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MODULATION OF EXPERIMENTAL PROLONGED PAIN AND SENSITISATION USING HIGH DEFINITION TRANSCRANIAL DIRECT CURRENT STIMULATION: A DOUBLE-BLIND, SHAM-CONTROLLED STUDY

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ABSTRACT

High definition transcranial direct current stimulation (HD-tDCS) targeting brain areas involved in pain processing has shown analgesic effects in some chronic pain conditions, but less modulatory effect on mechanical and thermal pain thresholds in asymptomatic subjects. This double-blinded study assessed the HD-tDCS effects on experimental pain and hyperalgesia maintained for several days in healthy participants. Hyperalgesia and pain were assessed during three consecutive days following provocation of experimental pain (nerve growth factor injected into the right hand muscle) and daily HD-tDCS sessions (20-min). Forty subjects were randomly assigned to Active-tDCS targeting primary motor cortex and dorsolateral prefrontal cortex simultaneously or Sham-tDCS. Tactile and pressure pain sensitivity were assessed before and after each HD-tDCS session, as well as the experimentally-induced pain intensity scored on a numerical rating scale (NRS). Subjects were effectively blinded to the type of HD-tDCS protocol. The Active-tDCS did not significantly reduce the NGF-induced NRS pain score (3.5±2.4) compared to Sham-tDCS (3.9±2.0) on Day3 and both groups showed similarly NGF-decreased pressure pain threshold in the right hand ($P<0.001$). Comparing Active-tDCS with Sham-tDCS, the manifestation of pressure hyperalgesia was delayed on Day1, and an immediate (pre-HD-tDCS to post-HD-tDCS) reduction in pressure hyperalgesia was found across all days ($P<0.05$).

Perspectives

The non-significant differences between Active-tDCS and Sham-tDCS on experimental prolonged pain and hyperalgesia suggest that HD-tDCS has no effect on moderate persistent experimental pain. The intervention may still have a positive effect in more severe pain conditions, with increased intensity, more widespread distribution, or increased duration and/or involving stronger affective components.

INTRODUCTION

The use of transcranial direct current stimulation (tDCS) to modulate brain regions involved in pain processing has been an area of interest for more than a decade[60]. Clinical recommendations for tDCS is available for fibromyalgia (level B of evidence: probable efficacy) and lower limb pain due to spinal cord injury (level C of evidence: possible efficacy)[50,60]. However, why tDCS alleviate these conditions specifically is unknown[50]. For tDCS to be used more broadly in clinical pain rehabilitation, more fundamental insight with controlled settings is needed[16,50] since the neurobiological mechanisms underlying the induced analgesic effect is not clear[46,64]. Better spatial resolution of the electrical field during stimulation has recently been introduced by increasing the number of electrodes from the conventional pair, to arrays of several electrodes[17,18,54]. This high definition transcranial direct current stimulation (HD-tDCS) technique allows the possibility to improve the specificity of which cortical areas are stimulated[4,17,18,42] and to stimulate several brain areas simultaneously. Multifocal stimulation has shown enhanced modulatory effects compared to the conventional tDCS montages with one cathode and one anode[17,26,42]. The acute effects of tDCS is a sub-threshold shift in the resting membrane potential of neurons in the outer cortical layers[46,70]. This changes the excitability of the neurons either towards depolarization using anodal stimulation or hyperpolarization using cathodal stimulation[70] which may last up to 24 hours, driven by changes of the GABAergic activity as well as plastic changes in glutamatergic synapses[64,70]. Despite the electrical field only reaching the neocortex, the stimulation may modulate deeper structures by initiating changes in connected brain areas[15,46,53,64,70] which supports the concept of using HD-tDCS to stimulate functionally connected areas, to modulate brain networks processing pain and somato-sensation.

The conventional tDCS montage frequently used in chronic pain conditions is anodal tDCS targeting the primary motor cortex (M1) with the cathode located over the contralateral supraorbital area[22,45,49,50,53,56,60,76]. The anodal tDCS targeting the dorsolateral prefrontal cortex (DLPFC) with the cathode located over the supraorbital area is also often used in pain conditions[5,20,45,50,60] potentially targeting affective pain components and inhibitory regulation. The M1 and DLPFC montages have shown promising results, but with high variability in the effect size and discrepancies in the specific configuration of the tDCS intervention in terms of stimulation intensity, duration and number of repeated sessions[17,50]. Few studies have yet investigated the effect of HD-tDCS of M1 and DLPFC simultaneously in relation to somatosensory processing. Positive

effects on pain intensity have been shown in fibromyalgia patients[14,76] and for experimentally induced heat pain in healthy subjects[23], but null-findings also exists[37,47].

Recent studies have shown that tDCS does not have the same effect on experimental pain and pain sensitivity in chronic pain patients compared to healthy subjects; likely due to sensitisation of central neuronal mechanisms due to persistent pain[40,52,56,59,65]. One way to bridge the gap between controlled trials in healthy subjects and clinical studies in chronic pain patients is to investigate the effects of HD-tDCS in healthy participants experiencing experimental pain for several days. A potential model of maintained pain for several days is based on intramuscular injection of nerve growth factor (NGF) causing movement-related pain and mechanical hyperalgesia as well as inducing cortical excitability changes[8,19,52,66] through peripheral and central mechanisms, mimicking the symptoms of chronic muscle skeletal pain[41,68,72].

This study aimed to investigate how a HD-tDCS intervention modulates the somatosensory effects of a prolonged pain model. In a double-blinded design, healthy subjects received either 1) Active-tDCS (anodal multichannel HD-tDCS targeting DLPFC and M1 simultaneously), or 2) Sham-tDCS (a placebo approach replicating the sensory perception of HD-tDCS) after induction of mechanical pain and hyperalgesia in a hand muscle maintained for several days by an NGF-injection. The HD-tDCS protocols was delivered for 20-min on three consecutive days. It was hypothesised, that the active HD-tDCS compared with sham HD-tDCS would decrease the perceived experimental muscle pain intensity as well as reducing mechanical hyperalgesia, immediately following administration and/or delayed to be evident on the subsequent study days.

MATERIALS AND METHODS

Participants

Forty healthy participants (20 male) aged 18-55 years were included in this study conducted at Center for Neuroplasticity of Pain (CNAP), Aalborg University, Denmark, between 01/08/2020 and 31/12/2020. The number of participants was estimated based on aims for detecting a small to medium effect size (0.3), with 80% power, a correlation between repeated measures of 0.5 and 0.05 as the alpha level[29]. The sample size calculation was aimed for the main analysis of the study (Repeated measures mixed model ANOVA with two groups and four assessment time points). The minimum number of subjects necessary to meet these statistical criteria was 36 for an effect size of 0.2, 18 for 0.3 and 12 for 0.4. The analysis was conducted using the software G*Power 3.1.9.2[25].

The effect size used in the sample size estimation was based on a meta-analysis of conventional M1-tDCS on quantitative sensory testing outcomes (QST) as no systematic reviews have been done on HD-tDCS[34]. This meta-analysis established a small effect in healthy subjects ($\eta p^2 = 0.16$) and a medium effect in chronic pain subjects ($\eta p^2 = 0.48$). As this study examined healthy subjects with induced pain the conservative expected ηp^2 of 0.3 was chosen for the sample size estimation.

Exclusion criteria included chronic pain conditions, sleep deprivation, pregnancy, addiction (e.g. drug or alcohol), caffeine intake that surpasses one cup of coffee within the last hour prior to the experiment, alcohol intake in the 24 hours prior to the experiment, having medical implants (e.g. pacemaker or surgical steel) or any current illnesses or ongoing pain conditions[9,12,13]. Use of all types of pain medication were restricted during and 48h before the experiment. Additionally, the participants were asked to refrain from activity that would produce muscle soreness during the three-day experimentation period.

Prior participation in other brain stimulation studies within the last three months were not allowed to avoid potential carry-over effect. All participants received written and verbal information about the study and signed a consent form before the first experimental session. Due to COVID19 restrictions in place during the study, the participants wore medical masks and the experimenter wore medical masks and medical gloves. 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).

Experimental design

This randomized double-blinded sham-controlled longitudinal study included two groups (Sham-tDCS: 20 min sham tDCS; Active-tDCS: 20 min simultaneous anodal multichannel tDCS of DLPFC and M1). Subjects participated in three consecutive days of HD-tDCS with QST (pressure pain thresholds, tactile detection thresholds, mechanical pain thresholds, provoked pressure pain scores) before and after each HD-tDCS protocol (Fig. 1). Sessions with HD-tDCS were separated by 24 h. Thus, a total of six assessment sessions were included (Day1pre, Day1post, Day2pre, Day2post, Day3pre, and Day3post). After Day1pre assessments, all participants 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 QST, and sat reclined in a medical bed during the HD-tDCS.

Insert Figure 1 here.

Participants were randomly allocated to one of the two groups (each N=20), received the same instructions before each session, and were blinded to which of the two stimulation protocols they received. The protocols were pre-configured in the HD-tDCS software and the device was set in 'double-blind mode' by a third party enabling the experimenter to administer the intervention blinded. Which stimulation protocol the subjects received, was revealed for the experimenter after the initial analysis was conducted.

Prolonged muscle pain model

Sterile solutions of recombinant human nerve growth factor (NGF) was be prepared by a pharmacy (Skanderborg Apotek, Denmark). After cleaning the skin over the muscle with alcohol swabs a dose of 5 μg (0.5 ml) NGF was given as a bolus injection into the muscle belly the right FDI muscle with a 2.5 ml syringe and a disposable needle (30Gx1/2). The injection of NGF is not painful per se, but pain will develop within hours[68,72]. Each day before the HD-tDCS session, participants were asked to rate the pain they felt in the FDI muscle of the right hand while at rest and while using the muscle on a numerical rating scale (NRS) from 0 to 10, with 0 being 'no pain at all' and 10 being 'the worst pain imaginable'. This assessment was done prior to the quantitative sensory testing and tDCS on each day, as the assessments of the muscle pain thresholds may induce acute soreness, which would confound the results. This is also the reason the assessment was not repeated post-tDCS. The results are labelled as NRS during muscle use and NRS during muscle rest (Day1, Day2 and Day3).

High definition transcranial direct current stimulation

HD-tDCS was administered using a 32-channel neuro-stimulation device (Starstim 32, Neuroelectrics, Spain) with 3.14 cm^2 Ag/AgCl gelled electrodes in a neoprene cap (NE056 Headcap R, Neuroelectrics, Spain). The HD-tDCS intervention took ~35 minutes; 15 minutes to apply conductive gel and electrodes in the cap, and 20 minutes of HD-tDCS. The electrode montage targeting left M1 and left DLPFC simultaneously were based on findings from earlier studies[16,26,42] based on the international 10-10 EEG system, with electrical current in the Active-tDCS protocol distributed between the electrodes as following: 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 mA. The electrode

montage and the estimated electric field distribution is shown in Fig. 2 (modelled by the Neuroelectrics stimulation software). The Active-tDCS ramped up to the target amplitude over 30 s, and stimulated continuously for 19 minutes before ramping down over 30 s. The Sham-tDCS used the same electrode montage as Active-tDCS, but was configured as a 'sham-stimulation' in the software, which made the current ramping up over 30 s, then automatically turned off for 19 minutes before it turned on again and ramped down over 30 s in the end of the stimulation. The sensory experience of the electrical current in the sham stimulation is supposed to be indistinguishable from the sensory experience of the active stimulation, as validated previously[11,31].

Blinding procedure

Participants were informed that they would be assigned to one of two groups, which would determine whether they would receive active or sham HD-tDCS. The participants received the same stimulation protocol on all three days, however the participants were not informed of this. It was informed that the sham-stimulation was designed to have no effects, but would provide the same physiological sensation as the active stimulation. The experimental design and blinding procedure has been suggested in parallel-group design studies where the subjects are naïve to the sensory experience of active tDCS[13,27,44,77]. After each session, participants were asked whether they thought they received sham or active stimulation; if believing they received Active-tDCS a Sham-trust-index was scored as 0, and if Sham-tDCS was assumed, the Sham-trust-index was scored as 1. The average Sham-trust-index across sessions was calculated (e.g. believing that they had received Active-tDCS the two first sessions and sham on the last, the mean Sham-trust-index was 0.33). This was done to investigate if the two groups differed in how often they believed that they had received Sham-tDCS. The 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. Additionally, the participants were asked how certain they were on their protocol assumptions on a scale from 0 to 10, where 0 being 'completely uncertain' and 10 being 'completely certain'. The average certainty-score was calculated across sessions.

Tactile sensitivity

The tactile detection threshold (TDT) was determined on the skin above the right FDI muscle using a set of Von Frey filaments (Touch Test® Sensory Evaluators, North Coast Medical Inc, USA), made by nylon fibre of various diameters. When pushed against the skin the filaments provide a

range of forces ranging from 0.02 g to 300 g. Stimulating with ascending and descending pressure intensities, the supra- and sub-detection thresholds were established. The final TDT was defined as the geometric mean of these five series[63]. TDT was only assessed on the right hand, that received a NGF injection to streamline the experimental design. Subjects unable to detect the 300g filament were excluded from the TDT analysis, as it was not possible to determine their TDT.

Mechanical pain sensitivity

The mechanical pain threshold (MPT) was determined at the skin above the right FDI muscle using a set of weighted pinprick stimulators (PinPrick, MRC Systems GmbH, Germany). The pinprick set consists of seven stimulators with a contact area of 0.25 mm tip diameter that exert forces between 8 mN and 512 mN. By stimulating with ascending and descending weight intensities, the needles that are perceived as painful, and the ones that are not, are identified to determine the average mechanical pain threshold. The final MPT was defined as the geometric mean of the five supra- and subthreshold readings[63]. Subjects with an average sub-pain threshold of 512 mN were excluded from the MPT analysis, as it was not possible to determine their MPT. MPT reflects cutaneous sensitivity at the targeted area[63]. MPT was only assessed on the right hand, that received a NGF injection to streamline the experimental design.

Pressure pain sensitivity

To record the pressure pain threshold (PPT) a hand-held pressure algometer (Somedic, Hörby, Sweden) with a 1-cm^2 probe was used. The pressure was increased gradually at a rate of 20 kPa/s. The participants indicated when the pressure sensation changed from strong pressure to a painful sensation by pressing a button, and the pressure applied was registered. The measurement was repeated three times at the FDI muscle of the right hand as well as the left hand as a control area. The PPT reflects the sensitivity of the deep muscle tissue[63]. The PPT of each hand used for analysis was defined as the mean value of three trials[63]. Subsequently, the pressure was increased gradually at a rate of 20 kPa/s to the participant's average PPT at which point they were asked to rate the pain intensity on a NRS defined as NRS@PPT (0 being 'no pain' and 10 being the 'worst pain imaginable'). The pressure was released the moment the participant responded with a pain rating. This assessment was done once at the FDI muscle on both hands. This is a fairly new assessment method and should be interpreted with care. Previous studies have performed similar assessments by applying pressure stimulation at 120% of the pressure pain threshold to investigate the effects of

suprathreshold pressure stimulation[21,58]. As the pressure is held at threshold level for a few seconds while posing the question of pain rating, it is expected that the assessment will produce a low-to moderate pain at baseline level, much like temporal summation at pain threshold level[10,36]. The NRS@PPT is expected to increase if the muscle is sensitized[10].

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. The reported effect size is partial η^2 (ηp^2). Significance was accepted at $P < 0.05$. Data were evaluated for normal distribution using the Shapiro-Wilk's test of normality. Non-normal distributed parameters were log-transformation for further analysis. The efficacy of the blinding was evaluated by independent samples t-test with *Groups* (Sham-tDCS and Active-tDCS) as the independent variable and the *Sham-trust-index*, the *Accuracy* and the *Certainty-score* as the dependent variables, respectively. Baseline values of all parameters were compared between groups (Sham-tDCS and Active-tDCS) by an independent samples t-test.

The experimental pain model was assessed by two-way mixed-model ANOVAs of NRS pain scores during muscle use and at rest. The analysis included the factors *Time* (Day2 and Day3) as within subject factors and *Group* (Sham-tDCS and Active-tDCS) as between group factor. Day1 was not included in this analysis as it was assessed prior to administration of the pain model. For the significant main effects and interactions, post-hoc analysis was conducted using a least significant differences (LSD). To identify subjects that responded positively to the HD-tDCS, the participants were subdivided based on the pain NRS score when using the FDI muscle on Day3. An NGF-injection in the smaller muscles (i.e. the FDI) is expected to induce a moderate pain intensity when using the muscle, defined as 4-7 on the NRS[8,39]. In the present study, a positive response to the HD-tDCS intervention is defined as experiencing a low pain intensity when using the muscle (0-3 on the NRS[36]). Thus, the groups were subdivided into responders (NRS <4) and non-responders (NRS $\geq$ 4) on Day3. A Chi^2 test of independence was conducted to test for association between the number of responders/non-responders and the stimulation paradigms (Sham-tDCS and Active-tDCS).

Instead of running a single mixed-model ANOVA with 6 time points, the analysis was split into two for ease of interpretation. The first mixed-model ANOVAs included the factors *Time* (Day1pre, Day1post, Day2post and Day3post) as within subject factors and *Group* (Sham-tDCS

and Active-tDCS) as between group factors. This analysis was conducted to investigate the overall effect of the tDCS over the duration of the entire intervention, and would potentially reveal both acute and delayed effects. The second analysis included the factors *Time* (Day1pre, Day2pre, and Day3pre) as within subject factors and *Group* (Sham-tDCS and Active-tDCS) as between group factor. This analysis was conducted to investigate whether a delayed effect of tDCS manifested, and would not have been contaminated by a potential acute effect of tDCS. For the modalities TDT and MPT the analysis included the factors *Time* and *Group* (Two-way mixed model ANOVA), while the analysis of PPT and NRS@PPT also included the factor *Side* (Right, Left) (Three-way mixed-model ANOVA). For significant main effects and interactions, post-hoc analysis was conducted using a LSD test. The post-hoc analysis is presented as mean difference and SEM based on estimated marginal means.

RESULTS

Forty healthy participants were included and completed the study (Table 1). Missing data happened for TDT (3 participants in both groups, either exceeding the maximum value or reported to have misunderstood the instructions in a prior session), and MPT data (one participant in each group, thresholds exceeding maximum value). No adverse effects of the experiment were reported. Day1pre somatosensory and pain sensitivity parameters were not significantly different between the Active-tDCS and Sham-tDCS groups (Fig. 2; right PPT: $t(38) = 0.26$, $P = 0.79$; left PPT: $t(38) = -0.63$, $P = 0.53$; TDT: $t(32) = -2.00$, $P = 0.054$; MPT: $t(36) = -0.58$, $P = 0.56$; right NRS@PPT: $t(38) = 0.64$, $P = 0.53$; left NRS@PPT: $t(38) = -1.17$, $P = 0.25$).

Insert Table 1 here.

Blinding

There was no significant difference in the assumptions of whether subjects received active or sham stimulation between the two groups with the Sham-trust-index of 0.48±0.33 (Active-tDCS) and 0.37±0.30 (Sham-tDCS, $P = 0.25$). Similarly, there was no significant difference in the accuracy of the Sham-trust-index between the two group 0.52±0.33 (Active tDCS) and 0.37±0.30 (Sham-tDCS, $P = 0.15$). These averages indicate that the Active-tDCS group believed that they had received Sham-tDCS 48% of all sessions, and that the Sham-tDCS group believed that they had received

Sham-tDCS 37% of all sessions. Furthermore, there was no significant difference in the certainty-score with 4.3±3.0 (Active-tDCS) and 4.1±3.0 (Sham-tDCS, P = 0.82).

Prolonged muscle pain model

There was no pain during muscle use or rest at Day1 immediately following the NGF injection in any participants. Pain during muscle use increased at Day2 and Day3 with NRS scores at 2.3±1.9 and 3.5±2.4, respectively, in the Active-tDCS group, as well as 2.6±1.6 and 3.9±2.0 in the Sham-tDCS group. The ANOVA of pain NRS scores are presented in Table 2. During muscle use the ANOVA showed a main effect for the factor *Time* with higher scores at Day3 compared with Day2 (P < 0.001), but no significant *Group* effect or interaction was found. Resting pain NRS scores at Day2 and Day3 were low, with 0.8 ± 1.5 and 0.5 ± 1.1, respectively, in the Active-tDCS group, and 0.3 ± 0.6 and 0.6 ± 1.1 in the Sham-tDCS group, without significant main effects of *Time*, *Group* or interaction in the ANOVA.

Based on the Day3 pain NRS scores during muscle use, 13 participants of 20 in the Active-tDCS group had NRS scores during muscle use of 3 or lower. The sham-tDCS group had 8 participants of 20 on Day3 with NRS scores during muscle use of 3 or lower. The Chi^2 test of independence revealed a non-significant relation ($X^2(1,40) = 2.51$, P = 0.11, Phi and Cramer's V=0.25) with participants in the Active-tDCS group having a tendency for being more likely to have pain NRS scores at 3 or lower than the Sham-tDCS group.

Somatosensory pain and detection thresholds after tDCS on each day

The results of the ANOVAs with the factors *Group* (Active-tDCS, Sham-tDCS), *Time* (Day1pre, Day1post, Day2post, Day3post) and *Side* (Right, Left) are presented in Table 2

Insert Table 2 here.

A three-way ANOVA of the PPT revealed that there was a *Time* and *Side* effect (Table 2). Moreover, there was a two-way interaction between *Side* and *Time*. Post-hoc analysis of the interaction between *Time and Side* showed that unrelated to *Group* the right PPT decreased between Day1pre and Day1post (-36.0±12.5 kPa, P < 0.01, Fig. 3), as well as between Day1pre and Day2post (-109.1±17.6 kPa, P < 0.001) and between Day1pre and Day3post (-98.3±27.5 kPa, P < 0.001). Unrelated to group the left PPT did not change significantly between Day1pre and Day1post (-1.6±10.5 kPa, P = 0.88), but decreased between Day1pre and Day2post (-29.6±14.5 kPa, P =

0.05), but not significantly between Day1pre and Day3post (-9.4±23.7 kPa, P = 0.69). The right PPT was not significantly higher than the left PPT at Day1pre (3.7±6.8 kPa, P =0.89), but lower at Day1post (-30.8±10.8 kPa, P = 0.001), as well as day Day2post (-75.8±8.7 kPa, P < 0.001) and Day3post (85.2±9.6 kPa, P < 0.001).

The three-way ANOVA of the NRS@PPT revealed an effect of the factors *Time* and *Sid*e (Table 2). There was a two-way interaction between *Time* and *Group* and between *Time* and *Side*, as well as between *Side* and *Group*. Post-hoc analysis of the interaction between *Time* and *Group* revealed that the Active-tDCS group had no significant difference in the NRS@PPT rating between Day1pre and Day1post (-0.1±0.3, P = 0.71), but an increase between Day1pre and Day2post (1.5±0.4, P < 0.001), as well as between Day1pre and Day3post (1.2±0.4, P = 0.002). The Sham-tDCS group had an increase between Day1pre and Day1post (1.6±0.3, P < 0.001), between Day1pre and Day2post (2.1±0.4, P < 0.001) and between Day1pre and Day3post (2.4±0.4, P < 0.001). On Day1pre the Active-tDCS group had a non-significantly higher NRS@PPT than the Sham-tDCS group, unrelated to the factor *Side* (0.2±0.6, P = 0.77), at Day1post the Active-tDCS group had a lower NRS@PPT than the Sham-tDCS group (-1.5±0.7, P = 0.03), at Day2post the Active-tDCS group had non-significantly lower NRS@PPT than the Sham-tDCS group (-0.5±0.7, P = 0.52), and the same was the case on Day3post (-1.0±0.8, P = 0.20). Post-hoc analysis of the *Time* and *Side* interaction revealed that unrelated to the factor *Group*, the right NRS@PPT increased between Day1pre and Day1post (0.9±0.2, P < 0.001), between Day1pre and Day2post (2.7±0.3, P < 0.001) and between Day1pre and Day3post (2.8±0.3, P < 0.001). The left NRS@PPT increased from Day1pre to Day1post (0.6±0.2, P = 0.02), between Day1pre and Day2post (0.9±0.3, P = 0.02) and between Day1pre and Day3post (0.8±0.3, P = 0.005). At Day1pre the right NRS@PPT was non-significantly lower than the left NRS@PPT (-0.1±0.1, P = 0.51), at Day1post the right NRS@PPT was non-significantly higher than the left NRS@PPT (0.2±0.3, P = 0.35), at Day2post the right NRS@PPT was higher than the left NRS@PPT (1.7±0.25, P < 0.001), as well at Day3post (1.9±0.27, P < 0.001). The post-hoc analysis of the interaction between *Side* and *Group* revealed that the Active-tDCS group showed higher NRS@PPT in the right than the left side (1.4±0.2, P < 0.001). The Sham-tDCS group also showed higher NRS@PPT rating in the right than the left side (0.5±0.2, P = 0.03). Unrelated to the factor *Time* the Active-tDCS group had non-significantly lower right hand NRS@PPT than the Sham-tDCS group (-0.3±0.7, P = 0.66) as well as the left hand NRS@PPT (-1.1±0.7, P = 0.12).

For TDT a two-way ANOVA revealed that there was there was no significant effect on any of the factors (Table 2). The same was the case for MPT.

Insert Figure 3 here.

Somatosensory pain and detection threshold before tDCS on each day

PPT, TDT, and MPT before the tDCS intervention on each day are presented in supplementary material (Table S1). In summary, the Active-tDCS group and the Sham-tDCS group responded differently in the left PPT, where the Active-tDCS group showed no difference over time, whereas the Sham-tDCS group showed decreased PPT on day Day3pre compared with Day1pre.

Immediate effects of tDCS on each day

Subtracting the Pre-tDCS PPT, TDT, MPT and NRS@PPT from the Post-tDCS results on each day establish the immediate effects (e.g. Delta PPT) of each of the tDCS sessions. Analysis of these immediate effects are presented in supplementary material. In summary, the Sham-tDCS group showed a lower Delta PPT than the Active-tDCS group. The right hand side Delta PPT were lower on Day1 than on Day2 and Day3, whereas the left hand side Delta PPT did not change significantly. For Delta NRS@PPT the Sham-tDCS group had a higher Delta NRS@PPT than the Active-tDCS group. The Sham-tDCS group had a higher Delta NRS@PPT on Day1 than on Day2 and Day3, whereas the Active-tDCS group had no significant differences between the days. No significant findings were seen for Delta TDT and Delta MPT.

DISCUSSION

This is the first double-blinded sham-controlled study investigating the effects of HD-tDCS targeting pain related cortical areas to modulate pain induced experimentally extending over several days. The experimental pain model demonstrated hyperalgesia to pressure stimulation and increased pain scores when using the muscle in the course of the three sessions. The active HD-tDCS administered for three days did not significantly attenuate the experimental pain model compared to sham-stimulation. However, the Active-tDCS group showed a delayed manifestation of NRS@PPT compared to the Sham-tDCS group; postponing the pressure evoked pain intensity increase from manifesting from Day1 to Day2. MPTs and TDTs were unaffected by the pain model.

Deep-tissue hyperalgesia and pain across days

No previous studies have investigated the somatosensory sensitivity of the FDI muscle following induction of experimental pain for several days. The FDI muscle was chosen as the hand muscles have a well-defined cortical representation, which is easily stimulated with non-invasive brain stimulation[57]. The pain and increased pain sensitivity induced by the NGF-injection in the present study is comparable to studies using other muscles. In the present study, the self-reported pain intensity during muscle use one day after the NGF-injection was 2.3±1.9 in the Active-tDCS group, and 2.6±1.6 in the Sham-group on a NRS. Similar study designs targeting other muscles have shown NGF-induced pain NRS ratings between 2 and 4.5 one day after injection[6,8,32,72,78]. In the present study, the pain peaked two days after injection with 3.5±2.4 in the Active-tDCS group and 3.9±2.0 in the Sham-tDCS group, which is in contrast to previous studies that report a slight reduction in the movement evoked pain and an increased PPT two days after NGF administration compared with one day after[8,68]. The subjects were only assessed for their self-reported pain rating during muscle use and during muscle rest before the tDCS, so it is unfortunately not possible to evaluate acute effects of the intervention on this modality. Interestingly the increased pressure evoked pain and decreased pressure pain thresholds were also present in the left FDI muscle although not to the same degree as the muscle with NGF. The hyperalgesia may originate from repeated pressure provocations from the pain assessments, or possibly due to sensitisation of central mechanisms caused by the pain model, inducing contralateral hyperalgesia[2,79].

Reduction of prolonged experimental pain by HD-tDCS

The applied HD-tDCS montage has one anode (C3) targeting the primary motor cortex and the secondary anode (F3) targeting the dorsolateral prefrontal area of the left brain hemisphere; both with cathodes placed in concentric rings around the anodes. This tDCS montage coined unihemispheric concurrent dual-site stimulation by Vaseghi et al. (2015) has shown improved modulatory effect (larger and longer lasting changes in cortical excitability) compared to conventional single-site M1 tDCS[75]. Similarly this tDCS configuration has shown improved effect over conventional tDCS in modulating motor learning[73], as well as improving motor function in patients with sub-acute stroke[1]. The multi-target tDCS is thought to have improved modulatory effect compared to conventional M1-tDCS due to the stimulation targeting functionally connected areas [75]. This is supported by fMRI studies, which show that the modulation induced by anodal-tDCS is not limited to the targeted area, but also affects functionally connected brain areas[43,48,75].

The two cortical areas targeted in the current study (M1 and DLPFC) are both involved in pain processing as part of a network that is referred to as the pain matrix[74]. The stimulation of DLPFC from the HD-tDCS montage is thought to modulate the excitability of the DLPFC-premotor-primary motor pathway and further affects the M1-cortical excitability[75]. Furthermore the C3 anode target the motor cortical representational area of the contralateral hand muscle[7,28], which was speculated to improve the modulatory effect of the sensory and nociceptive system on the right FDI muscle.

As configurations of HD-tDCS targeting M1 and DLPFC show anti-nociceptive properties in chronic pain conditions[16,45,50,60] but not in acute experimental conditions it may be hypothesized that an already perturbed cortical system as that of chronic pain patients may be more readily modulated than a healthy non-perturbed system. To test this hypothesis healthy subjects were exposed to pain for several days to imitate the initial somatosensory symptoms of chronic pain. No previous studies have examined the effects of HD-tDCS on prolonged experimental muscle pain. Few studies have however investigated the anti-nociceptive effect of HD-tDCS in a prolonged pain model, by applying topical capsaicin[24,37]. The capsaicin pain model has been shown to induce pain of similar severity (NRS: 2.5-5) to the NGF pain model, despite inducing pain for a shorter duration unless regularly reapplied[30,37,53,78]. Interestingly, Elsawy et al. (2018) and Gregoret et al. (2021) showed contradictory results despite the apparent similarities in study design[23,37]. Elsawy et al.[23] found a reduction in subjective pain scores and area of primary and secondary hyperalgesia after a single session (20 min, 2 mA) HD-tDCS targeting either M1 or insular cortex compared to sham-tDCS. Gregoret et al.[37] found no significant pain reduction in the group receiving Active-tDCS targeting M1 and its associated resting state brain regions (20 min, 2 mA, two sessions on consecutive days), compared to the sham-tDCS group. The active-tDCS group did however have a significantly altered conditioned pain modulation effect following the intervention, indicating that the stimulation did modulate a somatosensory function[37]. In conjunction with the present findings, these studies illustrate the variable effects of HD-tDCS in prolonged experimental pain.

Fundamental differences in the pain models may contribute to the contradictory findings. The topical capsaicin is often used as a surrogate model of changes observed in neuropathic pain patients[61], where tDCS interventions also have shown positive effects of pain reduction[60]. Intramuscular injection of NGF mimics the symptoms of prolonged musculoskeletal pain, which is not as readily modulated by tDCS[5]. Topical capsaicin primarily induces peripheral sensitisation through activation of transient receptor potential vanilloid 1 (TRPV1) receptors[61,67]. The

hyperalgesia induced from intramuscular injection of NGF is likely a result of both central and peripheral mechanisms[6,69,72]. Currently there is no evidence suggesting that tDCS preferentially modulates nociception from specific tissues[46], but there could be a difference between superficial and deep tissue. The outcome difference between the pain models may also be attributed to variations in psychological underpinnings of the models. Despite the topical capsaicin pain model and the NGF-pain model induce comparable pain intensities, the capsaicin model works more rapidly[51]. The hyperalgesia established by the intramuscular NGF injection establishes gradually over 24 hours, which may demand less salience than the quicker heat pain of the topical capsaicin. Additionally, the NGF-model requires activity of the affected muscle to be painful, which the participants can easily autonomously relieve[6], possibly resulting in less psychological strain or pain catastrophizing. The HD-tDCS may have better effects on pain conditions with more psychological strenuous effects/symptoms.

The NRS@PPT showed a baseline rating on average above 3. Such pain rating seems rather high considering, that the pressure is applied at the subjects' own pain threshold level. This finding is however, not uncommon as steady pressure induce progressively more intense pain over time, and persistent pressure is administered at pain threshold level while the pain rating is prompted[10,36]. The Active-tDCS group showed a delayed manifestation of the NGF-induced hyperalgesia, reflected in the NRS@PPT, but not in the PPT compared to the Sham-tDCS group. This is a novel finding, not reported in previous experimental prolonged pain studies. It indicates that the Active-tDCS had an acute effect that either induced a short-term analgesic effect or possibly delayed the central changes developing from day 1 to day 2. Unfortunately, the Active-tDCS group did not significantly differ from the Sham-tDCS group in the level of hyperalgesia on day 2 and day 3, suggesting that the relevance of this delayed hyperalgesia is low.

An immediate tDCS effect is supported by the analysis of the delta values between pre-tDCS and post-tDCS sessions, revealing that the Sham-tDCS group showed an increase in NRS@PPT on day 1 (from pre-tDCS to post-tDCS) that the Active-tDCS group did not, and also generally a reduction in PPT (from pre-tDCS to post-tDCS) across all days that the Active-tDCS group did not show, indicating an acute effect of the tDCS. The increase in pain sensitivity in the sham condition is probably linked with repeated pressure stimulation assessment of the hyperalgesic muscle. A possible explanation may be that the HD-tDCS induces an acute sub-threshold shift of the resting membrane potential of the neurons at the targeted cortical area, which reduces the neuronal spontaneous activity and excitability[35,45,62]. It is possible that these acute effects reduced the

perceived pain of the provoked pressure assessment. However, it is unclear why this were only evident in the NRS@PPT on the first day and not the subsequent two days. A potential reason is that NRS@PPT is the only assessment probing a supra-pain threshold, whereas the other QST assessments probe the pain thresholds. Suprathreshold assessments differ by potentially entailing a negative affective component in terms of inducing the displeasure of pain. Furthermore, the pressure stimulus administered for a longer duration is perceived more intense, resulting in a potentially low-level modulation of tDCS being more pronounced.

Chronic pain and the effects of HD-tDCS

The number of studies demonstrating an anti-nociceptive effect of HD-tDCS on various chronic pain conditions is growing. Villamar et al. (2013) and Castilllo-Saavedra et al. (2016) both reported that HD-tDCS of M1 reduced the pain intensity of fibromyalgia patients[14,76]. Interestingly, Villamar et al.[76] found that a single session of either anodal and cathodal HD-tDCS (20 min, 2 mA) reduced pain intensity, whereas Castillo-Saavedra et al.[14] estimated that a median of 15 anodal HD-tDCS sessions (20 min, 2 mA) are necessary to reach clinically meaningful outcomes. This is further supported by Geva et al. (2015), demonstrating a 50% pain intensity reduction in seven out of twelve fibromyalgia patients enrolled in the study following a minimum of 10 HD-tDCS sessions targeting M1[33].

The positive findings of the reduction of overall perceived pain in pain patients is in contrast to the present findings, where the experimental pain was not reliably attenuated on all three days. The large amount of repeated tDCS sessions used by Castillo-Saavedra et al.[14] may offer a possible explanation of the present null findings. Here only three consecutive days of tDCS sessions were administered, which may have been too few to induce the neuroplastic changes necessary for a significant pain reduction. Secondly, the high number of non-responders from Geva et al.[33] is informative. With the expected effects of the HD-tDCS being low to moderate, non-responders are detrimental to the power of statistical analysis. This underlines the importance of identifying non-responders and investigating the reason behind the heterogeneity and interpersonal tDCS effects.

Pain sensitivity and effects of HD-tDCS

High-definition tDCS has previously shown promise in modulating the somatosensory sensitivity. Borckardt et al. (2012) found that a single session of anodal HD-tDCS (20 min, 2 mA) of M1 decreased heat and cold sensory thresholds, decreased thermal wind-up pain, and had a marginal

hypoalgesic effect for cold pain thresholds in healthy subjects[11]. The stimulation did however not affect heat pain or mechanical pain thresholds. Grundmann et al. (2011) found that a single session of cathodal HD-tDCS of the primary sensory cortex (15 min, 1 mA) decreased the sensitivity to thermal perception changes, but did not significantly modulate the thermal pain thresholds or the mechanical pain and sensitivity thresholds in healthy subjects[38]. In contrast, Kold et al. (2021) found no significant changes in thermal or mechanical somatosensory thresholds in healthy subjects following three consecutive days of HD-tDCS (20 min, 2 mA) of M1 or M1+DLPFC compared to sham HD-tDCS[47]. Based on such modest findings, it was speculated that tDCS efficacy depends on sensitised central pain mechanisms, e.g. during prolonged pain conditions. This is supported by recent studies of chronic pain patients, in which M1-tDCS successfully modulated static and dynamic QST-thresholds in chronic pain patients[3,34,71]

The more common lack of effects by HD-tDCS on the somatosensory sensitivity in healthy participants is in line with the findings of the present study. Despite administering a prolonged pain model, the general effects seen in the Active-tDCS group is not significantly different to the Sham-tDCS group. The groups with active and sham tDCS responded similarly in PPT, TDT and MPT, and only NRS@PPT showed delayed manifestation in the Active-tDCS group compared to the Sham-tDCS group. The Active-tDCS group showed a step-wise increase in the MPT that the Sham-tDCS group did not, but due to the large variance this did not reach significance levels. It is unknown whether a larger sample size would have shown this to be an effect of the intervention or a statistical coincidence. The non-significant findings suggest that the pain model did not perturb the central pain mechanisms in healthy subjects to the degree of clinical persistent pain conditions, resulting them responding similarly to healthy subjects exposed to HD-tDCS.

Limitations

The results of the present study showed few tendencies that did not reach significance, possibly due to the sample size. This does not appear to be problematic for the ANOVA with N=20 in each group, but for the exploratory comparisons of responders and non-responders, in which the participants are further sub-grouped the analysis may be underpowered. Additionally, using a three-way ANOVA for PPT and NRS@PPT, may have underpowered the analysis as opposed to the two-way ANOVA used for other modalities. This is however less relevant as the effects of PPT and NRS@PPT were robust enough to be significant in the three-way design. A cross-over design instead of the used parallel-group design may have improved the statistical power sufficiently to

rule out sample size as a confounding factor. If the blinding method were ensured in a cross-over study, this design has merits for future research[13]. Another possible limitation is the duration of time the participants were investigated. The participants showed peak pain ratings at the last experimental session, which affords the possibility that the pain have further increased after completion of the experiment. The pain model, has been shown to induce hyperalgesia and increased sensitivity for more than five days after injection, however usually peaking after one day[6,32,68,72]. Similarly, it has been suggested that a five consecutive days of tDCS may be more efficient than three, due to the slow nature of the induced neuroplastic changes[16,35,55]. Following the participants longer, would have clarified this uncertainty.

CONCLUSION

The effects of multifocal HD-tDCS targeting M1 and DLPFC were investigated in healthy subjects with experimentally induced muscle pain for several days. The prolonged pain model successfully induced hyperalgesia and pain in the hand. The Active-tDCS did not reduce the experimental pain compared to Sham-tDCS, but did delay the establishment of hyperalgesia although not consistently in all outcome parameters. The central pain mechanisms of an individual exposed to experimental prolonged pain is probably less perturbed than the central mechanisms of an individual suffering from chronic pain. Thus, the effects of the HD-tDCS in the present experimental pain model are more similar to the ones seen in healthy subjects, than the analgesic effects shown in chronic pain patients.

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FIGURE AND TABLE LEGENDS

Figure 1. *The experimental protocol including quantitative sensory testing (QST) before (Pre) and after (Post) high-definition transcranial direct current stimulation (HD-tDCS). The timeline illustrates how the chronological duration of the session ~120 min was distributed between experimental components.*

Figure 2*. The electrode montages and the electrical field distribution of the stimulation paradigm targeting primary motor cortex (M1) and dorsolateral prefrontal cortex (DLPFC) simultaneously.*

All coloured electrodes are included in the HD-tDCS cap, whereas the blue electrodes are used for stimulation. The model is developed using the modelling program in Neuroelectrics stimulation software.

Table 1. *Distribution of participants between groups and mean (±SD) demographics.*

Table 2. *ANOVA statistics of the modalities; Experienced pain on a numerical rating scale during muscle use (NRS@Use), Experienced pain on a numerical rating scale during muscle rest (NRS@Rest), pressure pain threshold (PPT), mechanical pain threshold (MPT), tactile detection threshold (TDT) and the experienced pain on a numerical rating scale at the baseline pressure pain threshold (NRS@PPT) between the factors Group (Active-tDCS and Sham-tDCS), Time (Day1pre, Day1post, Day2post, Day3post) and Side (Right, Left). Significant results (P ≤ 0.05) is marked with bold text.*

Figure 3. *Mean (±SEM) pressure pain threshold (PPT), provoked pressure pain intensity (NRS@PPT), tactile detection threshold (TDT) and mechanical pain threshold (MPT) over 3 days before (open bars) and after (shaded, grey, and solid bars) active and sham tDCS. Significantly different from Day1pre based on a Time and Side interaction (*, P<0.05). Significantly different compared with the left hand based on a Time and Side interaction (#, P < 0.05). Significantly reduced in the Active-tDCS group compared with the Sham-tDCS group based on a Time and Group interaction (¤, P < 0.05).*

Group	Gender (N)		Handedness (N)		Age (years)	Height (cm)	Weight (kg)
	Male	Female	Right	Left			
Sham-tDCS	10	10	15	5	26.5±2.7	172.5±7.8	70.0±14.9
Active-tDCS	10	10	18	2	27.9±10.2	173.2±10.8	76.0±16.1

	Group	Time	Side	Group x Time	Group x Side	Side x Time	Group x Side x Time
NRS @Use	F(1, 38) = 0.38 P = 0.54 ηp^2 = 0.01	**F(1,38) = 16.83** **P < 0.001** **ηp^2 = 0.31**	-	F(1,38) = 0.04 P = 0.84 ηp^2 = 0.01	-	-	-
NRS @Rest	F(1, 38) = 0.62 P = 0.44 ηp^2 = 0.02	F(1, 38) = 0.01, P = 0.91 ηp^2 = 0.00	-	F(1,38) = 1.64 P = 0.21 ηp^2 = 0.04	-	-	-
PPT	F(1, 38) = 0.23 P = 0.63 ηp^2 = 0.001	**F(3, 114) = 30.59** **P < 0.001** **ηp^2 = 0.45**	**F(1, 38) = 62.09** **P < 0.001** **ηp^2 = 0.62**	F(3, 114) = 1.50 P = 0.22 ηp^2 = 0.04	F(1, 38) = 2.12 P = 0.15 ηp^2= 0.05	**F(3, 114) = 35.24** **P < 0.001** **ηp^2 = 0.48**	F(3, 114) = 1.95 P = 0.14 ηp^2 = 0.05
MPT	F(1, 36) = 0.001 P = 0.98 ηp^2 < 0.001	F(3, 108) = 0.58 P = 0.63 ηp^2 = 0.02	-	F(3, 108) = 1.43 P = 0.24 ηp^2 = 0.04	-	-	-
TDT	F(1, 32) = 1.83 P = 0.18 ηp^2 = 0.06	F(3, 96) = 1.92 P = 0.13 ηp^2 = 0.06	-	F(3, 96) = 0.79 P = 0.50 ηp^2 = 0.02	-	-	-
NRS @PPT	F(1, 38) = 1.14 P = 0.29 ηp^2 = 0.03	**F(3, 114) = 34.37** **P < 0.001** **ηp^2 = 0.48**	**F(1, 38) = 32.18** **P < 0.001** **ηp^2= 0.46**	**F(3, 114) = 5.79** **P = 0.004** **ηp^2 = 0.13**	**F(1, 38) = 6.10** **P = 0.02** **ηp^2 = 0.14**	**F(3, 114) = 29.61** **P < 0.001** **ηp^2 = 0.44**	F(1, 114) = 0.97 P = 0.40 ηp^2 = 0.03

Figure1

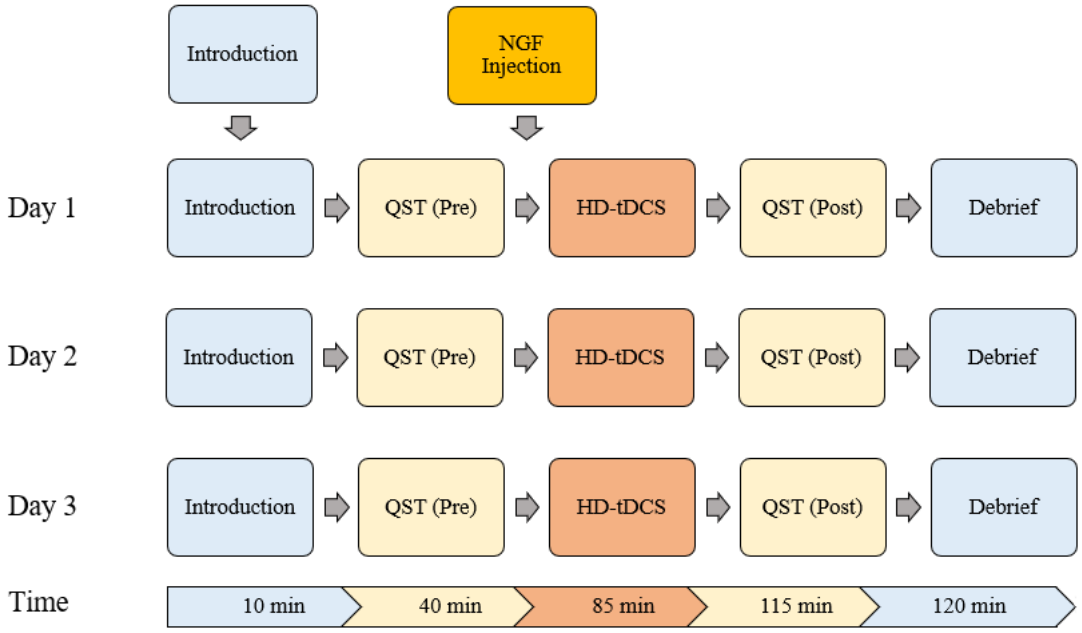

Figure2

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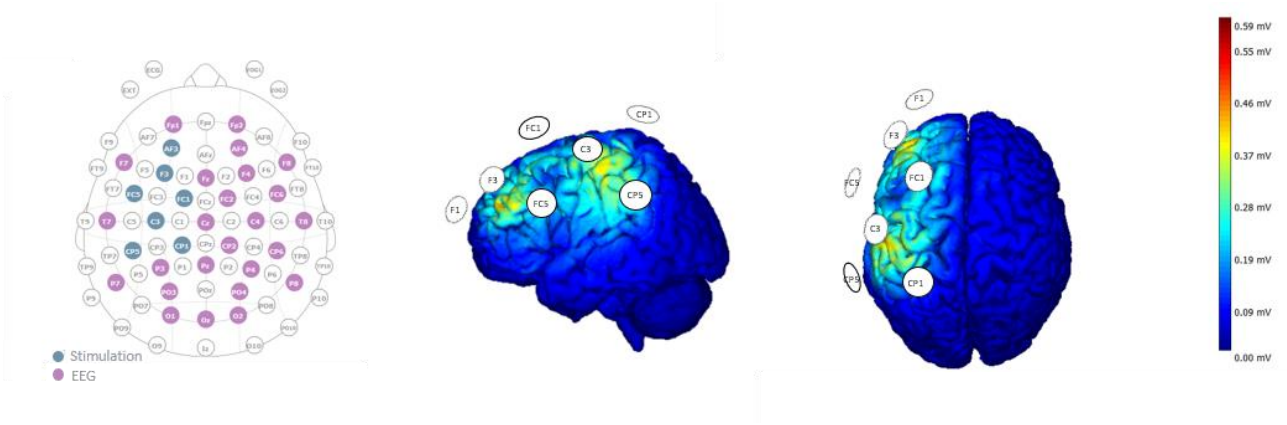

Figure3

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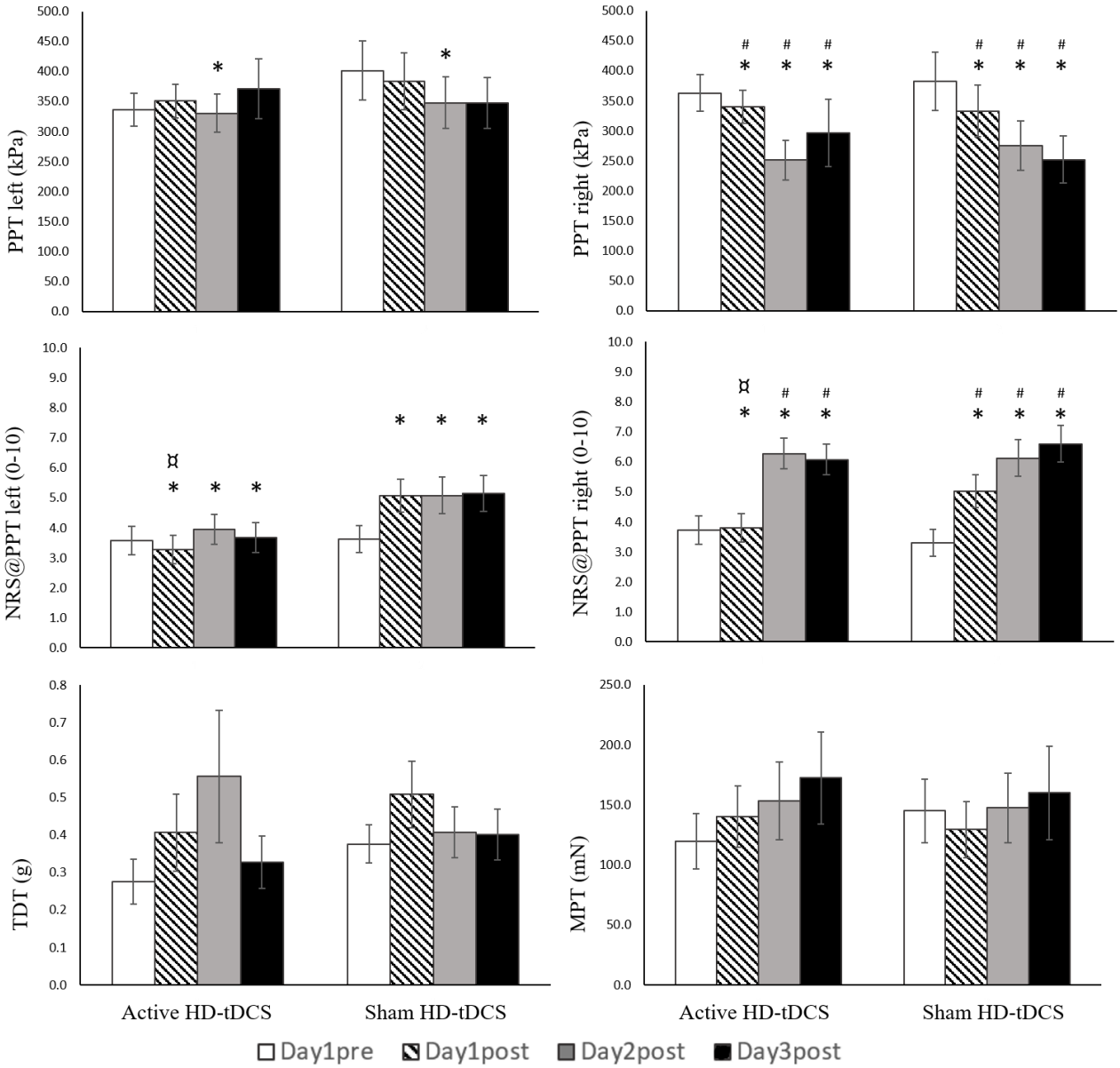

SUPPLEMENTARY MATERIAL TO:

MODULATION OF EXPERIMENTAL PROLONGED PAIN AND SENSITISATION USING HIGH DEFINITION TRANSCRANIAL DIRECT CURRENT STIMULATION

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RESULTS

Mean values of all modalities

The mean values of all modalities over time and across groups are presented in table S1.

Modality	PPT right (kPa)		PPT left (kPa)		TDT (g)		MPT (mN)		NRS@PPT right		NRS@PPT left	
Group	Active	Sham	Active	Sham	Active	Sham	Active	Sham	Active	Sham	Active	Sham
Day1pre	362.5 ±136.6	382.2 ±217.3	336.0 ±123.7	401.4 ±218.3	0.3 ±0.3	0.4 ±0.2	119.4 ±103.0	144.8 ±119.3	3.7 ±2.2	3.3 ±2.1	3.6 ±2.0	3.6 ±2.2
Day1post	339.7 ±122.2	333.0 ±193.5	350.8 ±125.3	383.5 ±210.7	0.4 ±0.5	0.5 ±0.4	140.1 ±114.2	129.5 ±104.4	3.8 ±2.1	5.0 ±2.5	3.3 ±1.8	5.1 ±2.6
Day2pre	235.5 ±141.7	273.0 ±162.2	333.8 ±153.8	361.3 ±175.6	0.4 ±0.5	0.4 ±0.3	121.8 ±99.9	118.7 ±96.9	5.8 ±2.1	5.8 ±2.6	3.5 ±1.9	4.5 ±2.7
Day2post	251.2 ±148.7	275.3 ±183.7	330.3 ±144.0	347.8 ±192.8	0.6 ±0.8	0.4 ±0.3	153.1 ±145.6	147.5 ±130.4	6.3 ±2.3	6.1 ±2.7	4.0 ±2.3	5.1 ±2.6
Day3pre	277.2 ±188.3	267.9 ±198.3	370.7 ±194.7	360.1 ±193.7	0.4 ±0.4	0.3 ±0.2	137.3 ±129.7	148.5 ±153.7	5.8 ±2.4	6.1 ±2.5	3.2 ±2.4	4.5 ±2.3
Day3post	296.1 ±250.7	252.1 ±177.6	371.5 ±223.5	347.13 ±189.0	0.3 ±0.3	0.4 ±0.3	172.3 ±172.0	159.8 ±175.1	6.1 ±2.3	6.6 ±2.7	3.7 ±2.5	5.2 ±2.7

Table S1. *Mean (±SD) somatosensory and pain sensitivity parameters before and after Active-tDCS or Sham-tDCS sessions on Day1, Day2, and Day3. Day1pre is baseline before induction of prolonged muscle pain. Pressure pain threshold (PPT), pain rating on a 1-10 numerical pain rating scale at the pressure threshold (NRS@PPT), tactile detection threshold (TDT), mechanical pain threshold (MPT).*

Mean results of the self-reported pain on a NRS (0-10) on each day is presented in table S2.

Modality	NRS (0-10) during muscle use		NRS (0-10) during muscle rest	
Group	Active	Sham	Active	Sham
Day1pre	0±0	0±0	0±0	0±0
Day1post	-	-	-	-
Day2pre	2.3±1.9	2.6±1.6	0.8±1.5	0.3±0.6
Day2post	-	-	-	-
Day3pre	3.5±2.4	3.9±2.0	0.5±1.2	0.6±1.1
Day3post	-	-	-	-

Table S2. *Mean (±SD) self-reported pain during muscle use and during muscle rest on a NRS (0-10) before Active-tDCS or Sham-tDCS sessions on Day1, Day2, and Day3. Day1pre is baseline before induction of prolonged muscle pain.*

Somatosensory pain and detection threshold before tDCS on each day

A three-way ANOVA (Table S3) of the PPT between *Group* (Active-tDCS and Sham-tDCS), *Time* (Day1pre, Day2pre and Day3pre), and *Side* (Right, Left) before the tDCS intervention on each day showed an effect of the factors *Time* and *Side* but non-significant for *Group*. There was no significant interaction between the factors *Side* and *Group* or between *Time* and *Group,* but between *Side* and *Time*. There was a three-way interaction. Post-hoc analysis of the three-way interaction revealed that the Active-tDCS group showed a decrease in the right PPT between Day1pre and Day2pre (-127.0±27.6 kPa, $P < 0.001$) and between Day1pre and Day3pre (-85.3±28.5 kPa, $P = 0.01$). The Sham-tDCS group showed a decrease in the right PPT between Day1pre and Day2pre (-109.2±27.6 kPa, $P < 0.001$) and between Day1pre and Day3pre (-114.4±28.5 kPa, $P < 0.001$). For the left PPT the Active-tDCS group showed no significant difference between Day1pre and Day2pre (-2.2±21.2 kPa, $P = 0.47$) as well as between Day1pre and Day3pre (34.7±28.5 kPa, $P = 0.32$). The Sham-tDCS group showed no significant difference between Day1pre and Day2pre (-40.1±21.2 kPa, $P = 0.08$) but a decrease between Day1pre and Day3pre (-41.4±28.5 kPa, $P = 0.05$). At Day1pre the Active-tDCS group had a non-significantly lower right PPT than the Sham-tDCS group (-19.7±57.4 kPa, $P = 0.79$), the same was the case on Day2pre (-37.5±48.2, $P = 0.58$) but non-significantly higher than the Sham-tDCS group on Day3pre (9.4±61.1, $P = 0.51$). At Day1pre the Active-tDCS group had non-significantly lower left PPT than the Sham-tDCS group (-65.5±56.1, $P = 0.53$), as well as on Day2pre (-27.5±52.2, $P = 0.78$) but non-significantly higher than the Sham-tDCS group on Day3pre (10.6±61.4, $P = 0.63$).

A three-way ANOVA of NRS@PPT (Table S3) between *Group* (Active-tDCS and Sham-tDCS), *Time* (Day1pre, Day2pre and Day3pre), and *Side* (Right, Left) before the tDCS intervention on each day revealed that there was a *Time* and *Side* but no significant *Group* effect. There was no significant interaction between the factors *Time* and *Group*, but there was an interaction between the factors *Time* and *Side* as well as between the factors *Side* and *Group*. There was no three-way interaction. Post-hoc analysis of the *Time* and *Side* interaction revealed that the right NRS@PPT increased between Day1pre and Day2pre (2.3±0.28, $P < 0.001$), as well as between Day1pre and Day3pre (2.4±0.3, $P < 0.001$). The left NRS@PPT did not change significantly change between Day1pre and Day2pre (0.4±0.3, $P = 0.21$) or between day Day1pre and Day3pre (0.3±0.3, $P = 0.34$). The right NRS@PPT were not significantly different from the left NRS@PPT at Day1pre (-0.1±0.1, $P = 0.51$), but higher at Day2pre (1.8±0.2, $P < 0.001$) and at Day3pre (2.1±0.3, $P < 0.001$). Post-hoc analysis of the interaction between *Side* and *Group* revealed that both the Active-tDCS group (1.7±2.3, $P < 0.001$) and the Sham-tDCS group (0.9±0.2, $P < 0.001$) showed higher NRS@PPT ratings in the right hand than the left hand. The NRS@PPT ratings were not

significantly different between the Active-tDCS and Sham-tDCS groups in the right hand (0.01±0.7, P = 0.99) and in the left hand (-0.8±0.6, P = 0.23).

For the TDT there was no significant effect of *Time*, *Group*, or interaction. For MPT there was no significant effect of *Time*, *Group*, or interaction.

	Group	Time	Side	Group x Time	Group x Side	Side x Time	Group x Side x Time
PPT	F(1, 38) < 0.001 P = 0.98 ηp^2 < 0.001	**F(2, 76) = 23.64** **P < 0.001** **ηp^2 = 0.38**	**F(1, 38) = 93.42** **P < 0.001** **ηp^2 = 0.71**	F(2, 76) = 2.99 P = 0.07 ηp^2 = 0.07	F(1, 38) = 0.75 P = 0.39 ηp^2 = 0.02	**F(2, 76) = 67.82** **P < 0.001** **ηp^2 = 0.64**	F(2, 76) = 3.67 P = 0.03 ηp^2 = 0.09
MPT	F(1, 36) = 0.06 P = 0.81 ηp^2 = 0.002	F(2, 72) = 0.20 P = 0.82 ηp^2 = 0.01	-	F(2, 72) = 0.32 P = 0.73 ηp^2 = 0.01	-	-	-
TDT	F(1, 32) = 2.32 P = 0.14 ηp^2 = 0.07	F(2, 72) = 0.20 P = 0.82 ηp^2 = 0.01	-	F(2, 64) = 1.11 P = 0.34 ηp^2 = 0.03	-	-	-
NRS @PPT	F(1, 38) = 0.02 P = 0.89 ηp^2 = 0.001	**F(2,76) = 45.17** **P < 0.001** **ηp^2 = 0.54**	**F(1, 38) = 63.04** **P < 0.001** **ηp^2 = 0.62**	F(2, 76) = 2.33 P = 0.10 ηp^2 = 0.06	**F(1, 38) = 6.03** **P = 0.02** **ηp^2 = 0.14**	**F(2, 76) = 53.83** **P < 0.001** **ηp^2 = 0.59**	F(2, 76) = 0.70 P = 0.50 ηp^2 = 0.18

Table S3. *ANOVA statistics before tDCS on each day of the modalities; pressure pain threshold (PPT), mechanical pain threshold (MPT), tactile detection threshold (TDT) and the experienced pain on a numerical rating scale at the baseline pressure pain threshold (NRS@PPT) between the factors Group (Active-tDCS and Sham-tDCS), Time (Day1pre, Day2pre, Day3pre) and Side (Right, Left). Significant results (P ≤ 0.05) is marked with bold text.*

Immediate effects of tDCS on each day

To investigate the immediate effects of the tDCS the delta values of all modalities were calculated by subtracting Day1pre from Day1post, Day2pre from Day2post, and Day3pre from Day3post. The results are labeled Delta_PPT_R, Delta_PPT_L, Delta_MPT, Delta_TDT, Delta_NRS@PPT_R, and Delta_NRS@PPT_L.

A three-way ANOVA was conducted on the delta values of PPT (Table S4) between *Groups* (Active-tDCS and Sham-tDCS), *Time* (Day1, Day2 and Day3) and *Side* (Right, Left). The analysis showed no significant main effect of the factors *Side*, or *Time*, but did show a main *Group* effect. There was an interaction between the factors *Side* and *Time*, but non-significant interactions between *Side* and *Group*, or between *Time* and *Group*. Similarly, there were no significant three-way interaction. Post hoc analysis of the main Group effect showed a lower Delta PPT in the Sham-tDCS group (-17.9±7.7 kPa) than the Active-tDCS group (3.96±7.7 kPa, P = 0.03). Post hoc analysis of the interaction between the factors *Side* and *Time* showed a lower right-hand Delta_PPT_R on Day1 (-36±12.5 kPa) than Day2 (9±8.6 kPa, P = 0.01), as well as than Day3 (1.5± 10.2 kPa, P = 0.04). This was not the case for the left-hand Delta_PPT_L which were non-significantly higher on Day1 (-1.6±10.5 kPa) compared to Day2 (-8.5±7.2 kPa, P = 0.32) as well as compared to Day3 (-6.1±9.2 kPa, P = 0.79).

A three-way ANOVA was conducted on the Delta NRS@PPT (Table S4) with factors *Group* (Active-tDCS and Sham-tDCS), *Time* (Day1, Day2 and Day3), and *Side* (Right, Left). The analysis revealed that there was no significant main effect of the factors *Side* or *Time*, but did show a main effect on the factor *Group*. There was a significant interaction between the factors *Time* and *Group*, but not between *Side* and *Time* or between *Side* and *Group*. Similarly, there was no significant three-way interaction. Post hoc analysis of the interaction between the factors *Time* and *Group* revealed that the Sham-tDCS group showed a decrease in Delta NRS@PPT from Day1 (1.6±0.3) to Day2 (0.5±0.3, P < 0.01) and Day3 (0.6± 0.2, P < 0.01), whereas the Active-tDCS showed non-significant increases from Day1 (-0.1±0.3) to Day2 (0.5±0.3, P = 0.11) and Day3 (0.4±0.2, P = 0.15). At Day1 the Delta NRS@PPT was higher in the Sham-tDCS than the Active-tDCS (P < 0.001), but not significantly different on Day2 (P = 0.94) and Day3 (P = 0.51).

A two-way ANOVA was conducted on the Delta TDT (Table S4) with factors *Group* (Active-tDCS and Sham-tDCS), *Time* (Day1, Day2 and Day3). The analysis showed no main effect of the factors *Time* or *Group*. Similarly, there was no significant interaction between the two factors.

A two-way ANOVA was conducted on the Delta MPT (Table S4) with factors *Group* (Active-tDCS and Sham-tDCS), and *Time* (Day1, Day2 and Day3). The analysis revealed that there was no main effect of the factor Time or Group. Similarly, there was no interaction between the two factors.

	Group	Time	Side	Group x Time	Group x Side	Side x Time	Group x Side x Time
Delta PPT	**F(1, 38) = 5.62** **P = 0.02** **ηp^2 = 0.13).**	F(2, 76) = 1.23 P = 0.30 ηp^2 = 0.03	F(1, 38) = 1.09 P = 0.30 ηp^2 = 0.03	F(2, 76) = 0.77 P = 0.47 ηp^2 = 0.02	F(1, 38) = 0.07 P = 0.80 ηp^2 = 0.002	**F(2, 76) = 6.82** **P = 0.002** **ηp^2 = 0.15**	F(2, 76) = 0.02 P = 0.98 ηp^2 < 0.001
Delta MPT	F(1, 36) = 1.51 P = 0.23 ηp^2 = 0.04	F(2, 72) = 0.61 P = 0.55 ηp^2 = 0.02	-	F(2, 72) = 2.81 P = 0.07 ηp^2 = 0.07	-	-	-
Delta TDT	F(1, 32) = 0.86 P = 0.36 ηp^2 = 0.03	F(2, 64) = 1.86 P = 0.16 ηp^2 = 0.06	-	F(2, 64) = 2.63 P = 0.08 ηp^2 = 0.08	-	-	-
Delta NRS @PPT	**F(1, 38) = 8.04** **P < 0.001** **ηp^2 = 0.18**	F(2, 76) = 0.73 P = 0.49 ηp^2 = 0.02	F(1, 38) = 0.02 P = 0.90 ηp^2 < 0.01	**F(2, 76) = 7.72** **P < 0.001** **ηp^2 = 0.17**	F(1, 38) = 0.33 P = 0.57 ηp^2 < 0.001	F(2, 76) = 1.36 P = 0.26 ηp^2 = 0.03	F(2, 76) = 0.08 P = 0.92 ηp^2 < 0.001

Table S4. *ANOVA statistics of the immediate effects of tDCS on each day of the modalities; delta pressure pain threshold (Delta PPT), delta mechanical pain threshold (Delta MPT), delta tactile detection threshold (Delta TDT) and the delta of the experienced pain on a numerical rating scale at the baseline pressure pain threshold (Delta NRS@PPT) between the factors Group (Active-tDCS and Sham-tDCS), Time (Day1, Day2, Day3) and Side (Right, Left). Significant results (P ≤ 0.05) is marked with bold text.*