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ORIGINAL ARTICLE

Modulation of central pain mechanisms using high-definition transcranial direct current stimulation: A double-blind, sham-controlled study

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Abstract

Background: The use of high-definition transcranial direct current stimulation (HD-tDCS) has shown analgesic effects in some chronic pain patients, but limited anti-nociceptive effects in healthy asymptomatic subjects.

Methods: This double-blinded sham-controlled study assessed the effects of HD-tDCS applied on three consecutive days on central pain mechanisms in healthy participants with ($N = 40$) and without ($N = 40$) prolonged experimental pain induced by intramuscular injection of nerve growth factor into the right hand on Day 1. Participants were randomly assigned to Sham-tDCS ($N = 20$ with pain, $N = 20$ without) or Active-tDCS ($N = 20$ with pain, $N = 20$ without) targeting simultaneously the primary motor cortex and dorsolateral prefrontal cortex for 20 min with 2 mA stimulation intensity. Central pain mechanisms were assessed by cuff algometry on the legs measuring pressure pain sensitivity, temporal summation of pain (TSP) and conditioned pain modulation (CPM), at baseline and after HD-tDCS on Day 2 and Day 3. Based on subject's assessment of received HD-tDCS (sham or active), they were effectively blinded.

Results: Compared with Sham-tDCS, Active-tDCS did not significantly reduce the average NGF-induced pain intensity. Tonic pain-induced temporal summation at Day 2 and Day 3 was significantly lower in the NGF-pain group under Active-tDCS compared to the pain group with Sham-tDCS ($p \leq 0.05$). No significant differences were found in the cuff pressure pain detection/tolerance thresholds or CPM effect across the 3 days of HD-tDCS in any of the four groups.

Conclusion: HD-tDCS reduced the facilitation of TSP caused by tonic pain suggesting that efficacy of HD-tDCS might depend on the presence of sensitized central pain mechanisms.

1 | INTRODUCTION

Transcranial direct current stimulation (tDCS) for chronic pain management has been a research focus for more than a decade (Fregni et al., 2006; Lefaucheur et al., 2008). Recent systematic reviews demonstrate conflicting findings with successfully induced analgesia in some clinical studies and no effect in others (Knotkova et al., 2021; Lloyd et al., 2020; Shirahige et al., 2016; Yu et al., 2020).

Development in the tDCS technology has introduced the possibility of using an array of electrodes contrary to the conventional tDCS with a single anode and cathode. The high-definition tDCS (HD-tDCS) increases the spatial focality of the current delivery (Datta et al., 2009; Villamar et al., 2013). HD-tDCS also permits targeting more brain areas simultaneously to modulate a functionally connected network. The cortical targets showing the most promising analgesic tDCS effect are primary motor cortex (M1) and dorsolateral prefrontal cortex (DLPFC) (Giannoni-Luza et al., 2020; Lefaucheur et al., 2017). Brain imaging studies suggest that the analgesic effect of M1-tDCS is driven by modulation of the endogenous pain control system (Castelo-Branco et al., 2019; DosSantos et al., 2016, 2018). The mechanism underlying the analgesic effects of DLPFC may be driven by changes in affective, cognitive or attentional aspects of pain processing (Sanchez-Lopez et al., 2021). DLPFC and M1 appear to be functionally connected as studies have shown that concurrent HD-tDCS of DLPFC and M1 has shown stronger modulatory effect of corticospinal excitability than conventional M1-tDCS in healthy subjects (Vaseghi et al., 2015, 2016). This suggests that the multifocal DLPFC+M1 tDCS stimulation may also modulate endogenous pain mechanisms.

The somatosensory system of chronic neuropathic pain patients has shown greater response to M1-tDCS compared to healthy subjects in terms of normalization of sensory and pain thresholds, indicating that the state of the central pain mechanisms prior to intervention is crucial to the outcome (Giannoni-Luza et al., 2020; Kold & Graven-Nielsen, 2021). Chronic pain patients with neuropathic or musculoskeletal pain often show altered central pain mechanisms (Arendt-Nielsen et al., 2018; Petersen et al., 2019). However, heterogeneity in the pain mechanisms between chronic pain patients is common, making experimental studies of this population difficult to interpret compared to studies of healthy subjects (Karakunnel et al., 2018; Kravitz et al., 2004). Moreover, in contrast to clinical studies, pain-free baseline measures are available. As a result, a design was devised in which active- and sham-tDCS would be administered to both groups of healthy subjects and groups of subjects who were administered a prolonged muscle pain model. This study was designed to (1) investigate the effects of tDCS on pressure sensitivity and central pain mechanisms and (2) provide insight into the influence of the state of the CNS in the effects of tDCS by provoking the system with prolonged experimental pain in half of the subjects.

A pronociceptive mechanism that can be assessed experimentally is temporal summation of pain (TSP), increasing neuronal output during a train of identical nociceptive stimuli (Arendt-Nielsen et al., 2018). Similarly, a pain inhibitory mechanism that can be assessed experimentally is conditioned pain modulation (CPM) (Corrêa et al., 2015; Goubert et al., 2015, 2017), which is an endogenous downstream capacity to inhibit diffuse painful stimuli (Graven-Nielsen & Arendt-Nielsen, 2010). It is hypothesized that the analgesic effect of DLPFC+M1 HD-tDCS may be driven through modulation of these endogenous pain control systems (DosSantos et al., 2018; Knotkova et al., 2013).

In this double-blinded randomized sham-controlled trial, we investigated the effects of DLPFC+M1 HD-tDCS on cuff pressure pain sensitivity, TSP and CPM in healthy subjects with and without experimentally induced muscle pain lasting for several days to mimic the initial phase of clinical pain conditions.

2 | MATERIALS AND METHODS

2.1 | Participants

Eighty healthy participants (38 females) aged 18–55 years were included in this study conducted at Center for Neuroplasticity of Pain (CNAP), Aalborg University, Denmark, between 18/12/2018 and 21/12/2020. Participants were originally enrolled in two studies focusing on static quantitative sensory testing (single-point mechanical and thermal thresholds) following HD-tDCS with (Kold & Graven-Nielsen, 2021) and without (Kold & Graven-Nielsen, 2022) prolonged experimental pain. The two studies followed identical inclusion and exclusion criteria and were conducted in the same test facility and by the same investigator that was trained in the assessment and tDCS methods. During enrolment, age, weight and handedness were recorded. This present study investigates the effect of HD-tDCS on cuff pressure pain sensitivity, TSP and CPM. The first forty subjects (Kold & Graven-Nielsen, 2021) were randomly assigned to either the Sham-tDCS group ($N = 20$) or active-tDCS group ($N = 20$). The subsequent forty subjects had tonic experimental pain induced (Kold & Graven-Nielsen, 2022) and were randomly assigned to the Pain-Sham-tDCS group ($N = 20$) or Pain-Active-tDCS group ($N = 20$). Randomization was done by a third party that prior to the experiment, had assigned the subject IDs to the four corresponding groups. The

sample size was based on detecting a small to medium effect size, with 80% power and an alpha level of 0.05, and is in line with similar studies (Flood et al., 2016; Giannoni-Luza et al., 2020; Ihle et al., 2014; Jiang et al., 2022; Pinto et al., 2018; Wan et al., 2021).

Exclusion criteria included prior participation in other brain stimulation studies within the last 3 months, any current pain conditions, sleep deprivation, pregnancy, drug or alcohol addiction, caffeine intake that surpasses one cup of coffee within the hour prior to the experiment, alcohol intake in the 24 h prior to the experiment, having magnetic or electrical medical implants (e.g. pacemaker), use of any type of pain medication or any current illnesses or ongoing pain conditions (Bikson et al., 2016; Bornheim et al., 2019; Brunoni et al., 2012). Additionally, the participants were asked to refrain from activity that would produce muscle soreness during the 3-day experimentation period. All participants received written and verbal information about the study and signed a consent form before the first experimental session. The study was performed according to the Helsinki Declaration, approved by the North Denmark Region Committee on Health Research Ethics (VN-20180085), and was registered at ClinicalTrials.gov (NCT04650048 (Kold & Graven-Nielsen, 2022) and NCT04165876 (Kold & Graven-Nielsen, 2021)).

2.2 | Experimental design

This randomized double-blinded sham-controlled longitudinal study included four groups receiving 20 min simultaneous anodal multichannel tDCS of DLPFC and M1 (Active-tDCS, Pain-Active-tDCS) or 20-min sham tDCS (Sham-tDCS, Pain-Sham-tDCS). Subjects participated in three consecutive days of HD-tDCS with assessments of cuff pressure pain detection and tolerance thresholds and dynamic quantitative sensory testing on the legs (CPM and TSP) before the HD-tDCS on Day 1, and after HD-tDCS on Day 2 and Day 3 (Figure 1). Sessions with HD-tDCS were separated by 24 h. After Day 1 assessments, 40 participants (Pain-Sham-tDCS, $N = 20$; Pain-Active-tDCS, $N = 20$) received an injection with nerve growth factor (NGF) into the right first dorsal interosseous (FDI) muscle. The participants sat in a chair during the NGF injection and sat reclined in a medical bed during the quantitative sensory assessments and the HD-tDCS. With the experimental design, it was intended to study the generalized effects of localized experimental long-term muscle pain on pronociceptive and anti-nociceptive mechanisms similar to the studies on the effects of prolonged topical pain models by Gregoret et al., 2021 as well as Hoeger Bement et al., 2020.

2.3 | Prolonged muscle pain model

Sterile solutions of recombinant human nerve growth factor (NGF) was produced by a pharmacy (Skanderborg Apotek). After cleaning the skin with alcohol swaps, 5 μg (0.5 ml) NGF was injected centrally in the right FDI muscle with a 2.5 ml syringe and a disposable needle (30 G × 1/2, 0.3 × 13 mm). Intramuscular injection of NGF produces a

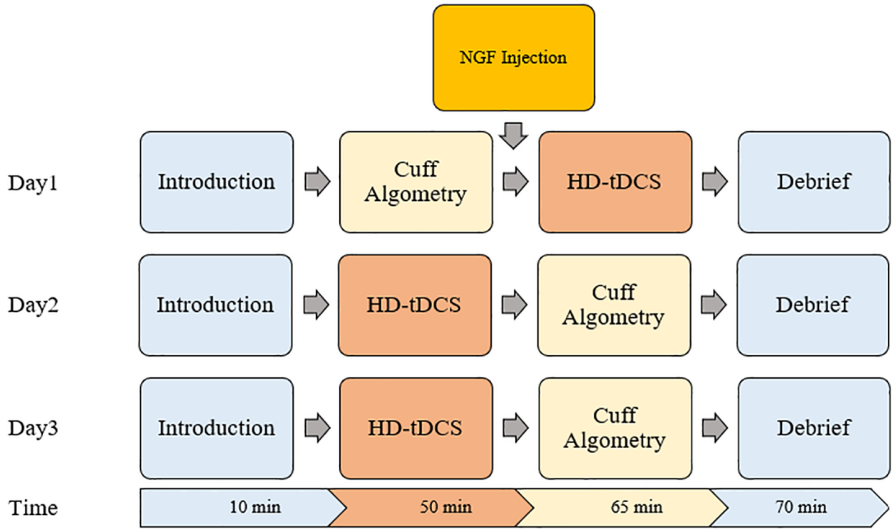

FIGURE 1 Illustration of experimental design. The experimental protocol with cuff algometry (assessment of pressure pain detection and tolerance thresholds, temporal summation of pain and conditioning pain modulation) before the transcranial direct current stimulation (HD-tDCS) intervention on Day 1 and after HD-tDCS on Day 2 and Day 3. The timeline illustrates how the chronological duration of the session approximately 70 min was distributed between experimental components. Induction of prolonged pain (NGF injection) was done after the first cuff algometry assessment on Day 1.

delayed soreness and evoked pain persisting for 3–14 days (Sørensen et al., 2019; Svensson et al., 2003). The intensity of the muscle pain was assessed by self-reported numerical rating scale (NRS, 0–10) scores during muscle use. Zero represented 'no pain' and 10 represented the 'worst pain imaginable'. The average pain NRS rating during muscle activity across Day 2 and Day 3 was extracted.

2.4 | High-definition transcranial direct current stimulation

HD-tDCS was administered using a 32-channel neurostimulation device (Starstim 32, Neuroelectrics) with 3.14 cm^2 Ag/AgCl gelled electrodes in a neoprene cap (NE056 Headcap R, Neuroelectrics). The HD-tDCS session took approximately 50 min; 20 min preparing the setup by applying conductive gel and electrodes in the cap, 4 min resting before and after the 20 min of HD-tDCS. The electrode montage was based on the international 10-10 EEG system. The stimulation montage targeting M1 and DLPFC simultaneously with cathodes placed in concentric rings around the anodes were based on findings from previous studies (Alam et al., 2016; Vaseghi et al., 2016). The Active-tDCS protocol had the stimulation electrodes distributed with anodes (C3 = 2.0 mA, F3 = 2.0 mA) and cathodes (AF3 = −0.8 mA, CP1 = −0.8 mA, FC1 = −0.8 mA, FC5 = −0.8 mA, CP5 = −0.8 μA). This montage generated an electric field distribution concentrated around M1 and DLPFC (Kold & Graven-Nielsen, 2021). The Active-tDCS ramped up to the target amplitude over 30 s, and stimulated continuously for 19 min before ramping down over 30 s. The Sham-tDCS had the same electrode montage and ramped up the current over 30 s, but then automatically turned off for 19 min before it turned on again and ramp down over 30 s in the end of the stimulation. This sham stimulation paradigm mimics the sensory experience of the Active-tDCS and has previously been validated (Garnett & den Ouden, 2015). The tDCS parameters were preconfigured by a third party and named *Stimulation A* and *Stimulation B* for the experimenter to run, based on the subject ID number. The build-in tDCS software (NIC2, Spain) was run in 'blind mode', which conceals the stimulation parameters for the experimenter, enabling that both the participant and the experimenter were blinded to the type of stimulation that was administered.

2.5 | Blinding procedure

Participants were informed that they would be assigned to either active or sham HD-tDCS. The participants received the same stimulation protocol on all 3 days but were not informed about this. It was explained that the sham stimulation was designed to have no effects, but would be indistinguishable to the active stimulation. After each session (Day 1, Day 2 and Day 3), participants were asked whether they thought they received sham or active stimulation; if they believed they had received Sham-tDCS a Sham-trust-index was scored as 1, and if Active-tDCS was guessed, the Sham-trust-index was scored as 0. The average Sham-trust-index across sessions was calculated (e.g. believing that they had received Active-tDCS at Day 1 and Day 2 and sham on Day 3, the mean Sham-trust-index was 0.33). Accuracy of the response to the Sham-trust-index was scored as 1 if the subject guessed correctly and 0 if the guess was incorrect (e.g. correctly guessing the stimulation type they had received on two of the three sessions the accuracy was 0.66).

2.6 | Cuff pressure algometry

A computer-controlled cuff pressure algometer (Nocitech) with a 13 cm wide inflatable tourniquet cuff (VBM, Germany) was used to assess pressure pain detection thresholds (PDTs) and pressure pain tolerance thresholds (PTTs) on both legs. Only the results of the right leg PDT and PTT are used for analysis. The left leg PDT and PTT were used for the conditioning stimulus in the CPM paradigm. The cuffs were mounted below the head of the gastrocnemius muscle on each leg. The pressure was increased at a rate of 1 kPa/s to a maximum of 100 kPa. The subjects were instructed to rate the cuff-induced pain using a handheld electronic 10 cm visual analogue scale (VAS, 0 cm meaning 'no pain' and 10 cm meaning 'worst pain imaginable'). The PDT was defined as the cuff pressure the first instance where the VAS exceeded 1 cm (Graven-Nielsen et al., 2015). When the subjects reached their maximum pain tolerance level, they were instructed to press a button, which immediately released the cuff pressure (defined the PTT). If the subjects did not stop the stimulation before 100 kPa, the PTT was estimated as 100 kPa for the further analysis.

2.7 | Temporal summation of pain

TSP was assessed on the right side immediately after the PDT/PTT recordings. Ten repeated cuff pressure stimulations (1 s cuff stimulation at the PTT intensity and 1 s interval with no cuff pressure) (Graven-Nielsen et al., 2015). Participants were instructed to continuously rate the pressure pain intensity on the electronic VAS during the sequential cuff stimulation. For each cuff stimulus, a VAS score was extracted. For analysis of TSP, the VAS score for each of the 10 pressure stimulations was extracted and

normalized by subtraction of the VAS scores from the first stimulus. The TSP effect was defined as the average of normalized VAS score from stimulations 8 to10 (McPhee & Graven-Nielsen, 2019).

2.8 | Conditioned pain modulation

CPM was assessed on the right side after TSP. The CPM effect reflects the change in pressure pain sensitivity that occurs, when being administered concurrently with a painful conditioning stimulus at another body site (Manresa et al., 2014). Here, the PDT and PTT of the right leg were assessed as test stimuli, while the left leg received a continuous conditioning cuff pressure stimulus at an intensity corresponding to 70% of the PTT (assessed on the left side). This CPM assessment design has previously been used (Graven-Nielsen et al., 2017). After the left leg cuff was rapidly inflated to a constant conditioning pressure level, the PDT and PTT were reassessed on the right leg; when reaching the PTT, the pressure of the cuffs on both legs was released. The CPM effect was calculated as the PDT during conditioning minus the PDT without and saved as CPM-PDT (and similar for PTT and defined as CPM-PTT) (Graven-Nielsen et al., 2017). Participants were excluded from CPM analysis if the PTT or PTT during conditioning were not reached before 100 kPa, as a CPM response would not be reliably detected.

2.9 | Statistics

Data are presented as mean and standard deviation (SD) in text and tables, and mean and standard error of the mean (SEM) in figures. Significance was accepted at $p < 0.05$. Data were evaluated for normal distribution using the Shapiro–Wilk's test of normality. A log-transformation was conducted of the non-normal distributed parameters (PDT and PTT) and used for further analysis. Baseline values of all parameters were compared between groups by one-way ANOVA with *Groups* (Sham-tDCS, Pain-Sham-tDCS, Active-tDCS and Pain-Active-tDCS). Two-way mixed-model ANOVA were performed on data normalized to the baseline (Day 1 results subtracted from Day 2 and Day 3 respectively) for each modality (ΔPDT, ΔPTT, ΔCPM-PDT, ΔCPM-PTT and ΔTSP). The analysis included the factors *Time* (Day 2, Day 3) as within subject factor and *Groups* (Sham-tDCS, Pain-Sham-tDCS, Active-tDCS and Pain-Active-tDCS) as between group factor. Additional two-way mixed-model ANOVA were performed for the blinding efficacy measurements (Sham-trust-index and Accuracy) with the factors *Session* (Day 1, Day 2 and Day 3) and *Groups* (Sham-tDCS, Pain-Sham-tDCS, Active-tDCS and Pain-Active-tDCS). For the significant main effects and interactions, post hoc analysis was conducted using the least significant difference (LSD) test to correct for multiple comparison. The post hoc analysis is presented as mean and SEM for the significant main effects and interactions.

3 | RESULTS

Demographics are shown in Table 1. One subject from the Pain-Sham-tDCS group was excluded from all assessments, as he reported having misunderstood the electronic VAS score after testing. Seven subjects (two from the Sham-tDCS group, two from the Pain-Sham-tDCS group, one from Pain-Sham-tDCS group and two from the Pain-Active-tDCS group) were excluded from the CPM analysis due to the PTT reaching the pressure ceiling, impairing the CPM assessment. Two subjects (one from the Pain-Sham-tDCS group and one from the Pain-Active-tDCS group) were excluded from the TSP analysis as they reported to have misunderstood the assessment protocol. One-way ANOVA revealed that the four groups did not differ significantly in pain sensitivity parameters at baseline recordings (Table 2).

3.1 | Blinding

A two-way ANOVA of the Sham-trust-index (Table 3) revealed no significant main effects or any interaction

TABLE 1 Distribution of participants between groups and demographics

	Gender (*N*)		Handedness (*N*)				
Group	**Male**	**Female**	**Right**	**Left**	**Age (years)**	**Height (cm)**	**Weight (kg)**
Sham-tDCS	12	8	16	4	26.5 ± 7.0	176.8 ± 9.1	76.8 ± 12.1
Pain-Sham-tDCS	10	10	15	5	26.5 ± 2.7	172.5 ± 7.9	70.0 ± 14.9
Active-tDCS	10	10	20	0	27.9 ± 6.9	173.2 ± 9.8	74.2 ± 17.5
Pain-Active-tDCS	10	10	18	2	26.7 ± 7.1	173.2 ± 10.8	76.0 ± 16.1

Note: Distribution of gender, handedness, as well as mean (±SD) age, height and weight of participants in the four groups.

TABLE 2 Baseline cuff algometry parameters

	PDT (kPa)	PTT (kPa)	CPM-PDT (kPa)	CPM-PTT (kPa)	TSP (cm)
Sham-tDCS	22.5 ± 11.0	49.8 ± 16.7	4.6 ± 9.8	0.4 ± 9.1	1.6 ± 1.6
Pain-Sham-tDCS	22.4 ± 7.7	55.2 ± 19.3	4.8 ± 10.3	3.5 ± 9.9	0.9 ± 1.2
Active-tDCS	20.8 ± 8.1	47.7 ± 13.5	3.8 ± 7.7	3.1 ± 6.3	1.4 ± 1.8
Pain-Active-tDCS	24.9 ± 7.3	54.3 ± 11.2	1.1 ± 9.4	−1.0 ± 4.3	1.5 ± 1.5

Note: Mean (±SD) cuff algometry parameters at baseline (Day1) before induction of prolonged muscle pain. Pressure pain detection threshold (PDT), pressure pain tolerance threshold (PTT), conditioned pain modulation effect on pressure pain detection threshold (CPM-PDT), conditioned pain modulation effect on pressure pain tolerance threshold (CPM-PTT) and temporal summation of pain (TSP) are illustrated.

TABLE 3 Mean (±SD) blinding parameters

Groups	Sham-trust-index (0–1)	Accuracy (0–1)
Sham-tDCS	0.33 ± 0.34	0.33 ± 0.34
Pain-Sham-tDCS	0.37 ± 0.29	0.37 ± 0.29
Active-tDCS	0.27 ± 0.26	0.73 ± 0.26
Pain-Active-tDCS	0.48 ± 0.33	0.52 ± 0.33

Note: Sham-trust-index and accuracy score of each group averaged across the 3 days. The sham-trust-index indicates how often out of the three sessions they were asked, whether they believed to have received sham stimulation. Accuracy indicates the success rate of their responses.

between the factors *Time* and *Group* (see statistics in Table 4). The two-way ANOVA of the accuracy (Table 3) revealed that there was a main effect of both the factor *Time* and the factor *Group*, but no significant interaction between the two. Post hoc analysis of the *Time* effect showed that the accuracy of the Sham-trust-index was higher on Day 1 (0.58 ± 0.5) than Day 2 (0.44 ± 0.05, $p = 0.04$) and Day 3 (0.44 ± 0.06, $p = 0.04$). Post hoc analysis of the *Group* effect showed that the Active-tDCS group had higher accuracy of the Sham-trust-index than the Sham-tDCS group ($p < 0.01$), the Pain-Sham-tDCS group ($p < 0.01$) and the Pain-Active-tDCS group ($p = 0.03$).

3.2 | Experimental prolonged pain

The NGF injection successfully induced pain in both the Pain-Active-tDCS group and the Pain-Sham-tDCS group, with average pain NRS scores across Day 2 and Day 3 at 2.8 ± 1.3 and 3.3 ± 2.0, respectively, although not significantly different.

3.3 | Cuff pressure pain sensitivity across days with and without pain and HD-tDCS

A two-way mixed model ANOVA of the ΔPDT and ΔPTT (Table 5) with the factors *Time*, and *Group* revealed that there were no significant main effects or interaction (Table 4).

3.4 | Temporal summation of pain across days with and without pain and HD-tDCS

A two-way mixed model ANOVA of the ΔTSP (Figure 2) with the factors *Time*, and *Group* revealed that there was a main effect of *Group* but no significant main effect of *Time* or interaction (Table 4). Post hoc analysis of the ΔTSP revealed that unrelated to the factor *Time*, the Pain-Sham-tDCS group (0.85 ± 0.36 cm) showed higher ΔTSP than the Sham-tDCS group (−0.70 ± 0.34 cm, $p < 0.01$), the Active-tDCS group (−0.13 ± 0.34 cm, $p = 0.05$) and the Pain-Active-tDCS group (−0.19 ± 0.35 cm, $p = 0.04$).

3.5 | Conditioned pain modulation across days with and without pain and HD-tDCS

A two-way mixed model ANOVA of the ΔCPM-PDT (Table 5) with the factors *Time*, and *Group* revealed that there were no significant main effects or interactions (Table 4). The same was the case the ANOVA of the ΔCPM-PTT.

4 | DISCUSSION

No significant differences were found in the pressure pain sensitivity and CPM over the 3 days, indicating that neither the prolonged experimental pain nor the HD-tDCS modulated these. However, the Pain-Sham-tDCS group showed facilitated TSP at Day 2 and Day 3 compared with Sham-tDCS, Active-tDCS and Pain-Active-tDCS groups. This indicates that the pain-related facilitation of TSP may be counteracted by the active HD-tDCS of M1 and DLPFC in the Pain-Active-tDCS group.

4.1 | Central pain mechanisms modulated by prolonged experimental pain

Impaired CPM and facilitated TSP are well documented in severe chronic musculoskeletal pain conditions and

TABLE 4 Two-way ANOVA statistics

	Time/Session	Group	Time × Group
PDT (kPa)	$F(1, 75) = 0.04$ $p = 0.84$ ES < 0.01	$F(3, 75) = 0.23$ $p = 0.88$ ES = 0.01	$F(3, 75) = 2.18$ $p = 0.10$ ES = 0.08
PTT (kPa)	$F(1, 72) = 2.67$ $p = 0.11$ ES = 0.04	$F(3, 72) = 0.39$ $p = 0.76$ ES = 0.02	$F(3, 72) = 0.30$ $p = 0.82$ ES = 0.01
TSP (VAS 0–10)	$F(1, 73) = 0.06$ $p = 0.81$ ES < 0.01	**$F(3, 73) = 3.33$** **$p = 0.02$** **ES = 0.12**	$F(3, 73) = 1.10$ $p = 0.35$ ES = 0.04
CPM-PDT (kPa)	$F(1, 75) = 0.71$ $p = 0.40$ ES = 0.01	$F(3, 75) = 0.31$ $p = 0.82$ ES = 0.01	$F(3, 75) = 0.31$ $p = 0.82$ ES = 0.01
CPM-PTT (kPa)	$F(1, 69) = 0.03$ $p = 0.86$ ES < 0.01	$F(3, 69) = 0.18$ $p = 0.91$ ES = 0.01	$F(3, 69) = 0.18$ $p = 0.91$ ES = 0.01
Sham-trust-index (0–1)	$F(2, 152) = 0.25$ $p = 0.78$ ES < 0.01	$F(1, 76) = 1.71$ $p = 0.17$ ES = 0.06	$F(6, 152) = 1.73$ $p = 0.12$ ES = 0.06
Accuracy (0–1)	**$F(2, 152) = 3.05$** **$p = 0.05$** **ES = 0.04**	**$F(3, 76) = 6.89$** **$p < 0.01$** **ES = 0.21**	$F(6, 152) = 0.79$ $p = 0.58$ ES = 0.03

Note: ANOVA statistics (F and p-values, ES: effect size) of the sensory modalities normalized to the Day 1 baseline: Pressure pain detection threshold (PDT), pressure pain tolerance threshold (PTT), conditioned pain modulation effect of the PDT (CPM-PDT), conditioned pain modulation effect of the PTT (CPM-PTT) and temporal summation of pain effect (TSP) between the factors *Time* (Day 2, Day 3) and *Group* (Sham-tDCS, Pain-Sham-tDCS, Active-tDCS and Pain-Active-tDCS). ANOVA statistics for the blinding efficacy tests sham-trust-index and accuracy between the factors Session (Day 1, Day 2 and Day 3) and Group. Significant results ($p \leq 0.05$) are marked with bold text.

TABLE 5 Results of normalized pressure thresholds and conditioned pain modulation effect

Modality	Time	Sham-tDCS	Pain-sham-tDCS	Active-tDCS	Pain-active-tDCS
ΔPDT (kPa)	Day 2	1.1 ± 9.34	2.46 ± 6.62	1.25 ± 7.26	−0.46 ± 7.91
	Day 3	2.66 ± 12.36	−0.17 ± 6.42	1.98 ± 8.29	0.36 ± 7.75
ΔPTT (kPa)	Day 2	0.03 ± 10.56	−1.25 ± 11.75	2.33 ± 8.27	−1.44 ± 6.51
	Day 3	0.75 ± 12.09	0.47 ± 14.37	2.89 ± 9.43	1.13 ± 8.05
ΔCPM-PDT (kPa)	Day 2	−1.82 ± 10.15	−3.45 ± 13.5	−3.93 ± 10.39	0.42 ± 14.71
	Day 3	0.02 ± 11.72	−0.14 ± 8.28	−3.08 ± 10.41	−0.4 ± 6.77
ΔCPM-PTT (kPa)	Day 2	2.4 ± 8.44	0.14 ± 10.74	−0.42 ± 6.89	4.49 ± 6.92
	Day 3	2.98 ± 13.01	−1.24 ± 13.16	−0.16 ± 7.47	4.32 ± 6.18

Note: Mean (±SD) pressure pain detection threshold (ΔPDT), pressure pain tolerance threshold (ΔPTT), conditioned pain modulation effect of PDT (ΔCPM-PDT) and conditioned pain modulation effect of PTT (ΔCPM-PTT) on Day 2 and Day 3 normalized to baseline (delta values).

is likely a result of maladaptive neuroplastic changes that occur from prolonged peripheral nociceptive drive to the CNS (Arendt-Nielsen et al., 2018; Graven-Nielsen et al., 2015; Holden et al., 2018; Petersen et al., 2019). In this study, it was attempted to induce similar symptoms in healthy subjects, by NGF-induced prolonged pain over multiple days. This pain model has previously been shown to induce facilitation of TSP (Hayashi et al., 2013; Nie et al., 2009). This facilitation may be driven by the prolonged peripheral input sensitizing dorsal horn neurons, which facilitate the wind-up mechanism (Nie et al., 2009).

These studies assessed TSP with pressure algometry at, or near the injection site, whereas this study assessed TSP using cuff pressure algometry at the legs, making direct comparisons between the studies difficult. Despite this, the facilitation of TSP seen in the Pain-Sham-tDCS group is similar to what was seen in the previous studies, with

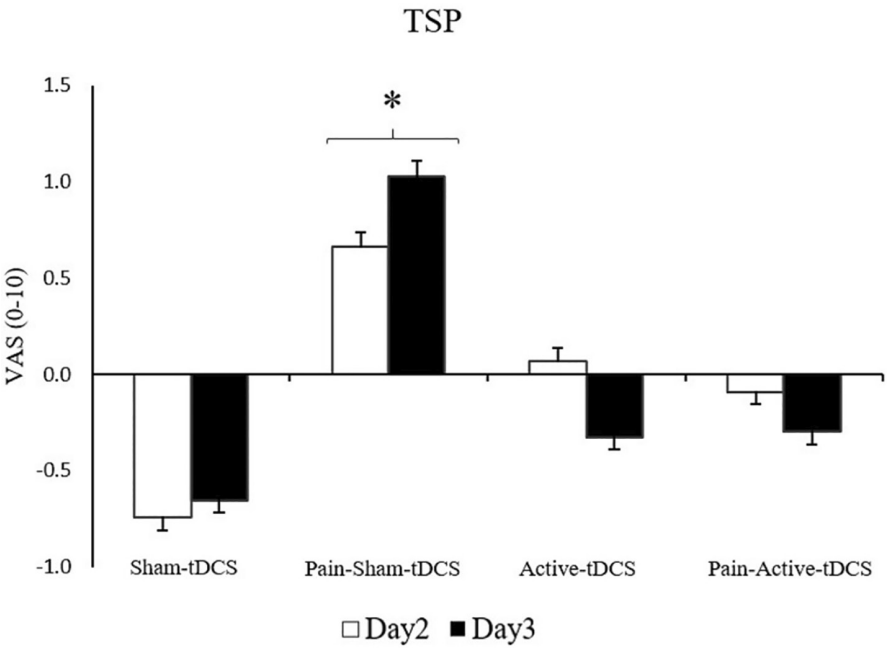

FIGURE 2 Results of normalized temporal summation of pain effect. Mean (±SEM) normalized to baseline temporal summation effect (VAS 0-10) (ΔTSP) on Day 2 and Day 3 for the four groups: Sham-tDCS, Pain-Sham-tDCS, Active-tDCS and Pain-Active-tDCS. Significant differences in the post hoc analysis of the two-way ANOVA is marked (*$p < 0.05$); unrelated to the factor *time*, the Pain-Sham-tDCS group showed facilitated TSP compared to the other groups.

approximately 0.5–1.5 cm higher TSP effect (0–10 cm VAS) following NGF injection, compared to baseline (Hayashi et al., 2013; Nie et al., 2009). Moreover, for the first time, the present experimental study demonstrated facilitated TSP away from the pain locus as also demonstrated in clinical studies of, for example, low back pain patients where the TSP was facilitated when assessed on the lower legs (McPhee & Graven-Nielsen, 2019). Importantly, the facilitation of TSP occurring as a result of the NGF-induced pain is of similar magnitude to what is seen in chronic pain patients, who also show an increased TSP-effect of approximately 1–2 cm (VAS 0–10 cm) compared to healthy subjects (Holden et al., 2018; Izumi et al., 2017).

The prolonged pain model did not modulate the CPM effect in the pain groups. This is in line with another study by Sørensen et al. (2020) demonstrating facilitation of TSP but no modulation of the CPM following NGF-induced prolonged pain (Sørensen et al., 2020). The inefficacy of experimental prolonged or subacute pain to impair the CPM mechanism in healthy subjects has been shown before, where the authors suggested that pain needs to undergo chronification to decrease the CPM efficacy (Valencia et al., 2012). Similar findings have been shown in a study of experimental temporomandibular joint pain by Oono et al. (2011), who also suggests that CPM deficiencies in pain conditions are likely more related to the duration of clinical pain than to the pain itself (Oono et al., 2011). However, pain provocation for 3 days in the present study appears to produce similar neuroplastic changes as NGF-induced pain for 21 days in terms of changes in the CPM and TSP response (Sørensen et al., 2020). The mild-to moderate pain intensity provoked in this study may also underlie the lack of CPM modulation. Bement et al. (2020) demonstrated that changes in experimental pain intensity influence the CPM response (Hoeger Bement et al., 2020).

4.2 | Modulation of central pain mechanisms by HD-tDCS

The Active-tDCS group, in which experimental pain was not induced but who received active HD-tDCS, showed no significant differences in the CPM and TSP over the 3 days compared to the Sham-tDCS group. This finding contrasts with the hypothesis that HD-tDCS would modulate central pain mechanisms of the asymptomatic subjects and is not in line with findings from previous studies. Three previous studies have reported an enhancement of the CPM in healthy subjects following anodal tDCS of M1, with Flood et al. (2016) using 10 min of 2 mA HD-tDCS, Wan et al. (2021) using 20 min of 2 mA HD-tDCS and latest Jiang et al. (2022) also using 20 min of 2 mA HD-tDCS (Flood et al., 2016; Jiang et al., 2022; Wan et al., 2021). The three studies demonstrating CPM improvement and this study are methodologically similar in terms of blinding, placebo-control and sample size. However, all three studies demonstrating a tDCS effect on CPM used a paradigm consisting of pressure pain thresholds with handheld pressure algometry as the test stimuli and cold water immersion as the conditioning pain stimuli (Flood et al., 2016; Jiang et al., 2022; Wan et al., 2021), whereas this study utilized cuff pressure algometry on one leg as test stimuli and tonic painful pressure on the contralateral leg as conditioning stimulus. The difference in CPM assessment, may have affected the contrasts in findings although as both CPM paradigms have previously been validated, it is unlikely (Graven-Nielsen

et al., 2017; Manresa et al., 2014). The average baseline CPM-PTT response across groups was lower, than what has previously been reported in a large cohort study of the CPM effect in approximately 2000 subjects (Skovbjerg et al., 2017). However, similar low baseline CPM effect on the pressure pain threshold was found in a study demonstrating significant effects of tDCS (Jiang et al., 2022), suggesting that the baseline CPM may be less relevant for the effect of tDCS. It is not clear whether a more homogenous sample population, in which all had a well-expressed CPM effect at baseline, would have affected the outcome.

Two studies have investigated the effects of single-session tDCS on TSP with Hughes et al. (2018) (Hughes et al., 2018) administrating 2 mA M1-tDCS for 20 min, and Gurdiel-Álvarez et al. (2021) (Gurdiel-Álvarez et al., 2021) using 2 mA multifocal HD-tDCS of M1 and DLPFC for 20 min. Gurdiel-Álvarez et al. (2021) found no significant effect of the stimulation on the TSP effect in the asymptomatic subjects, which is in line with the present findings (Gurdiel-Álvarez et al., 2021). Interestingly, Hughes et al. (2018) showed that the M1-tDCS inhibited TSP provoked by electrical stimuli at 20 Hz, but not on stimuli administered at 5 Hz (Hughes et al., 2018). In the present study, the stimuli of the TSP stimuli were delivered at 0.5 Hz with cuff pressure algometry, which is in line with the previous findings using the 5 Hz stimuli paradigm without a tDCS effect. It is unclear whether other TSP assessment methods may be modulated differently by HD-tDCS.

4.3 | HD-tDCS modulation of temporal pain summation during prolonged experimental pain conditions

The Pain-Sham-tDCS group showed facilitated TSP compared to the Pain-Active-tDCS group, indicating that the stimulation either antagonized the manifestation of these maladaptive neuroplastic changes or produced an inhibition of the ascending pain signals, decreasing the wind-up mechanism. Inhibition of ascending nociceptive signals has been suggested to be an underlying mechanism of tDCS analgesia (Knotkova et al., 2013). However, a generalized inhibition of these signals should arguably not only exclusively be seen in TSP mechanism but also in the PDT and PTT, which were not significantly modulated. This indicates that the HD-tDCS may have modulated the pronociceptive pain mechanism more directly. M1-tDCS has previously been demonstrated to modulate central pain mechanisms in various chronic pain populations (Giannoni-Luza et al., 2020; Pinto et al., 2018). The modulation of central pain mechanisms has been suggested to be a driving analgesic mechanism of M1-tDCS (Flood et al., 2016; Giannoni-Luza et al., 2020). Despite TSP primarily being a spinal mechanism (Arendt-Nielsen et al., 2011), the top-down modulation of tDCS may affect it. M1-tDCS has been shown to not only affect the targeted regions but also produce changes to functionally connected areas involved in endogenous pain modulation, such as the cingulate cortex, insula, thalamus and the brain stem (Flood et al., 2016; Giannoni-Luza et al., 2020). Additionally, DLPFC and M1 are functionally connected in the cortico-subcortical pain-related networks, which are involved in central pain processing (Giannoni-Luza et al., 2020; Knotkova et al., 2013). The modulation of these networks may in turn reverse or inhibit the pain-induced changes from establishing in the spinal cord and affecting the TSP mechanism. The mechanisms are however still not fully elucidated and is an important focus for future research.

The effects of tDCS on TSP have not been thoroughly investigated, however the present findings conflict a clinical study by Lewis et al. (2018) who assessed the modulatory effect of M1-tDCS on TSP in a group with chronic neuropathic pain and found no difference in TSP following 5 days of M1-tDCS (Lewis et al., 2018), This discrepancy may be driven by differences in the type of pain with Lewis et al. (2018) probing neuropathic pain patients, whereas the subjects in this study were administered an experimental persistent pain model. The current intensity may also influence the conflicting findings, as Lewis et al. (2018) used 1 mA as opposed to this study administering 2 mA tDCS. Hughes et al. (2020) demonstrated that anodal M1-tDCS can reduce both dynamic mechanical allodynia and mechanical pain sensitivity provoked from an experimental pain model, which support that tDCS can inhibit pain-induced perturbation of central and possibly peripheral pain systems (Hughes et al., 2020).

It is interesting that the differences in TSP were not observed between the two groups that were not administered NGF (Active-tDCS and Sham-tDCS). A possible explanation is that the experimental pain provocation perturbed the central nervous system, rendering the pain groups more susceptible to modulation than the non-pain groups (Antal et al., 2008; Thapa et al., 2018; Wittkopf et al., 2021). This theory is in line with previous findings from experimental studies (Horvath et al., 2015; Kold & Graven-Nielsen, 2021, 2022), and is supported by a meta-review examining the modulatory effect of tDCS on sensory thresholds, which showed that the modulatory effect is larger in pain patients with sensitized central nervous systems than healthy subjects (Giannoni-Luza et al., 2020).

4.4 | Limitations

This study was conducted over a relatively long period of time with subjects being included in two clusters

(2019–2020 and 2020–2021). Despite both sub-studies being conducted by the same experimenter, variations may have occurred in the procedure due to the time gap. The inclusion criteria used in the two studies were also identical, however, the second block of the study included the experimental pain model, which may have influenced the characteristics of the recruited population. This difference may have caused a selection bias, even though there were no significant differences in the characteristics at baseline.

A second limitation of this study is that CPM and TSP were only assessed after HD-tDCS on Day 2 and Day 3. The primary interest of this study was the persistent neuroplastic modulation that HD-tDCS may produce. However, not assessing pre-tDCS data restricted the possibility for exploratory analysis of immediate effects of the HD-tDCS on central pain mechanisms. Additionally, it is possible that the tDCS-evoked modulation of the CNS was not yet fully established at the last study session on Day 3. Neuroplastic changes is usually a progressive process, which is why clinical studies of neurostimulation are commonly investigated over several weeks. Previous studies have however showed relative fast neurological changes as a result of pain induction (Martino et al., 2018) and of non-invasive brain stimulation (Nitsche & Paulus, 2001), which supports the decision of a 3-day study. A future experimental design could use multiple days of tDCS before induction of experimental pain.

The sample size of 20 subjects in each group is another possible limitation. Despite the a priori power calculation establishing this as a sufficient population, the effects of CPM may have been more elusive than first hypothesized, adding the risk of the statistical test being underpowered. A cross-over design instead of a parallel group design would have increased the power of the statistical analysis, but may have weakened the effectiveness of the blinding protocol as the subjects would gain familiarity with the sensory experience of tDCS (Fonteneau et al., 2019; Wallace et al., 2016).

A per-protocol analysis was utilized, which entailed that the analysis was only conducted on the subjects that adhered to the protocol. This may have exaggerated the treatment effect. However, including subjects that exceeded the baseline tolerance threshold of the pressure algometry would have ruined the interpretability of the results (e.g. not possible to have a further inhibition if maximum pain tolerance is reached), and as a result, an intention-to-treat analysis was not chosen.

A final limitation is that this study did not include a control group that only received M1-tDCS, which would allow investigation of which cortical target drives the TSP facilitation. With the current design, it is not possible to infer whether the effects can be attributed to the stimulation of M1, DLPFC or the fact that the two cortical targets are stimulated simultaneously.

5 | CONCLUSION

The effects of multifocal HD-tDCS or sham-tDCS targeting M1 and DLPFC on central pain mechanisms were investigated in 80 healthy subjects, of which 40 were administered experimentally induced muscle pain for 3 days. The experimental prolonged pain model successfully induced pain for several days, which facilitated the TSP but not the cuff pressure pain sensitivity or CPM. The active HD-tDCS inhibited the TSP facilitation caused by tonic pain, suggesting that the efficacy of HD-tDCS might be linked with the presence of sensitized central pain mechanisms. This study adds to the current literature demonstrating that the effects of tDCS are influenced by the state of the central nervous system and demonstrates that the pronociceptive pain mechanisms are affected by prolonged experimental pain.

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CONFLICT OF INTEREST

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