain during a 24-hour period. Specifically, compared to Day1-baseline, a general reduction of MEPs was observed at Day1-post-cap in both groups, which was significant after 24 hours of pain (Day2) only in the sham group. Previous studies exploring the inhibition of corticomotor excitability during experimental pain suggested that a transitory depression in corticomotor output is an adaptive mechanism in order to preserve the limb from further damage. This inhibition is part of a compensatory mechanism of the nervous system, which balances the facilitation of rapid protective spinal reflexes (Urban et al., 2004) during capsaicin-induced pain (Farina et al., 2003). It is important to mention that, even though a transient corticomotor reduction is beneficial in the initial stages of pain, long-lasting corticomotor reduction can be detrimental for motor behaviour (Summers et al., 2020) and contribute to the development and maintenance of

motor dysfunction (Trost et al., 2012; Vlaeyen and Linton, 2012) when experiencing long-lasting pain. Indeed, the aim of the present study is to characterize the effects of tDCS during a prolonged pain model to understand if it can provoke advantageous plasticity. The results of this study show that two sessions of active network-tDCS normalized the MEPs to baseline levels during pain, demonstrating that multifocal tDCS targeting the motor network can modulate pain-related inhibition of corticomotor excitability. Studies in healthy individuals showed that voluntary movements normalize reduced corticomotor excitability due to a session of cathodal M1 tDCS (which is known to reduce corticomotor excitability), whereas fails to do so following experimentally induced muscle pain (Schabrun et al., 2018) emphasizing the relevance of non-invasive brain stimulation to restore normal corticomotor output during pain. It is relevant to mention that, in contrast to our hypothesis, tDCS-driven normalization of corticomotor output could induce a negative effect if such normalization, results in exacerbated motor dysfunction, or given their protective nature, reduced nociceptive reflex responses. Future work should address the effects of tDCS on motor behaviour, fear avoidance as well as spinal protective reflexes during prolonged pain.

Moreover, previous research in healthy pain-free individuals demonstrated that network-tDCS (Fischer et al., 2017), bilateral M1 tDCS (Maezawa et al., 2019), single-site M1 tDCS (Nitsche et al., 2005), and repeated-TMS (rTMS) to M1 increased corticomotor excitability above baseline values. Results are also in agreement with rTMS studies in experimental pain (Fierro et al., 2010; De Martino et al., 2018) and chronic disorders (De Andrade et al., 2011; Mhalla et al., 2011; Moisset et al., 2016) where pain-related changes in corticomotor excitability were reverted to normal levels, but not beyond them, when targeting DLPFC or M1. These pieces of evidence suggest that tDCS effects on the corticomotor excitability show a state dependency (i.e. healthy or pain) prior to the intervention (Huang et al., 2017). Moreover, it supports the notion that the corticomotor excitability is influenced by homeostatic regulation, in particular through the reticular formation (Fossataro et al., 2020; Martins and Tavares, 2017), which tunes corticomotor output in order to maintain a stable internal environment (Thapa et al., 2018) and may keep corticomotor changes within a physiologically defined range (Moisset et al., 2016).

Two sessions of network-tDCS normalize CPM-effects during prolonged experimental pain

The present study demonstrates that experimental tonic pain reduces PDT CPM-effect in both groups at Day1-post-cap before tDCS, when compared to Day1-baseline. Whereas mild movement-evoked pain as observed in delayed onset muscle soreness exhibits insignificant

CPM reduction (Mcphee and Graven-Nielsen, 2019), Bement et al. (2020) showed that mild-to-moderate capsaicin-induced pain for 3 hours reduced PDT CPM-effect, highlighting the relevance of both, the intensity and duration of self-reported pain on this outcome. While Bement and colleagues found a tendency for reduced PDT CPM-effect after 1 hour of capsaicin-induced pain, the present study obtained a significant reduction at similar time points; factors such as slightly higher self-reported pain in the present study and larger sample size could account for such differences. Analysis of the 24-hour topical application of capsaicin (Day2) revealed no significant effects in PDT CPM-effect when comparing to Day1-baseline or Day1-post-cap most likely due to different effects in the two groups (tendencies for reduced CPM in the sham network-tDCS and normalized CPM in the active network-tDCS group) and as such influenced by the sample size. PTT CPM-effect was reduced at Day2 only in the sham group showing that prolonged capsaicin-induced pain hinders CPM expression as also shown for PDT in the present study and by others (Bement et al., 2020). Pain catastrophizing and sleep quality were exacerbated only in the sham group. These results are in conformity with previous research, which indicates that poor sleep quality impairs CPM function (Torp et al., 2019) and catastrophic thinking negatively correlates with CPM expression (Elliot and Dorit, 2008).

Interestingly, the two sessions of active network-tDCS restored PTT CPM-effect to baseline values (see Fig. 3B) suggesting that tDCS modulates central mechanisms including descending inhibitory pathways despite the prolonged and moderate-to-severe self-reported pain on Day2. Prior studies reported CPM enhancement after M1 tDCS in a healthy population (Flood et al., 2016; Reidler et al., 2012), osteoarthritis patients (Suchting et al., 2018), and post-surgical pain patients (Ribeiro et al., 2017). For example, two sessions of M1 tDCS prior to an orthopaedic surgery improved CPM and reduced analgesics intake after surgery compared to sham (Ribeiro et al., 2017). A possible underlying mechanism explaining these results may be due to indirect cortical influences from M1 to areas involved in the endogenous pain inhibitory pathway (i.e. PAG and ACC) as suggested by Meeker et al. (2019) using anodal M1 tDCS compared to sham tDCS and found enhanced activation of descending modulatory network during pain-induced facilitated central mechanisms (BOLD-evoked responses). Moreover, previous research shows that M1 tDCS indirectly influences activity in the ACC and can possibly enhance deficient endogenous pain modulatory activity in chronic patients by indirectly activating the rostral ACC (Reidler et al., 2012). Whereas these studies targeted solely the left M1, the present study targeted the resting state motor network with bilateral M1 as anodes, which might have had additive effects on the CPM-

effect, as other studies targeting bilateral motor cortices suggested (Maezawa et al., 2019; Tremblay and Lafleur, 2016).

Effects of network-tDCS on capsaicin-induced prolonged pain

Application of topical capsaicin on Day1 and Day2 induced tonic burning pain during 24 hours. Current NRS pain ratings and averaged NRS pain ratings increased over time compared to Day1-post-cap and were comparable in both groups, attributable to a tonic and prolonged peripheral nociceptive drive through the application of topical capsaicin (Bement et al., 2020; Farina et al., 2001; Fierro et al., 2010; Lo Vecchio et al., 2018). Interestingly, two sessions of network-tDCS did not produce significant changes in self-reported pain intensity. These results are in line with recent findings during one-hour capsaicin-heat induced pain (Meeker et al., 2019) and chronic pain (Lefaucheur et al., 2017), suggesting that single or dual sessions of tDCS does not elicit significant pain reduction even in a sensitized nociceptive system either due to moderate experimental or clinical pain. Increasing the number of repeated network-tDCS sessions over several days should be investigated to elucidate if this paradigm can exert analgesic effects during prolonged experimental or clinical pain.

No significant effects of network-tDCS on pain sensitivity

Pain sensitivity to thermal, mechanical, and cuff-pressure stimuli were assessed outside the area of capsaicin in order to assess central pain mechanisms. The lack of modulation indicates that capsaicin-induced pain did not produce widespread hyperalgesia in line with prior findings (Bement et al., 2020; Sacco et al., 2014). Similarly, two sessions of network-tDCS may not be able to modulate the general pain sensitivity. Previous results on tDCS analgesia are controversial (Mylius et al., 2012). Whereas a recent study showed that one session of anodal M1 tDCS reduced dynamic mechanical allodynia and secondary mechanical hyperalgesia (Hughes et al., 2020) due to 1% capsaicin, suggesting that tDCS may act on sensitized pathways, a number of tDCS (Borckardt et al., 2012; Jürgens et al., 2012; Lefaucheur et al., 2017) and rTMS (Bradley et al., 2016; Sacco et al., 2014) studies found that one session of non-invasive brain stimulation does not genuinely elicit analgesia in healthy individuals. Importantly, the present study was not powered to investigate pain sensitivity as a primary outcome. Furthermore, even though participants were naïve to the study design and did not know whether they were receiving two identical or different tDCS protocols in the two days of the experiment, potential learning of placebo effects cannot be

ruled out. Future tDCS studies should assess baseline expectations (Rabipour et al., 2018) as a possible influence on the examined outcomes. The combination of double blinding, randomization, sham control, and neutral instructions, however, was used to account for possible placebo effects. Therefore, if tDCS exerts analgesia it should be elicited through high-order networks influencing cognition and emotion on pathologically affected or strongly sensitized pathways.

No significant effects of network-tDCS on temporal summation of pain

The present lack of main effects of time and interactions in TSP-effect suggest that capsaicin-induced cutaneous pain for 24 hours does not intensify TSP despite a prolonged nociceptive drive. In accordance with these results, Bement et al. (2020) found that TSP remained unchanged following 1 hour, 3 hours and 24 hours of capsaicin application using similar methodology as in the present study. A main group effect was however observed, denoting possibly the variability of TSP-effect since no group differences were observed in the other outcomes. Furthermore, two sessions of network-tDCS did not significantly modulate TSP-effect. An exploratory study indicated, by contrast, that M1 tDCS (Hughes et al., 2019) reduced TSP of the noxious withdrawal reflex (NWR) at supra-threshold but not at threshold-level of noxious transcutaneous electrical stimulation on the sural nerve in healthy participants. The contrasting results between the exploratory (Hughes et al., 2019) and the present study are the modality of the stimulation and the number of included participants.

Limitations

A number of limitations need to be noted. This study used the electrode configuration for the network-tDCS paradigm developed by Fischer and colleagues (Fischer et al., 2017), which is based on fixed electrode positions using the 10-10 international EEG system. This standard configuration disregards individual anatomical differences and therefore it may not target the optimal motor representation (hotspot) of the assessed muscle, although it is important to note that C3 and C4 positions are associated to the hand representation in the motor cortex. Second, it cannot be discharged that the observed effects of facilitated CPM-effects after two sessions of network-tDCS could be also found after traditional M1 anodal tDCS during prolonged tonic pain. Finally, this study was powered to assess corticomotor excitability, thus limiting the interpretation of non-significant effects of the secondary parameters (NRS pain ratings, CPM, thermal skin sensitivity, mechanical pain sensitivity, TSP).

Conclusion

The current double-blinded study examined the anti-nociceptive effects of multifocal-tDCS targeting the resting state motor network and showed the multifocal network-tDCS to be an effective approach for modulating the corticomotor consequences and impaired endogenous pain inhibitory function associated with prolonged pain.

Conflict of interest

There is no conflict of interest to report.

Authorship

All authors provided significant contributions to this manuscript; LG contributed to conception and design of the experiment, data acquisition and analysis, interpretation of data and elaboration of manuscript, AZ contributed to conception and design of the experiment, interpretation of data and elaboration of manuscript, TGN contributed to conception and design of the experiment, interpretation of data and elaboration of manuscript.

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TABLES

Table 1. *Demographics of participants*

	Active network-tDCS	Sham network-tDCS
Group size	19	19
Sex	8 females	8 females
Age (years)	26.0±4.2	27.1±2.7
Height (m)	1.74±0.12	1.75±0.09
Weight (kg)	79.8±12.8	73.1±15.3

Table 2. *Mean (± SD, N=19) Positive Affect, Negative Affect, Pittsburgh Sleep Quality Index, and Pain Catastrophizing Scale (D) at Day1-baseline and Day2 in the active and sham network-tDCS groups. Significantly changed compared with Day1-baseline (*, p<0.05).*

	Active network-tDCS		**Sham network-tDCS**	
	Day1-baseline	Day2	Day1-baseline	Day2
Positive Affect	31.1±5.9	27.6±9.1*	31.6±7.9	25.6±8.6*
Negative Affect	20.6±8.8	20.7±10.1*	17.6±4.9	21.7±7.3*
Pittsburgh Sleep Quality Index	5.0±2.3	4.7±2.5	4.9±2.6	6.8±3.8*
Pain Catastrophizing Scale	15.3±7.5	16.5±9.9	15.2±8.3	23.2±12.6*

Table 3. *Mean (±SD, N=19) warm detection thresholds (WDT), heat pain thresholds (HPT), mechanical pain thresholds (MPT), cuff pressure pain detection thresholds (PDT) and cuff pressure tolerance thresholds (PTT) for the left and right leg, conditioned pain modulation effect (CPM-effect) for the right leg, and temporal summation of pain effect (TSP-effect) in the Active network-tDCS and Sham network-tDCS groups. Significant group difference (#, $p<0.05$).*

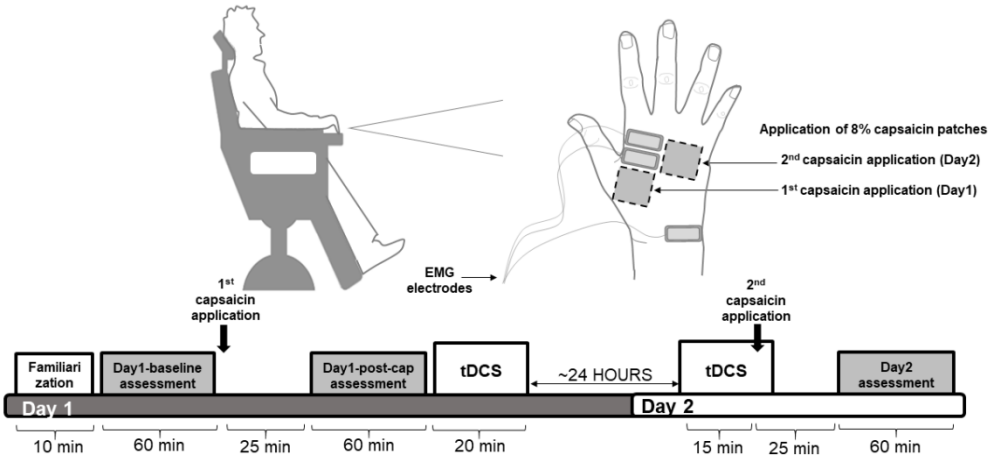
	Active network-tDCS			**Sham network-tDCS**		
	Day1-baseline	Day1-post-cap	Day2	Day1-baseline	Day1-post-cap	Day2
WDT [°C]	34.2±0.8	34.1±0.7	34.2±0.8	34.4±0.6	34.4±0.6	34.3±0.7
HPT [°C]	43.7±3.3	43.4±3.3	43.4±2.9	44.4±2.6	43.9±2.9	43.7±2.6
MPT [mN]	224.3±142.3	209.2±124.6	214.4±145.5	250.7±225.5	220.2±197.0	239.7±214.3
PDT left leg [kPa] (unconditioned)	22.6±11.6	21.8±11.4	22.3±10.5	24.4±10.0	22.9±12.8	22.1±11.9
PDT right leg [kPa] (unconditioned)	25.0±15.5	24.7±12.7	25.5±13.0	24.1±8.5	25.3±13.4	25.3±12.1
PTT left leg [kPa] (unconditioned)	53.8±22.9	49.38±23.5	55.0±24.0	60.2±21.6	58.0±23.5	55.2±16.6
PTT right leg [kPa] (unconditioned)	62.2±23.1	57.6±24.2	58.8±24.6	66.5±21.0	64.4±21.1	65.2±19.7
TSP-effect [cm]	7.9±15.0	7.7±9.1	3.9±8.2	11.1±8.9#	12.1±12.4#	12.8±11.2#

FIGURE LEGENDS

Figure 1. *Subjects sat relaxed in a chair with electromyographic (EMG) electrodes mounted above the first dorsal interosseous (FDI) muscle. First capsaicin was applied adjacent to the electrodes immediately after Day1-baseline assessment. Second capsaicin patch was applied adjacent to the first patch 15 minutes after the onset of 2nd tDCS session at Day 2. Assessments were performed at baseline (Day1-baseline), after 25 min of the application of the 1st capsaicin patch (Day1-post-cap), and after 25 minutes of the application of the 2nd capsaicin patch (Day2), respectively. Sessions of transcranial direct current stimulation (tDCS) started immediately after the assessment of Day1-post-cap and at the beginning of day 2.*

Figure 2. *Mean (±SEM, N=19) rest motor threshold (RMT, **A**) and motor evoked potential (MEP) amplitudes (**B**) recorded on Day1-baseline, Day1-post-cap, and Day2 in the active network-tDCS (solid bars) and sham network-tDCS groups (open bars). Significantly reduced compared with Day1-baseline (*, $p<0.05$) and between groups at Day2 (#, $p<0.05$).*

Figure 3. *Mean (± SEM, N=19) conditioned pain modulation effect (CPM-effect) using PDT (**A**) and PTT (**B**) in active network-tDCS (solid bars) and sham network-tDCS (open bars) groups. Significantly decreased compared with Day1-baseline (*, $p<0.02$) and compared with the active network-tDCS group (#, $p<0.02$).*



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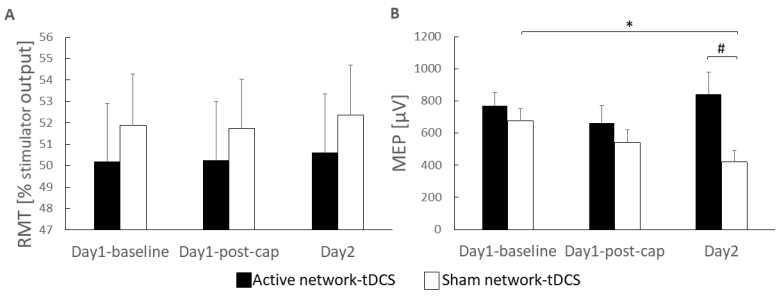

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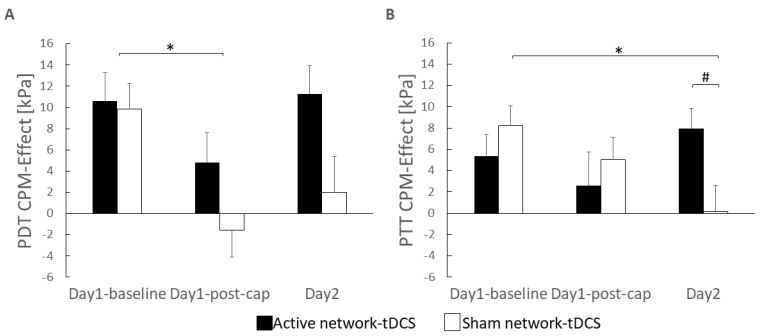

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