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MODULATING THE SOMATOSENSORY SYSTEM USING HIGH-DEFINITION TRANSCRANIAL DIRECT CURRENT STIMULATION

BY
SEBASTIAN KOLD

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DENMARK

MODULATING THE SOMATOSENSORY SYSTEM USING HIGH-DEFINITION TRANSCRANIAL DIRECT CURRENT STIMULATION

PHD THESIS

by

Sebastian Kold

AALBORG UNIVERSITY
DENMARK

Dissertation submitted

.

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PhD supervisor: Prof. Thomas Graven-Nielsen
Aalborg University

PhD committee: Associate Professor Steffen Frahm
Aalborg University, Denmark

Professor Michael Nitsche
Leibnitz Research Centre for Working Environment and Human Factors, Germany

Associate Professor Aleksandra Vuckovic
University of Glasgow, UK

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CV

In 2018, Sebastian received a Master's in psychology with a specialization in neuropsychology from Institute of Communication and Psychology, Aalborg University, Denmark. With a long-standing passion for health research, he worked as a research assistant at Aarhus University Hospital and at Aalborg University during his University education. Hereafter he enrolled as a PhD fellow, working at the Center for Neuroplasticity and Pain (CNAP) under the supervision of Professor Thomas Graven-Nielsen. Sebastian's main research focus has been on the effects of transcranial direct current stimulation (tDCS) on the somatosensory system in healthy people. In this respect, the physiological responses to various tDCS configurations have been explored, to determine the potentials of tDCS to be utilized in clinical pain rehabilitation. During his PhD, Sebastian has been involved in student project supervision, lecturing at a PhD course, reviewing papers for various international peer-reviewed journals, and presenting results at international pain conferences.

PREFACE

This PhD thesis provides an overview of work performed at the Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University, Denmark, in the period from September 2018 to December 2021. It has been financially supported by Aalborg University and the Danish National Research Foundation (DNRF121).

The purpose of the thesis was to investigate the effect of high-definition transcranial direct current stimulation (HD-tDCS) on the somatosensory system in healthy humans. This was achieved through experimental studies, determining the sensory profiles of healthy volunteers with quantitative sensory testing before and after administration of HD-tDCS for several days. The thesis is organised as an extended summary of the background, methodology, results and discussion of the studies conducted during the PhD. It presents content from three journal articles.

Throughout the thesis, these articles are referred to as:

Study I: **Kold S**, Graven-Nielsen T: Effect of anodal high-definition transcranial direct current stimulation on the pain sensitivity in a healthy population: a double-blind, sham-controlled study. Pain 162:1659–68, 2021.

Study II: **Kold S**, Graven-Nielsen T: Modulation of experimental prolonged pain and sensitization using high-definition transcranial direct current stimulation a double-blind, sham-controlled study. J Pain S1526-5900(22)00034-7, 2022.

Study III: **Kold S**, Graven-Nielsen T: Modulation of central pain mechanisms using high-definition transcranial direct current stimulation: a double-blind, sham-controlled study. Submitted, 2022.

ENGLISH SUMMARY

Chronic pain conditions pose an immense societal problem, being a leading cause of disability globally and severely impacting the patients' quality of life. The existing pain management options are insufficient, with only 40-60% of patients experiencing a favourable outcome from pharmacological treatments. The majority of currently available treatments, including antidepressants, opioids and topical anaesthetics, have limited long-term effectiveness and are often associated with moderate or, in some cases, severe adverse effects. As a result, the search for new therapeutic methods to alleviate pain conditions is highly relevant. High-definition transcranial direct current stimulation (HD-tDCS) has shown analgesic efficacy in a number of chronic pain conditions, but the modulatory effects are not fully elucidated, and systematic research in controlled settings is necessary.

The aim of the present thesis was to investigate the modulatory potential of HD-tDCS on the somatosensory system in healthy humans. Two double-blinded sham-controlled experiments were designed and conducted on healthy subjects. Experiment I investigated the effects of three different active HD-tDCS protocols compared to Sham-tDCS (N=20 in each group) on peripheral somatosensory-and pain detection thresholds assessed through a battery of quantitative sensory testing applied over three days (QST). Experiment II investigated the effects of multifocal HD-tDCS compared to Sham-tDCS on experimental pain and hyperalgesia maintained for three days. Somatosensory-and pain detection thresholds, as well as hyperalgesia, were assessed on each day following provocation of experimental pain (injection of nerve growth factor into the right-hand muscle). Concurrently with the peripheral somatosensory testing, the central pain mechanisms (temporal summation of pain and conditioned pain modulation) were assessed in both studies. The effects of HD-tDCS on the central pain mechanisms were compared between the two studies, which constituted the subject of *Study III*.

In *Study I*, none of the three active HD-tDCS protocols induced significant changes in detection or pain thresholds compared with the Sham-tDCS. This led to the conclusion that healthy subjects respond differently to tDCS than chronic pain patients have previously been shown to respond. Possibly due to a ceiling effect or endogenous homeostatic mechanisms counteracting the exogenous modulation of stimulation.

In *Study II*, the experimental pain model successfully induced sustained hyperalgesia and pain in both the group that received active HD-tDCS and the group that received Sham-tDCS. The HD-tDCS did not modulate the perceived experimental pain intensity. The active stimulation did, however, delay the establishment of hyperalgesia, although not consistently in all outcome parameters. This led to the conclusion that the effects of the HD-tDCS following experimental persistent pain provocation are more similar to the ones seen in healthy subjects than the analgesic effects shown in

chronic pain patients. Possibly due to the central pain mechanisms of an individual exposed to experimental prolonged pain being less perturbed than the central mechanisms of an individual suffering from chronic pain.

In *Study III*, the experimental prolonged pain model facilitated TSP but did not perturb the cuff-pressure pain sensitivity or CPM. The active HD-tDCS inhibited the pain-related facilitation of TSP compared to Sham-tDCS, suggesting that the efficacy of HD-tDCS might be linked with the presence of sensitized central pain mechanisms.

Overall, HD-tDCS did not modulate the somatosensory pain and detection thresholds but was able to delay the establishment of hyperalgesia and modulate endogenous pain facilitatory mechanisms in healthy subjects with pain-perturbed nervous systems. This indicates that the modulation of the somatosensory system effects may be driven by changes in the pro-nociceptive pain processing mechanisms. When taken together, the findings from the three studies suggest that the effects of HD-tDCS are highly dependent on the state of the central nervous system.

DANSK RESUME

Kroniske smertelidelser udgør et enormt samfundsmæssigt problem ved at være den hyppigste årsag til invaliditet globalt, og i høj grad have negativ indvirkning på patienternes livskvalitet. Det eksisterende tilbud af konventionel smertebehandling er utilstrækkeligt, hvor kun 40-60% af patienterne oplever en et positivt udbytte ved den farmakologiske behandling. Majoriteten af de tilgængelige behandlinger, herunder antidepressiver, opioder og topikal anæstetika har en yderst begrænset langsigtet effekt og er ofte forbundet med moderate eller i nogle tilfælde svære bivirkninger. Af disse årsager er søgen efter nye behandlingsmetoder særdeles relevante. Høj-opløsnings transkraniel jævnstrømsstimulation (HD-tDCS) er før blevet vist at kunne have en analgesisk effekt ved flere kroniske smertelidelser, men den modulatoriske effekt er ikke fyldestgørende afdækket og systematisk forskning under kontrollerede forhold er dermed nødvendigt.

Formålet med indeværende afhandling var at undersøge det modulatoriske potentiale af HD-tDCS på det somatosensoriske system i raske mennesker. To dobbelt-blindede sham-kontrollerede forsøg blev designet og udført på raske forsøgspersoner. Forsøg 1 undersøgte effekten af tre forskellige aktive HD-tDCS protokoller sammenligned med Sham-tDCS (N=20 i hver gruppe) på de perifære somatosensoriske-og smertedetektionstærskler, afdækket gennem et batteri af kvantitative sensoriske undersøgelser (QST) påført over tre dage. Forsøg II undersøgte effekten af multifokal HD-tDCS sammenligned med Sham-tDCS på eksperimentel smerte og hyperalgesi vedholdt i tre dage. Somatosensoriske-og smertedetektionstærskler såvel som hyperalgesia var undersøgt over tre dage efter påførelsen af den eksperimentelle smerte (injektion af nervevækstfaktor i en muskel på den højre hånd). Samtidig med de perifære somatosensoriske undersøgelser blev der foretaget undersøgelser af centrale smerte mekanismer (temporal summation af smerte og betinget smertemodulering) i begge forsøg. Effekten af HD-tDCS på de centrale smertemekanismer blev sammenlignet på tværs af de to studier, hvilket konstituerede undersøgelsesemnet af forsøg III.

I forsøg I medførte ingen af de tre aktive HD-tDCS protokoller signifikante ændringer i detektions-eller smertetærsklerne sammenlignet med Sham-tDCS. Dette ledte til konklusionen, at raske mennesker responderer anderledes på HD-tDCS end hvordan det tidligere er blevet vist, at kroniske smertepatienter responderer. Dette skyldes potentielt en lofteffekt eller at endogene homeostatiske mekanismer modvirker den eksogene modulation af HD-tDCS.

I forsøg II inducerede den eksperimentelle smertemodel vedvarende hyperalgesi og smerte succesfuldt i både gruppen, der modtog aktiv HD-tDCS og i gruppen der modtog Sham-tDCS. Oplevelsen af intensiteten af den eksperimentelle smerte blev ikke moduleret af HD-tDCS. Den aktive stimulation medførte til gengæld en

forsinkelse af etableringen af hyperalgesi, dog ikke systematisk i alle parametre. Dette ledte til konklusionen at effekten af HD-tDCS følgende den eksperimentelle vedvarende smerte provokation er mere lignende den, der ses i raske mennesker end den analgesiske effekt, der er blevet vist i kroniske smertepatienter. Dette skyldes måske, at de centrale smertemekansime ved individer, der er blevet udsat for eksperimentel vedvarende smerte bliver mindre forstyrrede end de centrale smertemekanismer ved individer, der lider af kroniske smerter.

I forsøg III faciliterede den eksperimentelle vedvarende smertemodel TSP, men påvirkede ikke lancet-tryksmertetærsklen eller CPM. Den aktive HD-tDCS hæmmede den smerterelaterede facilitering af TSP i sammenligning med Sham-tDCS, hvilket indikerer at virkningsgraden af HD-tDCS potentielt er forbundet med tilstedeværelsen af sensibiliserede centrale smerte mekanismer.

Generelt modulerede HD-tDCS ikke de somatosensoriske smerte-og detektionstærskler, men var i stand til at forsinke etableringen af hyperalgesi og modulere endogene smertefaciliterende mekanismer i raske mennesker, der havde smertepåvirkede nervesystemer. Dette indikerer at modulationen af det somatosensory system potentielt bliver drevet af forandringer i de endogene smerteprocesseringsmekanismer. Anskues fundene af de tre studier samlet indikeres det at effekten af HD-tDCS i høj grad er afhængig af tilstanden af det centrale nervessystem.

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I would like to thank the following people who supported me personally and academically throughout the PhD without whom I would not have been able to complete this thesis.

The team at Center for Neuroplasticity and pain, especially to my supervisor Thomas Graven-Nielsen, whose experience, insight, and knowledge steered me through the studies and whose encouragement kept my hopes up on long days. And special thanks to Daniel Ciampi de Andrade and Peter Bang, whose support allowed me to go the extra mile on the thesis.

The many people who took the time to undergo the bit tedious and occasionally painful experiments that I have conducted during the research and without whom I would have no content for my thesis.

A big thanks to my family for all the support you have shown me through this PhD. And my biggest thanks go to my wife, Cathrine; thank you for all your support, patience, and loving encouragement, without which the writing process would have been insufferable. Our son is my greatest accomplishment in my time spent on the PhD.

ABBREVIATIONS

CDT – Cold detection threshold
CNS – Central nervous system
CPM – Conditioned pain modulation
CPT – Cold pain threshold
DLPFC – Dorsolateral prefrontal cortex
DNIC – Diffuse noxious inhibitory control
EEG - Electroencephalography
EMG – Electromyography
FDI – First dorsal interosseous muscle
HDT – Heat detection threshold
HD-tDCS – High-definition transcranial direct current stimulation
HPT – Heat pain threshold
LTD – Long-term depression
LTP – Term potentiation
M1 – Primary motor cortex
MEPs – Motor-evoked potentials
MPT – Mechanical pain threshold
NIBS – Non-invasive brain stimulation
NRS – Numerical rating scale
PDT – Pressure detection threshold
PPT – Pressure pain threshold
QST – Quantitative sensory testing
rTMS – Repetitive transcranial magnetic stimulation
SO – Supraorbital cortex
tDCS – transcranial direct current stimulation
TDT – Tactile detection threshold
TMS – Transcranial magnetic stimulation
TSP – Temporal summation of pain
VAS – Visual analogue scale
VDT – Vibration detection threshold

TABLE OF CONTENTS

TABLE OF FIGURES

CHAPTER 1. INTRODUCTION

1.1. SOMATOSENSATION AND THE CENTRAL NERVOUS SYSTEM

Somatosensation is a collective term for the bodily sensations and comprises some of the most important ways to perceive our body and the physical world around it. By sensing the physical properties of our surroundings as well as important bodily sensations, somatosensation form the basis for interacting with the environment[1]. A nudge on the arm, the heat radiating from a flame, the distinct edges of a keychain are all sensory features that can guide our behaviour and form our motor actions[1]. The biological processes underlying the sense of touch, temperature and pain comprise a complex system of sensory neurons and neural pathways that respond to changes inside and on the surface of the body[1]. The sensory afferents react to different stimuli depending on the nerve receptor type, which allows for subcategorization of somatosensation into thermoception (temperature), mechanoreception (vibration, discriminatory touch and pressure), proprioception (position and movement of our own body), equilibrioception (balance) and nociception (pain)[2,3]. The different nerve types propagate their signals to the brain, where they are integrated and processed to form the coherent conscious experience; *the perception of a physical self in immediate contact with the world around it.*

Perhaps the most crucial feature of somatosensation is to prevent bodily harm and maintain homeostasis[4]. When a physical stimulus reaches an intensity threshold that may cause tissue damage, nociceptors will propagate the signal to the spinal cord and brain, which will initiate immediate neural responses aiding in withdrawing from the stimulus and limiting the damage[5,6]. Nociception, in combination with mood, cognition, and other biopsychosocial factors, constitutes the experience of acute pain[7,8]. The otherwise unpleasant experience of acute pain serves a vital evolutionary function in facilitating quick avoidance of harmful stimuli[5]. Similarly, persisting pain facilitates immobility and rest to promote healing processes[9]. However, in some cases, the pain persists after tissue damage is no longer present and risks becoming chronic. This is called pain chronification and poses a major burden on the quality of life for the affected individual[4,10–13].

1.1.1. CHRONIC PAIN – AN ONGOING PANDEMIC

The international association of pain (IASP) defines pain as "*An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage*" and is considered chronic when it persists or recurs for more than 3 months[12,14]. Chronic pain has recently been added to the ICD-11 as a pathology in itself[4], and conditions such as low-back pain, neck pain, migraine and

osteoarthritis are the primary causes of "years lived with disability", with 1/5 adults suffering from pain globally[11,13]. Because of this, pain management in terms of rehabilitation or hindering pain chronification through early detection should be global health priorities[11]. Unfortunately, the effects of the typical biomedical interventions for several pain disorders (e.g., opioid medication, surgery) have risks of adverse effects, highly variable outcomes and lack long-term benefit for many patients[10,15]. Research and development of pain management interventions are thus major focuses of the health industry, with new pharmaceuticals and health technologies being developed rapidly. One area of development showing promise is non-invasive brain stimulation (NIBS). NIBS interventions aim to alleviate chronic pain disorders at the root of their pathogenesis through direct modulation of the underlying maladaptive neuroplasticity[15–17].

1.1.2. NEUROPLASTICITY AND PAIN

Chronic inflammatory and muscular pain disorders involve a constant barrage of nociceptive inputs from the affected tissues via the nociceptive pathways[18,19]. These prolonged nociceptive inputs provoke dysfunctional plastic changes in terms of both peripheral and central sensitization[20]. While nociceptors in the healthy peripheral nervous system have high thresholds for activation, repeated stimulation increases nociceptor excitability[20]. Inflammatory molecules are released from the tissue injury, and pronociceptive receptors in the periphery are upregulated, and as a result, the subject experience hyperalgesia[20]. The persistent transmission of pain signals from the periphery further provokes sensitization of the central nervous system (CNS) by increasing the excitability of second-order neurons in the dorsal horn, which propagates nociceptive signals to the brain[20]. In the process of pain chronification structural reorganization of synapses, cells and circuits also occur at various anatomical and temporal scales[19,21]. These system-wide changes leave chronic pain patients with a completely altered nociceptive system; often with localized and widespread hyperalgesia[21,22], dysfunctional descending pain control[23–25] facilitated temporal summation of pain[24,26–28], and occasionally even allodynia[20]. Fortunately, the neuroplasticity that underlies these dysfunctional changes leaves hope that the pathologies can be diminished, or even reversed, by treatments specifically targeting these mechanisms. All modern treatments for chronic pain act via peripheral or central mechanisms that aim to counteract the pain-related maladaptive changes, be it pharmacological or non-pharmacological[19,29]. There are several approaches to modulating the nervous system non-pharmacologically, e.g. physical therapy[30,31], psychological therapy[32], neurofeedback[33] and, of course, neuromodulation[34]. Neuromodulation differs from the aforementioned methods by directly targeting the peripheral or CNS with electrical stimulation. One of the most recent non-invasive brain stimulation techniques is transcranial direct current stimulation (tDCS)[35].

1.2. AIM OF THE PHD PROJECT

As chronic pain conditions constitute an immense burden on patients and society, attempts to modulate the somatosensory system to provide analgesia and rehabilitate the underlying pathology have been made[15,36,37]. Interestingly tDCS has shown promising potential as a clinical tool to alleviate pain in certain conditions[15,16,37]. Constant development in the technology and lack of agreement on intervention parameters have, however, resulted in considerable methodological heterogeneity between studies. This heterogeneity may drive the inconsistent findings of tDCS research in terms of outcome and efficacy. Meta reviews and guidelines in the field call for systematic research with rigorous methodology and larger sample sizes[15,16,37]. The therapeutic effects of tDCS are, despite its use for more than twenty years, still unknown. The neuronal mechanism has largely been uncovered through studies done in vitro, in animals and through computational modelling[38–45]. However, the functional effect of tDCS on somatosensation and pain processing remains elusive[46–49]. For this reason, the aim of the PhD project was to investigate the effects of high-definition transcranial direct current stimulation (HD-tDCS) on the somatosensory system. The studies were conducted on healthy subjects as previous studies have shown highly variable effects of tDCS between clinical populations[15,37,50].

To investigate this aim, three research questions and subsequent hypotheses were formed:

Research question 1: What areas of the brain are most effective to stimulate with HD-tDCS to modulate the somatosensory system in healthy subjects?
Hypothesis 1: In an experimental study design, the most efficient tDCS configuration will modulate the somatosensory sensitivity and pain thresholds in healthy subjects to a larger extent than both less efficient configurations and sham-tDCS (*Study I*).

Research question 2: Is it possible to alleviate experimentally induced long-term pain and muscle hyperalgesia in healthy subjects using HD-tDCS?
Hypothesis 2: Since HD-tDCS has been shown to alleviate chronic pain, it may also alleviate experimentally induced pain, providing a translational model for the research of the analgesic effects of tDCS in otherwise healthy subjects (*Study II*).

Research question 3: Is it possible to modulate the central pain mechanisms in healthy subjects and subjects with experimental persistent pain using HD-tDCS?
Hypothesis 3: Active tDCS may modulate the endogenous pronociceptive and antinociceptive mechanisms more efficiently than Sham-tDCS. This modulation may differ between healthy subjects and subjects with experimental persistent pain (*Study I & II*).

These questions and hypotheses were investigated through two double-blinded randomised controlled studies condensing the research questions into testable experiments, which were disseminated through three research papers. An overview of the experimental studies and the content of the research papers is illustrated in Fig. 1.

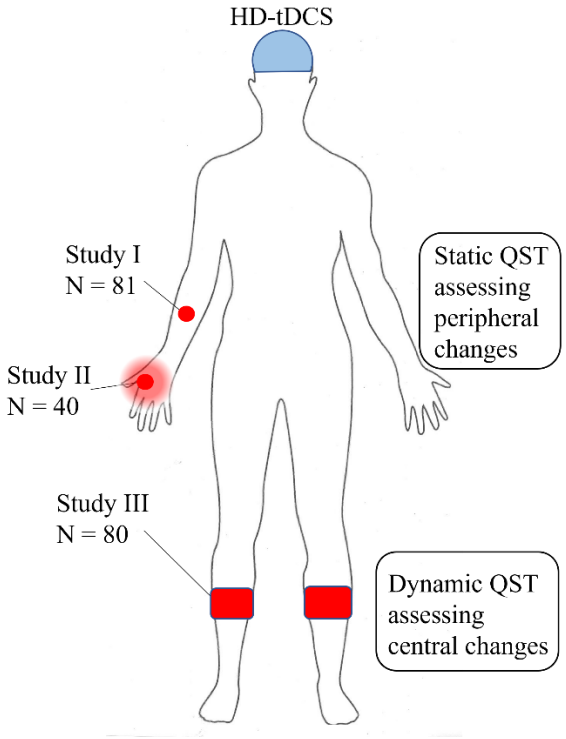


Figure 1. Overview of the dissertation studies. Study I assessed the effects of three different active HD-tDCS configurations and sham-tDCS (each group N = 20) on somatosensory and pain thresholds in healthy subjects assessed with quantitative sensory testing (QST). Study II assessed the effects of HD-tDCS compared to sham-tDCS on the somatosensory and pain thresholds assessed with QST in healthy subjects with perturbed nervous systems, provoked by an experimental pain model (each group N = 20). Study III assessed central pain mechanisms with dynamic quantitative sensory testing in healthy subjects (N=40) and subjects administered the experimental pain model (N=40).

1.3. PAPERS ASSOCIATED WITH THE DISSERTATION

Paper 1: Kold S, Graven-Nielsen T: Effect of anodal high-definition transcranial direct current stimulation on the pain sensitivity in a healthy population: a double-blind, sham-controlled study. Pain 162:1659–68, 2021.

Paper 2: Kold S, Graven-Nielsen T: Modulation of experimental prolonged pain and sensitization using high-definition transcranial direct current stimulation a double-blind, sham-controlled study. J Pain S1526-5900(22)00034-7, 2022.

Paper 3: Kold S, Graven-Nielsen T: Modulation of central pain mechanisms using high-definition transcranial direct current stimulation: a double-blind, sham-controlled study. Submitted to EJ Pain, 2022.

1.4. DEMOGRAPHIC CHARACTERISTICS OF INCLUDED SUBJECTS

An overview of the demographic characteristics of the included subjects in the three papers is shown in Table 1.

Recruited and tested	***Paper I***	***Paper II***	***Paper III***
Included in the main analysis (N)	81	40	80
Gender (male)	41	20	42
Dominant hand (right hand)	73	33	69
Age (years)	25.1±5.6	27.2±7.4	26.7±7.1
Height (cm)	174.1±10.1	172.9±9.3	173.9±9.4
Weight (kg)	72.5±14.0	73.0±15.6	74.2±15.2

Table 1. Demographic characteristics of all participants in the experimental studies. The table shows the number of subjects included in the main analysis, the number of males included, the number of subjects that were right-hand dominant, as well as the average age (years), height (centimeters), and weight (kilograms) of all subjects included (Mean±St.d.).

CHAPTER 2. BACKGROUND: TRANSCRANIAL DIRECT CURRENT STIMULATION

2.1. A BRIEF HISTORY OF NON-INVASIVE ELECTRIC BRAIN STIMULATION

The first attempts at using transcranial electrical stimulation as a medical treatment date all the way back to ~year 1-50 AD, when physicians in ancient Rome tried to utilize the electric discharge of certain fish to alleviate headaches[51,52]. Recently caught black torpedo fish were placed on the cranial surface of headache patients, where the fish emitted the electrical discharge, which ceased the pain[51]. This method is thought to rely on the paralyzing shock that an electrical discharge can produce and not purposeful brain modulation[53]. However, there are reports of bioelectric fish being used to treat various neurological conditions up through the eighteenth century, such as depression, epilepsy and chronic pain conditions, indicating that non-invasive brain stimulation was intended[51,54]. The first formal research in changing cerebral excitability by applying weak, technology-generated, transcranial electric currents began in the 1950s and was primarily used in animal research[55–57]. In this time period, it was established that cathodal stimulation generally decreases the excitability of the affected neurons, while anodal stimulation increases the excitability[58–60]. Furthermore, it was established that the excitability changes produced from the electrical stimulation persisted after ended stimulation and that these after-effects are dependent on changes in the transmission characteristics of the synapses[40,61]. What specifically drove these after-effects were at the time unknown and are still not fully uncovered[38,44,62,63]. In the 1960s- and ‘70s, systematic tDCS studies began in human subjects, primarily within the psychiatric field, to treat patients suffering from treatment-resistant depression or schizophrenia[64–66]. In the late 1990s, the interest in modulating the human motor cortex began[67–69], laying the groundwork for the research investigating modulation of the human somatosensory-and motor systems using low-intensity electrical transcranial stimulation, such as tDCS[67].

2.2. TRANSCRANIAL DIRECT CURRENT STIMULATION: METHOD AND MECHANISMS

tDCS is conventionally administered by mounting two saline-soaked sponge electrodes on the surface of the head[67]. One anode and one cathode. Any electrode from which current enters the body is an anode, and any electrode where current exits the

body is a cathode[70]. The electrodes are typically connected to a battery-driven stimulator that provides a low-intensity constant direct current. The lower-case *t* in the abbreviation *tDCS* emphasizes that tDCS, used across human trials, only characterizes fixed sustained direct current[70].

The current density at the electrode is calculated by dividing the applied current of an electrode by the electrode surface area. The dose of a single tDCS session is usually reported by electrode size [cm^2], electrode position [EEG 10-10 system], stimulation intensity [A] and duration [s][70,71]. A clear understanding of the tDCS dose is imperative to the design of tDCS studies, as the effects are highly dose-dependent and can even lead to the opposite effect of what was intended if administered inappropriately[41,62,71–74].

The electrical field [V/m] that reaches the brain is defined by the current density multiplied by local tissue resistivity[70]. The properties of the electric field predict the modulatory effect more meaningfully than current density, but it is sensitive to assumptions on local tissue resistivity[70,75]. An early tDCS study estimated that ~50% of the current applied to the scalp reached the cortical neurons of monkeys [76]. Modern computational models of the human head and brain have since contributed immensely to estimating the electrical field generated by various tDCS configurations[77–80]. The electrical field strength in the cortex of computational head models often falls between 0.2 and 0.5 V/m per 1 mA current delivered[73,81–83]. Neuromodulatory effects have been reported with administration of electrical field intensities as low as 0.1 V/m *in situ*[83].

A computational model (SimNIBS 3.2.6) of the electrical fields generated by a conventional 2 mA tDCS M1-montage is shown in Fig 2. This tDCS montage with 25 cm^2 sponge-electrodes over the primary motor cortex (M1) (anode) and the supraorbital cortex (SO) (cathode) is the most frequently used in clinical research of the somatosensory system[84].

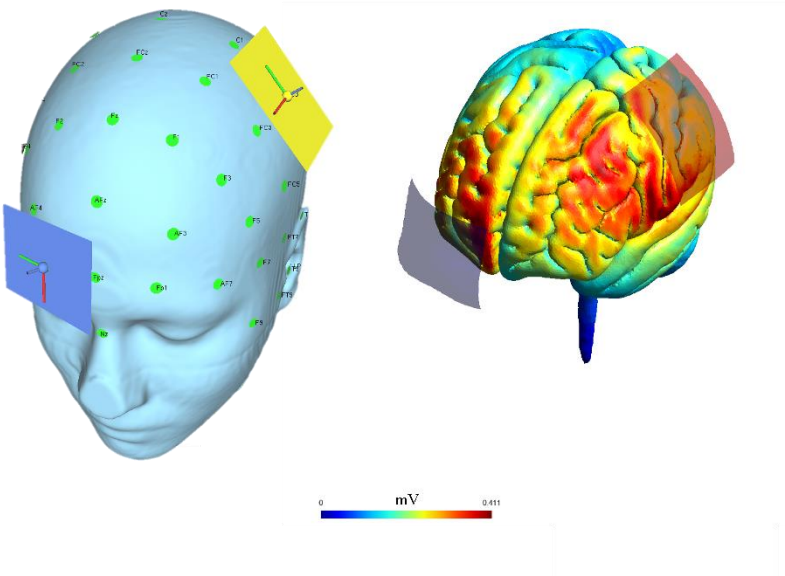


Figure 2. Computational model (SimNIBS 3.2.6) of conventional M1-tDCS configuration and corresponding electrical field distribution in millivolts. The anode (yellow) is located over the primary motor cortex, and the cathode (blue) is located over the supraorbital cortex.

2.3. FROM CONVENTIONAL tDCS TO HD-tDCS

The need to stimulate several cortical targets simultaneously and computational modelling of the electrical field generated by tDCS has driven technological advancements in the electrode configuration and design[77,78,80,85]. The conventional sponge electrodes generated a large electrical field at the cortical level, which was not focal to the intended target[78]. This led to the development of using multiple smaller electrodes in arrays, which was termed high-definition tDCS (HD-tDCS)[86,87]. The HD-tDCS provide the opportunity to stimulate more areas simultaneously and more precise focality[78,80,85,88–97]. Studies have demonstrated that this provides better and longer-lasting modulation of corticospinal excitability and functional outcomes[85,88,90,92,94,95,98,99]. In all tDCS configurations utilized in the present studies, the stimulation was delivered 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) (Figure 3).

Figure 3. Starstim 32 system (Neuroelectrics, Spain) mounted on a NE056 Headcap R (Neuroelectrics, Spain).

In all active HD-tDCS configurations of the three studies, the anodes delivered a 2 mA current for 20 min. The montages and corresponding electrical fields (modelled using the NIC 2.0 software, *Neuroelectrics, Spain*) are shown in Fig. 4.

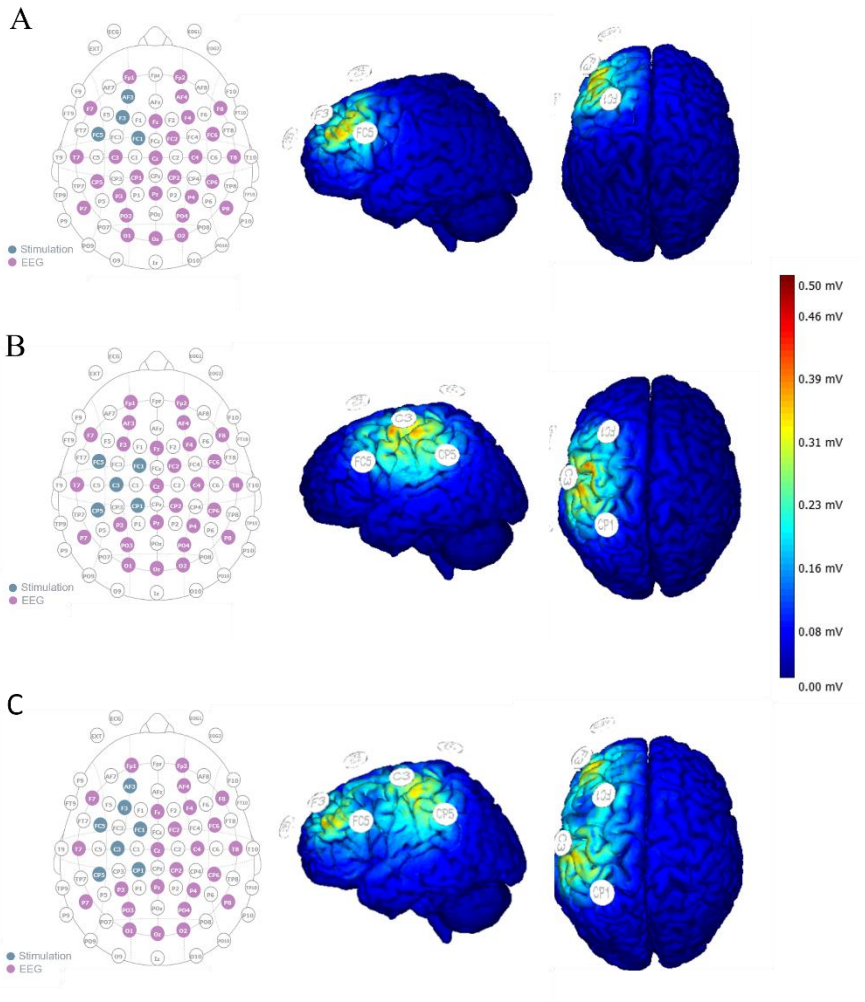


Figure 4. HD-tDCS electrode configurations with corresponding electrical field distributions in millivolts (mV). Targeting (A) dorsolateral prefrontal cortex (DLPFC), (B) primary motor cortex (M1) and (C) DLPFC and M1 simultaneously. The pink circles represent electrodes for recording electroencephalography (EEG), and the blue circles represent the stimulating and receiving electrodes. Modelled using NIC 2.0, Neuroelectrics, Spain.

Three active HD-tDCS configurations were utilized in *Study I*. The DLPFC-tDCS configuration (A on Fig. 4) consisted of F3 (2 mA) as anode, AF3 (-0.66 mA), FC5 (-0.66 mA), and FC1 (-0.66 mA) as cathodes. The M1-tDCS configuration (B on Fig. 4) consisted of C3 (2 mA) as anode, FC5 (-0.5 mA), FC1 (-0.5 mA), CP5 (-0.5 mA) and CP1 (-0.5 mA) as cathodes. The DLPFC+M1-tDCS configuration (C on Fig. 4) consisted of C3 (2 mA) and F3 (2 mA) as anodes and CP5 (-0.8 mA), FC5 (-0.8 mA), AF3 (-0.8 mA), FC1 (-0.8 mA) and CP1 (-0.8 mA) as cathodes. Only the DLPFC+M1-

tDCS configuration was utilized in *Study II & III*. The reference electrode was placed on the right earlobe in all configurations. These stimulation configurations have been utilized before[92,93,95,96,98,100,101]. Comparing the M1-configurations of conventional tDCS (Fig. 2) and HD-tDCS (Fig. 4, B), it is evident that the HD-tDCS is stimulating M1 more focally.

2.4. TOLERABILITY AND SAFETY

The current intensity of tDCS is so weak that with appropriate impedance, the sensation of the stimulation on the skin is only noticeable for the initial duration (~30 s) of the stimulation[102,103]. This makes tDCS tolerable, even for patients with hypersensitive skin or a sensitized pain system[81,104]. Additionally, very few adverse effects of tDCS have been reported from clinical studies[37,81]. The most frequent being skin burns, if the electrodes are dysfunctional or if they are improperly applied. In the present studies, no serious adverse effects were observed in any of the 372 sessions that were included in the studies. The most frequently reported sensations of the stimulations were tingling, itching, pricking or mild burning in the first minutes of the sessions. This is in line with what is seen in other clinical studies[70,81,104].

2.5. ACUTE MECHANISM

tDCS differs from other brain stimulation techniques, such as transcranial magnetic stimulation (TMS) and electroconvulsive therapy, in that it does not elicit neuronal action potentials due to the low intensity of the stimulation[105]. Anodal tDCS (1–2 mA) is not strong enough to depolarize the membrane potential of neurons to the firing threshold[67,106]. Instead, the immediate effect is a subthreshold shift of the resting membrane potential of targeted neurons[37,56,107–110]. The simplified mechanism often described in clinical studies is that the stimulation produces a shift of the membrane potential, which changes the excitability and spontaneous firing rate of the neurons[67,106,111]. The effects of tDCS of the cortex are polarity dependent: Anodal tDCS conventionally increases the excitability, and cathodal tDCS decreases the excitability of targeted neurons[67,89,105,106,112]. However, within the electrical field produced by the tDCS, each feature of a single cell is affected differently[113–115]. Structural cellular components at the cathode are subject to depolarization, whereas those facing the anode are more prone to hyperpolarization[114,116]. The effect of the mechanistic model of anodal and cathodal stimulation on membrane polarization is shown in Fig. 5.

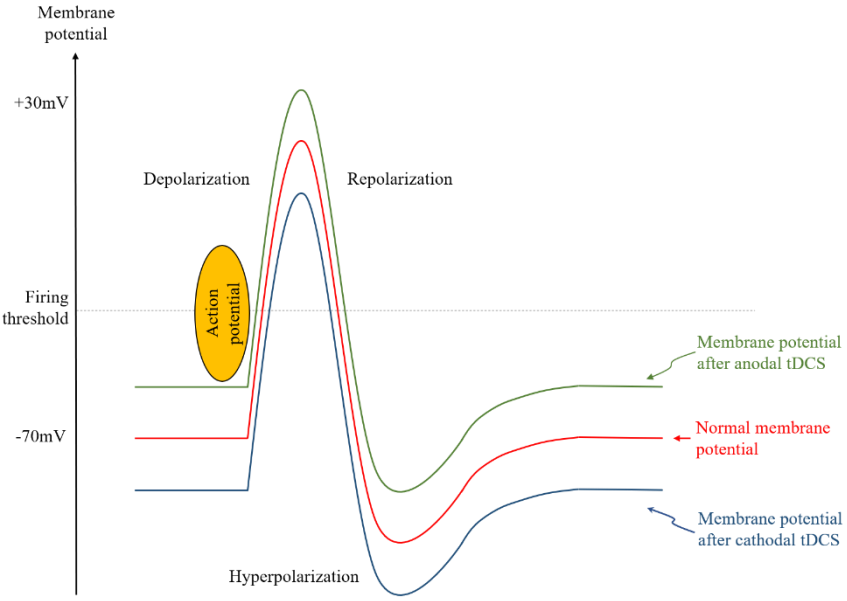


Figure 5. Effect of tDCS on resting membrane potential. Schematic diagram of changes in neuron membrane potentials by anodal and cathodal tDCS. mV, millivolts; tDCS, transcranial direct current stimulation.

2.6. ASSESSING THE ACUTE CENTRAL EFFECTS OF tDCS

The modulatory effect of the neuronal excitability is most frequently assessed by investigating the amplitude of TMS-elicited motor-evoked potentials (MEPs) recorded on the skin overlying the muscle of interest using surface electromyography (EMG) sensors[43,67,106,117–119]. Using this method, Gregoret et al. (2021) demonstrated that the M1 HD-tDCS configuration (utilized in *Study I* and *Study II*) increases excitability[120]. Another means of assessing the direct neuromodulatory effects of brain stimulation is recording electroencephalography (EEG)[98,109,121–123]. This has previously been done, investigating the effects of tDCS of the DLPFC[121] and the posterior parietal cortex[123,124]. These studies showed that tDCS could modulate the spontaneous oscillatory brain activity frontocentrally[121,123] and within frontoparietal networks[124]. Assessing the effects of tDCS on neural excitability was out of the scope of the present thesis.

2.7. PERSISTING EFFECTS OF tDCS

HD-tDCS can produce excitability changes within a short duration of stimulation (seconds), but to produce neuroplastic changes that persist, longer stimulation duration (several minutes) is necessary[37,125]. The persisting effects are similar to long-term potentiation (LTP) and long-term depression (LTD) plasticity and can extend to interconnected cortical and subcortical structures[41,44,74,126–128]. The duration of the after-effects appears to increase with repetition of tDCS sessions, with reported beneficial effects for up to 16 weeks after 10 consecutive sessions of tDCS (administered over two weeks)[129]. The recommended protocol to induce sustained effects is repeated stimulation sessions over at least three consecutive days[37,45].

The modulatory effect on neural activity by HD-tDCS has been investigated in regard to several functional properties, including modulation of working-memory function[98,130,131], attention[132–134] and motor learning[99,135]. Clinically, tDCS has been investigated in terms of its rehabilitation potential for pathologies such as Parkinson's disease[37,136,137], depression[138–140], anxiety disorders[141,142] and more[37]. These functional properties and pathologies all share core mechanisms in terms of being driven by neuroplastic changes in the CNS[37]. The biological mechanism underlying the persisting effects of tDCS is not fully uncovered, but several hypotheses have been proposed[49]. One theory is that calcium-dependent synaptic plasticity of glutamatergic neurons plays a key role in the persisting neuroplastic tDCS mechanism of action[37,143]. This was established as anodal tDCS of the cerebral cortex and hippocampus increases the intracellular Ca^{2+} concentration[39], and dextromethorphan, a NMDA receptor antagonist, can prevent the induction of after-effects of tDCS[144]. Dopaminergic receptors may also be involved in this mechanism, as pharmacological blockage of D2 receptors, which participate in NMDA-receptor-dependent neuroplasticity, inhibits the induction of tDCS after-effects[49,144,145].

Another hypothesis is that the after-effects of tDCS rely on the modification of intracortical neurotransmitter concentrations[39,49]. Using magnetic resonance spectroscopy, several studies have investigated the modulation of cortical neurotransmitters induced by tDCS[63,112,135,146]. These suggest that the local gamma-aminobutyric acid (GABA) concentration and glutamatergic neuronal activity can be modulated by tDCS[63,112,135]. These neurotransmitters are heavily involved in synaptic plasticity and LTP-and LTD-like changes in the neocortex and so may be driving the persisting effects of tDCS[49,112]. Other neurotransmitters involved in neuroplasticity, such as acetylcholine and serotonin, have also been suggested to be involved in the tDCS effects[39,147].

Almost all tissues and cells are sensitive to electric fields, so tDCS might elicit changes in non-neuronal tissues in the brain, such as glial cells, lymphocytes or various proteins[37,44,148]. For example, tDCS has been shown to modulate brain-derived

neurotrophic factor (BDNF), which is also highly involved in neuroplasticity[42]. Specifically, anodal tDCS can induce BDNF-mediated priming of synaptic plasticity, making synapses more susceptible to LTP induction in the hippocampus of rats[149].

Beyond local effects, network effects of tDCS have gotten increasing attention with the advent of HD-tDCS. Francis et al. (2003) demonstrated that neuronal networks are more sensitive to tDCS compared to single neurons[108]. This finding suggests that targeting functionally connected areas may be more meaningful than specific cortical areas and inspired the incorporation of a multi-target tDCS montage (DLPFC+M1-tDCS) in the three studies of the thesis.

2.8. SUMMARY AND DISCUSSION

Non-invasive electrical brain stimulation has been used for centuries but has in the last decades shown massive development and increase in use-cases. Advancement of the tDCS technology has allowed more flexibility in the design of the produced electrical field, with the use of arrays of stimulation electrodes (HD-tDCS). This has increased the focality and possibly the efficacy of the intervention. The intervention is safe and tolerable when administered correctly and no serious adverse effects were seen in any of the tested subjects in the three studies.

The acute neurophysiological mechanisms of tDCS are well established, although the persisting and functional effects are complex and less understood. There is no linear relationship between the intensity of the administered electrical stimulation and the functional outcome, which inhibits the predictability of a given intervention.

CHAPTER 3. CLINICAL RESEARCH OF HD-tDCS

Using non-invasive brain stimulation to rehabilitate pathologies rooted in the CNS has been a focus of researchers for decades[35,150]. Clinical studies of chronic pain patients have demonstrated that tDCS can provide analgesia in certain chronic pain conditions[15,16,37]. Primarily fibromyalgia, neuropathic pain, and spinal cord injury-related lower limb pain has -shown systematic positive effects[15,17,37,151–155]. A few studies and case reports have also demonstrated positive effects of tDCS in regard to migraine[156], refractory cancer pain[157], knee osteoarthritis[158,159], central post-stroke pain[160], vulvodynia[161] and more chronic pain conditions[37,162]. However, these positive findings have not been reliably replicated to reach evidence levels sufficient for clinical recommendation[37,163]. The reason that some conditions gain analgesic benefit while others do not have not been fully uncovered[15,48].

The tDCS configurations that have been used most in clinical studies are conventional anodal tDCS of M1 with a single cathode over the contralateral SO and anodal tDCS of the DLPFC with a single cathode over SO[84,163,164]. A few studies have used cathodal M1-tDCS[165–167] with anode over SO, and others have tried anodal tDCS of primary sensory cortex[168]; these montages appear to be less promising[16,164,169]. The heterogeneity in study designs, assessment methods, population samples and tDCS- protocols may have influenced the varying results in the tDCS literature[37].

The physiological mechanisms that mediate the analgesic effects of the positive tDCS-montages are unclear[45,48,170]. One theory is that the pain relief following anodal M1-tDCS is driven by a general inhibition of the neural processing of afferent sensory- and pain signals[48]. The inhibition may be driven by activation of endogenous opioid systems[47,171–173], motor-cortex-driven inhibition of the somatosensory cortex or modulation of pain-related thalamic activity[48]. Another theory is that the analgesic effect of tDCS is driven by enhancement or restoration of the endogenous inhibitory pain pathways[84,170], which are dysfunctional in many chronic pain conditions[45,84,170]. This may be a result of the tDCS producing widespread changes in networks involved in endogenous pain mechanisms, including subcortical structures such as thalamus, cingulate gyrus, periaqueductal grey, subnucleus reticularis dorsalis, among others[84,170]. The theories are not mutually exclusive but likely constitute parts of a more complex and integrative neurological mechanism.

The tDCS targeting the DLPFC has, on the other hand, been suggested to modulate emotional, affective or cognitive aspects of pain[48,170,174]. The DLPFC has a central role in pain processing[175]. The DLPFC is targeted both in the DLPFC-tDCS montage (*Study I*) and concomitantly with M1 in the DLPFC+M1-tDCS montage (*Study I &*

II). Targeting both sites simultaneously was hypothesized to improve the analgesic effect of the stimulation as more pain processing mechanisms would be modulated.

Additionally, M1 and DLPFC appear to be functionally connected, so multimodal HD-tDCS stimulating both sites simultaneously has shown increased modulatory effect, both in terms of corticospinal excitability and functional changes[88,95,176].

3.1. SHAM-STIMULATION

In experimental and clinical research, it is imperative to compare the effects of an intervention to a control condition to assess whether the observed effects can be contributed to the experimental condition or are a result of confounding factors[177]. For example, the expectancy of treatment and the caring attention of healthcare professionals can provide positive clinical effects in various conditions[178]. Implementing a control condition in pharmaceutical trials is fairly simple, as placebo medication (ingested or injected) can be produced indistinguishable from active medication[179]. However, in brain stimulation studies, the placebo model needs to mimic the sensory quality of real stimulation in order to be convincing. Due to the mild and transient sensory experience produced by active tDCS, the placebo model, named sham-tDCS, ramps up to the target stimulation intensity over a short duration (30 s) and then turns off completely to not produce neuromodulatory effects[102]. This model was first used by Gandiga (2006)[102] and has since been validated in conventional tDCS[180,181] and HD-tDCS[104,182]. However, some issues regarding the sham-tDCS model have been raised when the subjects have prior experience with the sensation of active tDCS[183–185]. As a result, tDCS naïve subjects in parallel group designs may be better suited for experimental studies than cross-over designs.

In *Study I* and II, the sham-tDCS configuration had the same electrode montage as the active configuration (DLPFC+M1 tDCS-montage, montage C in Fig. 2) and ramped up the current over 30 s but then automatically turned off for 19 minutes before it turned on again and ramped down over 30 s at the end of the stimulation. The sham-stimulation was preconfigured and blind to the experimenter. Unknown to the participants, they received the same stimulation protocol on all three days. It was explained that the sham-stimulation was designed to have no effects but would be indistinguishable from the active stimulation. The Sham-tDCS blinding efficacy was assessed through a questionnaire after each session, asking whether the participants believed they had received real or sham stimulation (Sham-trust index explained in detail in Paper 1)[186]. In *Study II*, a follow-up question was added in which the subjects could rate the certainty of their response from 0 (not sure at all) to 10 (completely sure). The sham-tDCS successfully blinded the participants in both studies, with no significant difference in the response rates between the groups receiving sham-tDCS and active-tDCS (Table 2.). Overall, subjects were more prone to believing they received active-tDCS than sham-tDCS, resulting in higher accuracy rates in the groups that received active-tDCS (Table 2).

Study	tDCS Group	N	Sham-trust index (0-100%)	Certainty (0-10)	Accuracy (0-100%)
Study 1	Sham	20	33%		33%
	DLPFC	21	30%		70%
	M1	20	22%		78%
	DLPFC+M1	20	27%		73%
Study II	Pain Sham	20	37%	4.3	37%
	Pain DLPFC+M1	20	48%	4.1	52%

Table 2. Average sham-trust-index, Certainty and Accuracy across days for each group in Study I and Study II. Sham-trust-index (0-100%) describes the percentage of sessions at which the subjects estimated that they had received sham-tDCS. Certainty (0-10) describes their confidence in their estimation (0, not certain at all; 10, completely certain). Accuracy (0-100%) describes the percentage of sessions the subjects estimated correctly, whether they received active or sham-tDCS.

3.2. ASSESSING THE FUNCTIONAL EFFECT OF HD-tDCS

The present thesis aims to provide greater insight into the effects of active HD-tDCS compared to sham-tDCS by assessing tDCS-induced changes in general somatosensory function (*Paper I & II*) and in endogenous central pain mechanisms (*Paper III*).

The majority of research on the analgesic effect of HD-tDCS has been investigated in chronic pain populations[162]. However, conducting research on heterogeneous patient groups with varying quality, intensity, duration, and aetiology of their pain conditions may be detrimental to the scientific outcome. At first glance, investigating the analgesic effect of a treatment without the presence of pain may appear contradictory, but the analgesic potential of an intervention can be inferred by assessing the effects on broader somatosensory function[187,188]. As a result, it was hypothesised that the modulatory effect of HD-tDCS on the somatosensory system would be reflected functionally through increased pain-and sensory thresholds in a healthy population. An issue with this approach may be that the state of the subject's CNS may influence the response to the HD-tDCS, so healthy subjects respond differently than chronic pain patients that have an altered nociceptive system[21–25]. To explore this issue experimentally, the healthy subjects of *Study II* were administered an experimental prolonged

pain model aimed to induce persistent muscle soreness and provoke perturbation of their CNS[189,190].

The effects of the HD-tDCS were investigated methodologically identically between the populations. An outline of the experimental components for *Study I* and *Study II* is illustrated in Figure 6.

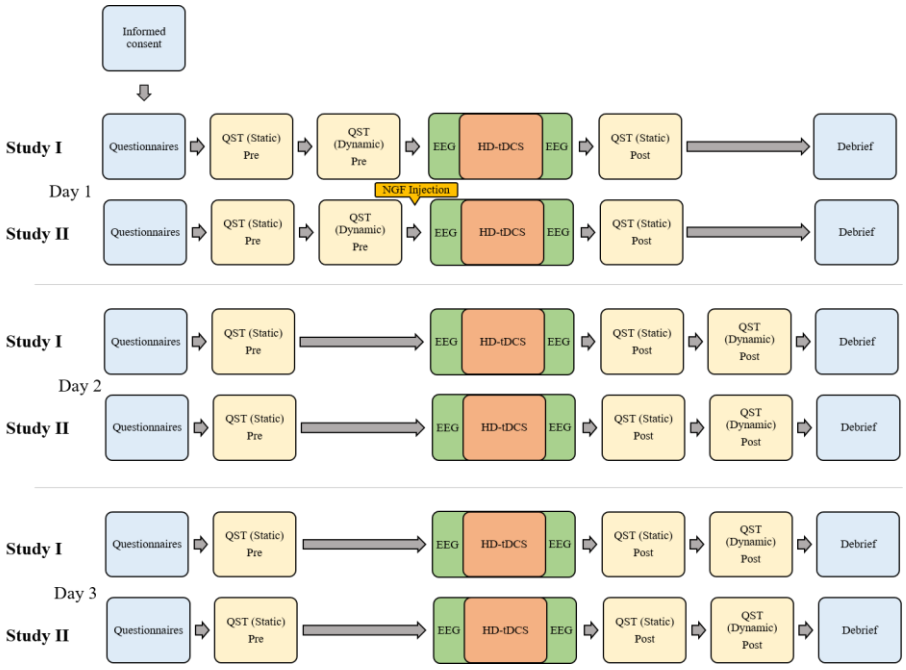


Figure 6. Experimental components of Study I and Study II. The model describes the experimental components of Day1, Day2 and Day3 for both Study I and Study II. The questionnaires consisted of demographic information and a safety screening tool for non-invasive brain stimulation. Quantitative sensory testing (QST) was conducted before and after the electroencephalography (EEG) and high-definition transcranial direct current stimulation (HD-tDCS) procedures. The assessments of the static QST are described in Figure 7. The assessments of the dynamic QST are described in Figure 13. In the debrief, participants were questioned about adverse effects and were asked whether they believed they had received active or sham-tDCS.

3.1. SUMMARY AND DISCUSSION

tDCS have shown analgesic potential in various chronic pain populations. Especially M1 and DLPFC appear to be promising targets for tDCS due to their involvement in pain processing, however the underlying physiological mechanisms remain unclear.

Studies of the analgesic effects have primarily been conducted on chronic pain patients, but heterogeneity in pathology may be detrimental to the interpretation of these. Therefore, more basic research with systematic methodology is needed.

The importance of utilizing effective placebo controls (sham-stimulation) has been underlined, as expectancy of treatment outcome is a powerful mechanism. The sham-tDCS configurations used in *Study I & II* successfully blinded the subjects to the type of tDCS they were administered. However, across groups, there was a tendency to estimate that active-tDCS were administered more frequently than sham-tDCS, resulting in the groups that received active-tDCS having higher accuracy than the groups receiving sham-tDCS.

In the following chapters, the assessment methodology and subsequent results of the modulatory effects of the HD-tDCS on the somatosensory system are presented. The results are presented in the order that the experimental studies were conducted; first, the results of healthy pain-free subjects in *Study I* (N=80) and *Study III* (N=40 pain-free) and secondly from the subjects administered the experimental persistent pain model in *Study II* (N=40) and *Study III* (N=40 with induced experimental pain).

CHAPTER 4. MODULATING THE SOMATOSENSORY SYSTEM OF HEALTHY PAIN-FREE SUBJECTS USING HD-tDCS

4.1. MODULATING SOMATOSENSORY THRESHOLDS IN HEALTHY PAIN-FREE SUBJECTS USING HD-tDCS

It was hypothesised that the effects of the administered HD-tDCS would be reflected through changes in pain-and sensory thresholds in a healthy population. To assess changes in pain-and sensory thresholds, a comprehensive battery of static quantitative sensory testing (QST) was utilized[191]. This test battery is designed by the German Research Network on Neuropathic Pain (DFNS)[192] and is one of the most frequently used clinical tools to assess somatosensory function in humans[21,187,193]. QST comprises a group of procedures that assess the characteristics of different nociceptive and non-nociceptive modalities, some of which are subserved by different groups of afferent nerve fibres and central pathways[192,194]. The assessments are conducted by estimating the perceptual responses to systematically applied and quantifiable sensory stimuli[187]. QST can provide an understanding of aspects related to pain transduction, transmission, and perception, with information of the entire neural axis regarding large myelinated A-beta, thinly myelinated A-delta, and small unmyelinated C fibre function and their corresponding central pathways[21,187]. In *Study I*, sensory-and pain thresholds of thermal and mechanical modalities were investigated in healthy, pain-free subjects[186]. The sensory testing conducted in *Study I* is illustrated in Fig. 7.

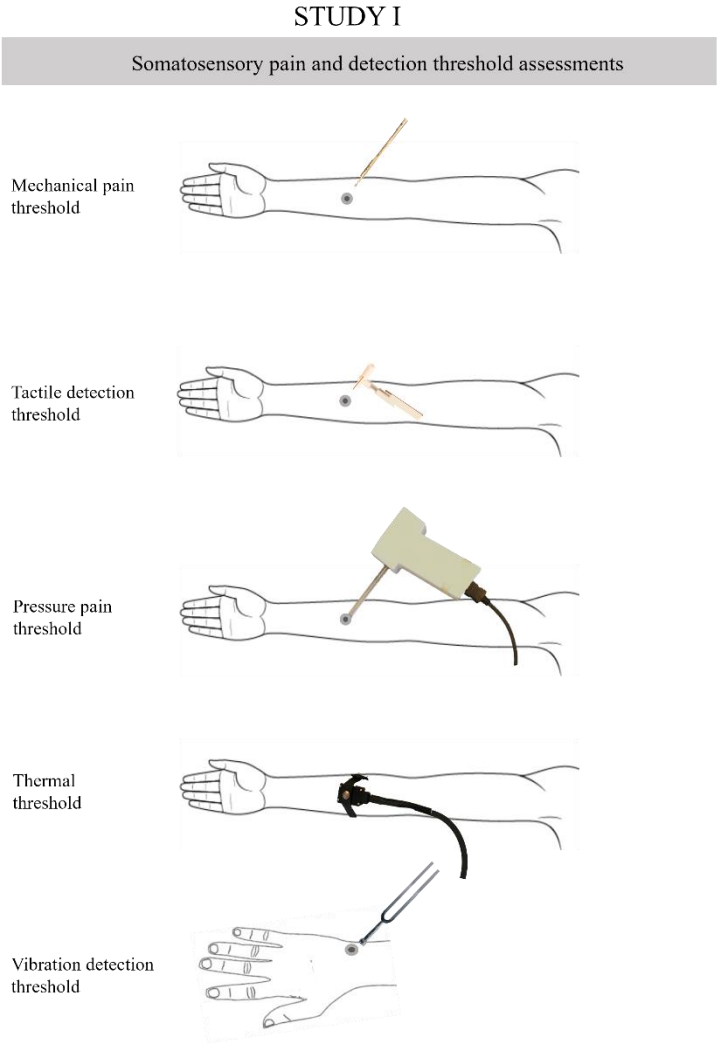


Figure 7. Static quantitative sensory testing protocols of Study I.

The mechanical pain thresholds, tactile detection thresholds, pressure pain thresholds and thermal thresholds were determined on the skin above the flexor carpi radialis muscle of the right arm. The vibration detection thresholds were assessed over the prominence of the distal part of the ulna in the right arm.

Mechanical pain thresholds (mN) were assessed using PinPrick (MRC Systems GmbH, Heidelberg, Germany). The tactile detection thresholds (g) were determined using a set of Von Frey filaments (Touch Test Sensory Evaluators, North Coast Medical Inc, Morgan Hill, CA). The pressure pain thresholds (kPa) were determined using

a hand-held pressure algometer (Somedic, Hörby, Sweden) with a 1-cm^2 probe. The thermal pain and detection thresholds (°C) were determined using the PATHWAY – Pain & Sensory Evaluation System with a 3 x 3-cm (9-cm^2) contact thermode (Medoc Advanced Medical Systems, Ramat Yishay, Israel). The vibration detection thresholds were determined with a Rydel–Seiffer tuning fork (64 Hz, 8/8 scale) (Uniplex, Sheffield, United Kingdom). Detailed descriptions of the static QST procedures are presented in *Paper I*[186].

4.1.1. PRESSURE PAIN THRESHOLD

The PPT represents the threshold at which the subject identifies the pressure stimulation as painful. A PPT assessment with a 1 cm^2 contact surface preferentially activates deep afferents in terms of A-delta-and C fibres[187,195]. The assessment method has shown excellent reliability coefficients and decent test-retest reliability[187,196,197]. Assessing pressure sensitivity has clinical value due to chronic pain patients having lower PPTs than healthy controls, which can be attributed to hyperalgesia of deep tissue[187,198]. The PPTs of the four groups assessed in *Study I* are presented in Fig. 8.

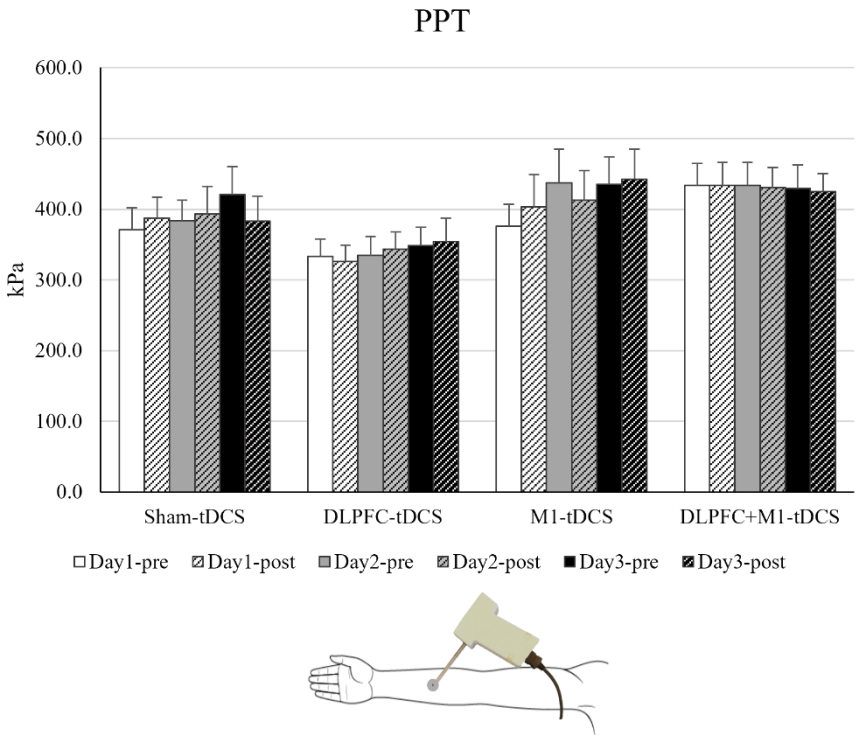


Figure 8. Mean (+SEM) pressure pain thresholds. Pressure pain threshold (PPT), over 3 days before (nonshaded) and after (shaded) HD-tDCS, for the four groups in Study I. tDCS, Transcranial direct current stimulation; SEM, standard error of the mean.

In *Study I*, a significant time effect was seen unrelated to the groups, which showed that the PPTs generally increased from Day1-pre to Day3-pre. This could indicate that habituation to the assessment occurred over the repeated sessions. Habituation to PPT testing following repeated assessments has been reported before and may be a result of the novelty and salience of the stimuli decreasing when repeated[199–201]. Alternatively, the decreased pressure sensitivity seen across groups could be a result of a placebo-effect, as the decreased pressure sensitivity was also seen in the Sham-tDCS group. Interventions targeting psychophysical properties have a high risk of being influenced by cognitive and affective components, facilitating the placebo effect[177,178]. A number of previous studies have demonstrated similar placebo effects of sham-protocols of non-invasive brain stimulation[172,177,202–204].

In *Study I*, there were no significant differences between groups in the PPTs over the course of the six assessments (estimated with a two-way mixed model ANOVA[186]).

This indicated that the active HD-tDCS protocols targeting M1, DLPFC or DLPFC+M1 simultaneously were not significantly more effective than sham-stimulation in modulating the pressure pain sensitivity.

PPT is one of the most frequently used assessments of somatosensory function in the tDCS literature; however, the findings of the studies are often conflicting[84,162]. Previous studies have reported anodal tDCS-driven inhibition of pressure sensitivity in healthy subjects, which contradicts the PPT findings of *Study I*[100,205]. The conflicting findings may be driven by differences in study design and methodology. Reidler et al. (2012)[205] administered conventional M1-tDCS with a single cathode over the contralateral supraorbital area, while Flood et al. (2016)[100] utilized a HD-tDCS montage similar to the M1-tDCS montage in *Study I*, but repeated the intervention on seven days instead of three. Conventional and HD-tDCS may differ in efficacy, despite sharing mechanisms[78,93]. Additionally, these studies were cross-over designs, with a one-week break between the Sham-tDCS and Active-tDCS sessions, while *Study I* was a parallel-group design. Cross-over studies generally have higher power due to the lower inter-subject variability[206] but may also have problems with blinding[207]. Although the number of participants in *Study I* was predetermined by sample size calculations, insufficient statistical power may drive the negative findings of *Study I*.

Several studies have reported PPT findings in line with *Study I* in healthy subjects[201,208,209]. While Jürgens et al. and Lerma-Lara utilized the conventional M1-tDCS montage[209], both Wan et al.[210] and Jiang et al.[208] utilized HD-tDCS montages identical to the M1-tDCS montage of *Study I*. The overall conflicting findings make it difficult to draw conclusions on the modulatory efficacy of HD-tDCS on PPTs in healthy subjects. However, the most recent studies[186,201,208,211] all point towards HD-tDCS not being significantly more efficient than Sham-tDCS at modulating pressure sensitivity.

4.1.2. MECHANICAL PAIN THRESHOLD

Mechanical pain thresholds (MPT) were assessed with PinPrick (Fig. 7). MPT quantify the function of cutaneous mechanical nociceptors[194]. The pinprick predominantly activates intraepidermal nerve endings in the thin epidermis, as the needle is small and light enough not to cause deformations of deeper tissue[195]. The epidermis is dense with free nerve endings of A-delta- and C-fibre nociceptors that are sensitive to very low forces and propagate the afferent nociceptive signals of pinprick[187,195,212]. Pinprick has generally been used clinically as a surrogate marker of functional integrity of somatosensory pathways[213–217]. The MPTs of the four groups assessed in *Study I* are presented in Fig. 9.

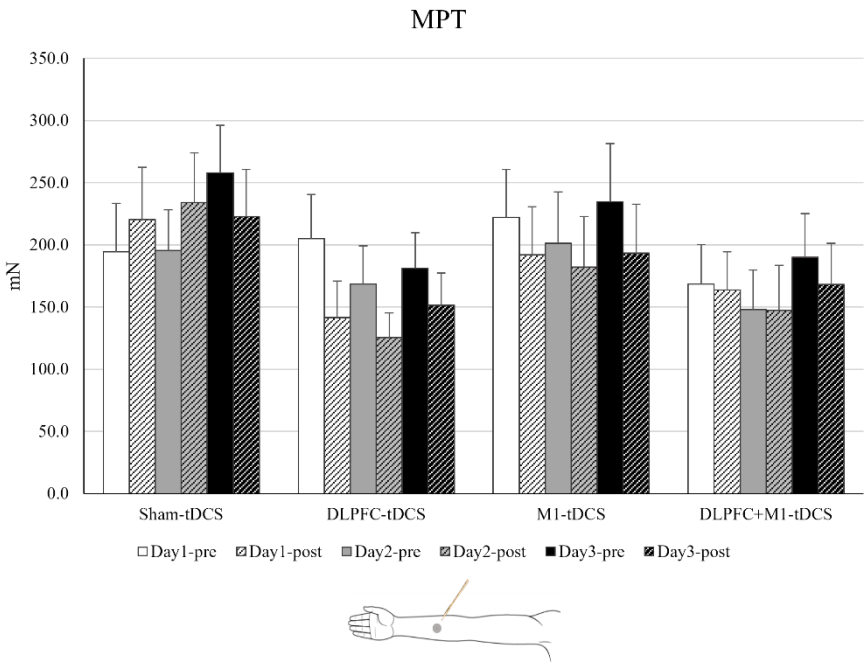


Figure 9. Mean (±SEM) mechanical pain threshold. Mechanical pain threshold (MPT), over 3 days before (nonshaded) and after (shaded) HD-tDCS, for the four groups in Study I. The MPT represent the threshold at which the participants identified the pressure and mechanical stimulation as painful. tDCS, Transcranial direct current stimulation; SEM, standard error of the mean.

Like the results of PPTs, the MPTs in *Study I* showed a significant time effect unrelated to the groups. This time effect can also be attributed to habituation to the assessment over time or possibly a placebo effect. There were no significant differences in the development of MPTs over the course of the six assessments between the Active-tDCS groups and the Sham-tDCS group, indicating that the HD-tDCS were not significantly more effective than sham stimulation in modulating mechanical pain sensitivity. tDCS modulation of MPTs in healthy subjects has been investigated in few other experimental studies. Bachmann et al. (2010) demonstrated positive tDCS effects on the MPT[218]. In this study, a single session of 15 min, 1 mA cathodal M1-tDCS with reference electrode over the right orbit significantly increased MPTs compared to both Sham-tDCS and anodal M1-tDCS. This protocol differed considerably from the HD-tDCS protocols of *Study I*, and may explain the conflicting results[218]. Other studies have demonstrated null-findings of the tDCS modulation of MPT, in line with the present findings[86,209]. Both Jürgens et al. (2012) (single session of 15 min, 2 mA conventional anodal M1-tDCS)[209] and Borckardt et al. (2012) (single session of 20 min, 2 mA HD-tDCS of M1)[86] demonstrated no significant difference in

the modulation of MPTs compared to Sham-tDCS. These studies utilized tDCS protocols in line with the M1-tDCS protocol of *Study I* and demonstrated similar non-significant effects.

4.1.3. TACTILE AND VIBRATION DETECTION THRESHOLD

Tactile detection thresholds (TDT) were assessed with Von Frey filaments (Fig. 7), and vibration detection thresholds (VDT) were assessed with a 64 Hz tuning fork (Fig. 7). Assessments of TDT and VDT differ from the PPT and MPT modalities by not assessing pain, but rather quantifying the perception thresholds for low-intensity mechanical stimuli[192]. The TDT and VDT are primarily mediated by A-beta fibres and together can provide a profile of large fibre function[192,219]. TDTs were assessed in both *Study I* and *Study II*, while VDTs were only assessed in *Study I*, as the intramuscular pain model used in *Study II* was presumed to not modulate this modality. The TDTs and VDTs of the four groups assessed in *Study I* are presented in Fig. 10 and 11 respectively.

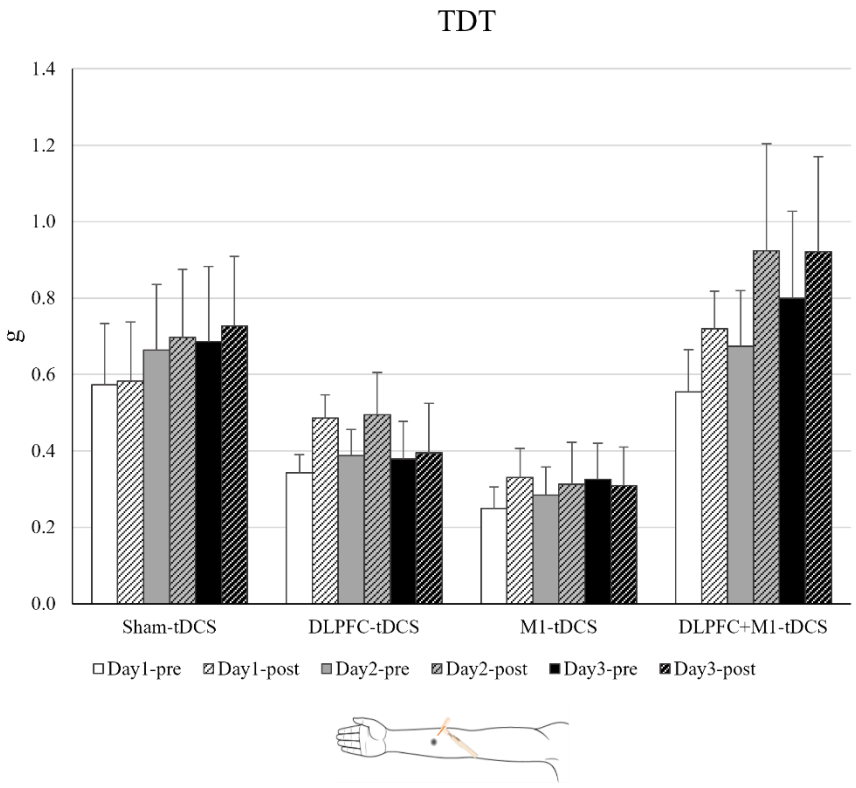


Figure 10. Mean (±SEM) tactile detection threshold. Tactile detection threshold (g) *(TDT), over 3 days before (nonshaded) and after (shaded) HD-tDCS, for the four groups in Study I. TDT represents the pressure stimulus intensity needed for the participants to detect the touch of the Von Frey filament. tDCS, Transcranial direct current stimulation; SEM, standard error of the mean.*

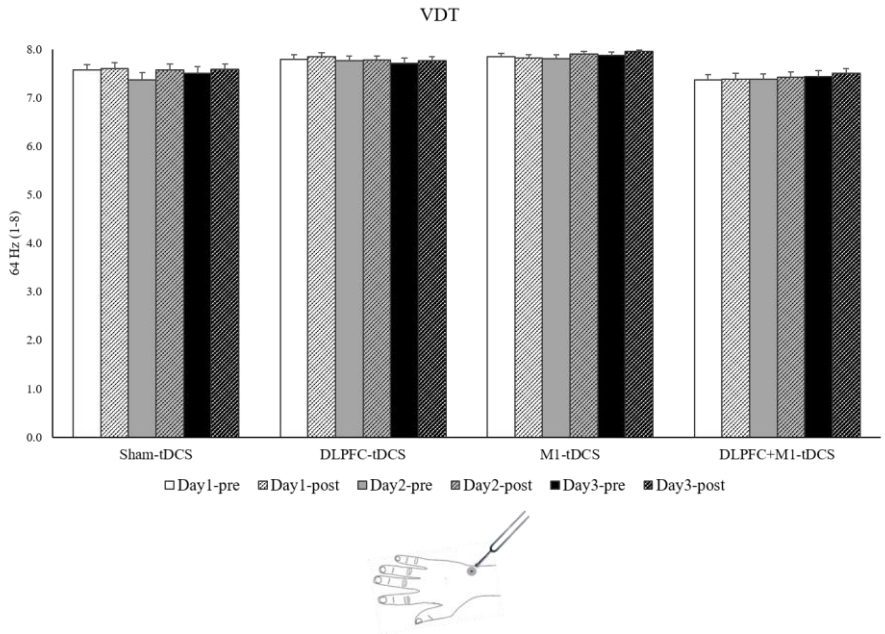
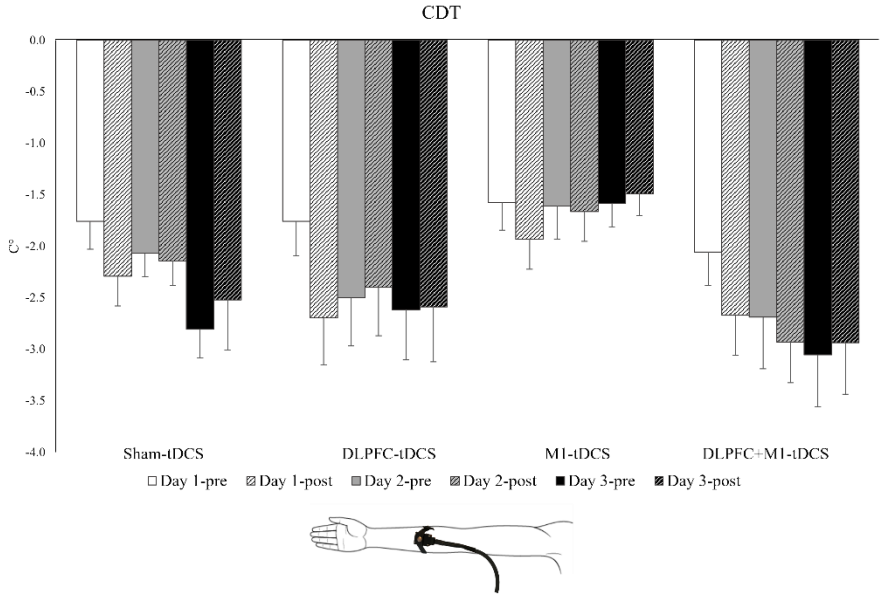


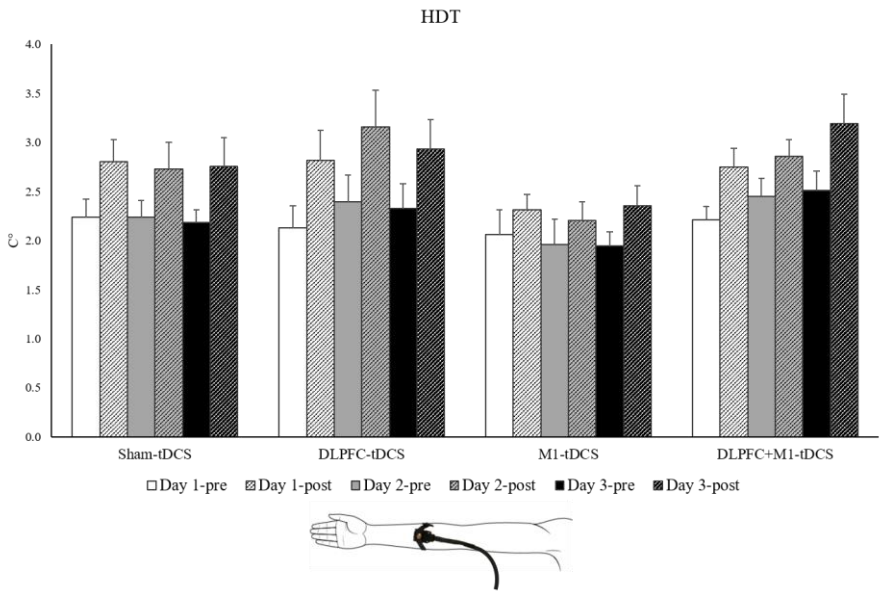
Figure 11. Mean (±SEM) vibration detection threshold. Vibration detection threshold (1-8) (VDT), over 3 days before (nonshaded) and after (shaded) HD-tDCS, for the four groups in Study I. Vibration detection threshold represents the amplitude of vibration of a tuning fork at which the participants could no longer detect the vibration. tDCS, Transcranial direct current stimulation; SEM, standard error of the mean.

In *Study I* unrelated to the HD-tDCS type, the TDTs increased over time like the MPTs and PPTs. This reflects an attenuated tactile sensitivity and may be attributed to habituation to mechanical stimuli or possibly a placebo effect, as previously described in section 4.1.1. There were no significant differences between the groups receiving active HD-tDCS and the sham-tDCS group in the development of the TDT or VDT over the course of the six assessments. This indicates the HD-tDCS were not significantly more effective than sham stimulation in modulating the tactile and vibration detection sensitivity. Previous studies have assessed modulation of TDT and VDT using tDCS. Jürgens et al. (2012)[209] demonstrated results in line with *Study I*, with no significant differences in TDT or VDT (single session 20 min, 2 mA M1-tDCS). Bachmann et al. (2010)[218] similarly identified no differences in the VDT between the Active-tDCS groups, receiving either anodal or cathodal M1-tDCS (15 min, 1 mA) and the Sham-tDCS group. However, Bachmann et al. did demonstrate increased TDTs in their study in the group that received cathodal M1-tDCS[218]. The same study demonstrated increased MPTs, which may indicate that a general decrease in mechanical sensitivity was induced, contrary to the present study. As suggested in section 4.1.2, the conflicting findings may be driven by the differences in utilized tDCS protocols.

4.1.4. THERMAL DETECTION AND PAIN THRESHOLDS

Thermal thresholds were assessed using the MEDOC PATHWAY – Pain & Sensory Evaluation System (Fig. 7). Thermal assessments provide information about the function of small diameter unmyelinated C-nerve fibres and thinly myelinated A-delta nerve fibres[187]. In the healthy nervous system, thermal stimuli can lead to the formation of highly acute perceptions, with the threshold for detecting temperature changes by the human hand being <0.5 °C[220]. The thermal assessments include cold detection thresholds (CDT), heat detection thresholds (HDT), cold pain thresholds (CPT) and heat pain thresholds (HPT), as each test probes different aspects of thermal perception function[194,219]. Clinically thermal sensory testing is used to identify small fibre nerve damage, which can manifest as thermal hypesthesia (raised perception thresholds) or hyperalgesia (lowered pain thresholds)[221]. The reliability of thermal quantitative sensory testing is generally on par with mechanical sensory testing[197,222,223], although large variances in CPT are often reported[187,224,225]. Thermal threshold testing was only conducted in *Study I*, as the utilized pain model of *Study II* was presumed to not affect the thermal modalities due to their superficial cutaneous histology[220].


CDT
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
C°
Sham-tDCS
DLPFC-tDCS
M1-tDCS
DLPFC+M1-tDCS
Day 1-pre
Day 1-post
Day 2-pre
Day 2-post
Day 3-pre
Day 3-post


HDT
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
C°
Sham-tDCS
DLPFC-tDCS
M1-tDCS
DLPFC+M1-tDCS
Day 1-pre
Day 1-post
Day 2-pre
Day 2-post
Day 3-pre
Day 3-post

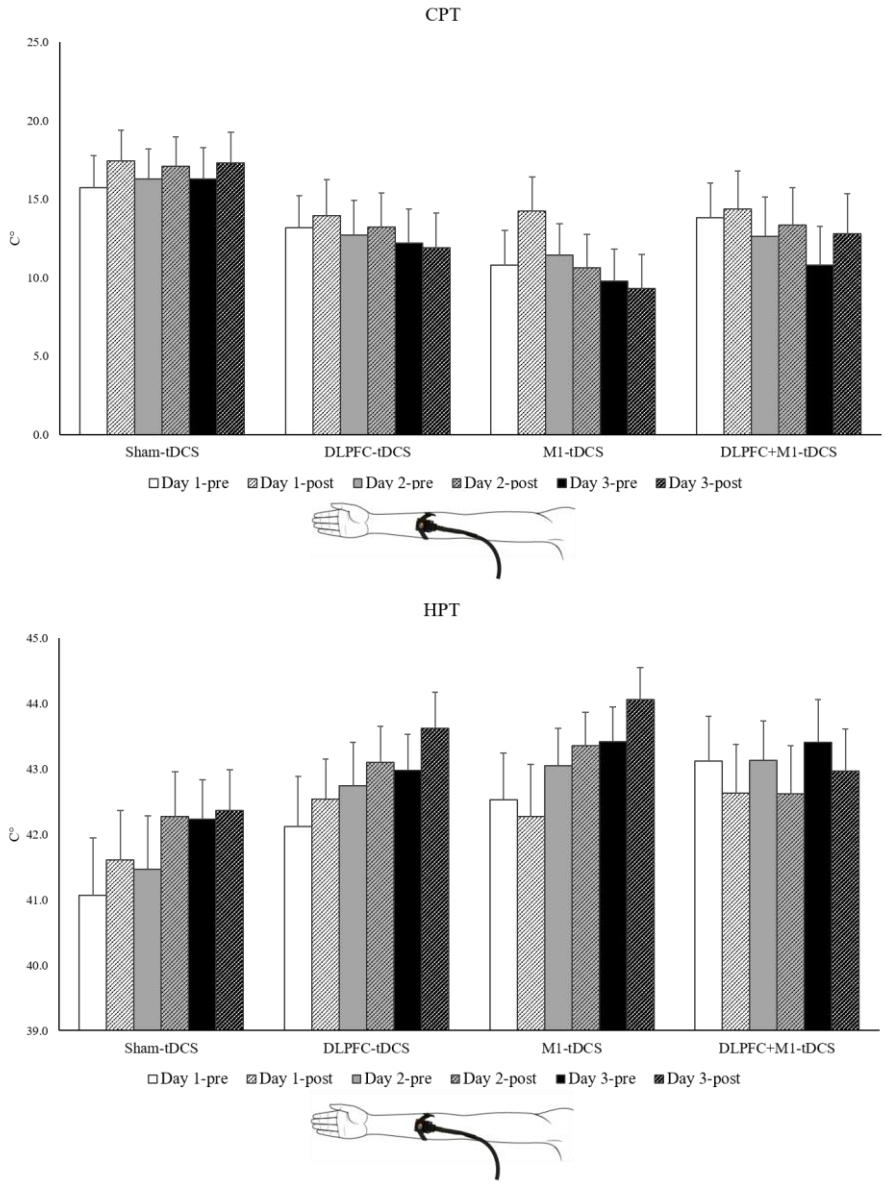


Figure 12. Mean (±SEM) cold detection threshold (CDT), heat detection threshold (HDT), cold pain threshold (CPT), and heat pain threshold (HPT) over 3 days before (nonshaded) and after (shaded) HD-tDCS of the four groups in Study I. CDT and HDT represent the temperature change required for the participants to notice an increase or decrease in temperature from the baseline (32 C°). CPT and HPT represent the temperature at which the participants identified the temperature (C°) as painful. tDCS, transcranial direct current stimulation; SEM, standard error of the mean.

In *Study I*, unrelated to the HD-tDCS type, the thermal sensitivity decreased over time (CDT, HDT, CPT and HPT), similar to what was demonstrated in the mechanical modalities (Section 4.1.1, 4.1.2). This reflects an attenuated thermal sensitivity and may be attributed to habituation to thermal stimuli or a placebo effect, as previously described in section 4.1.2. The analysis revealed that there were no significant differences between the groups receiving active HD-tDCS and the sham-tDCS group in the development of the thermal thresholds over the course of the six assessments. This indicates the HD-tDCS were not significantly more effective than sham stimulation at modulating the thermal detection and pain sensitivity. Modulation of thermal thresholds with tDCS has been a focus of several experimental and clinical studies[84,162]. Positive effects with thermal hypesthesia following a tDCS-intervention in healthy subjects have been demonstrated by Zandieh et al. (2013)[226] and partially by Grundmann et al. (2011)[168] and Borckardt et al. (2012)[86]. Zandieh et al. (2013) demonstrated increased detection and pain thresholds to cold stimuli in the group that received M1-tDCS (15 min. 2 mA conventional anodal tDCS) compared to the sham tDCS group, indicating thermal hypesthesia[226]. The findings of Grundmann et al. (2011) and Borckardt et al. (2012) are more complex to interpret. Grundmann et al. demonstrated increased CDT and HDT following tDCS of the primary sensory cortex (15 min. 1 mA cathodal tDCS with reference electrode over the right orbit) but saw no significant differences in the CPT and HPT compared to the sham tDCS group[168]. Additionally, the study demonstrated no significant modulation of the thermal thresholds following anodal tDCS of S1[168]. Similarly, Borckardt et al. demonstrated positive findings of increased CDT, HDT and CPT but no significant modulation of HPT following HD-tDCS of M1 (20 min. 2 mA, concentric ring configuration)[86]. These findings conflict with *Study I*, indicating that modulation of thermal thresholds may be possible with different tDCS configurations but that the individual thermal modalities may respond differently.

Despite these positive findings, most recent studies of the modulatory effect of tDCS on thermal thresholds are in line with the finding of *Study I*. Jürgens et al. (2012), assessing CDT, HDT, CPT and HPT[209], Ihle et al. (2014) assessing HPT[227], Braulio et al. (2018) assessing CPT[228], Wan et al. (2021) assessing CPT[201] and Jiang et al. (2022) assessing CPT[208] all demonstrated no significant modulation of the respective thermal thresholds following tDCS-interventions. All of these studies administered anodal tDCS to M1 (2 mA), although Jürgens et al., Ihle et al. and Braulio et al. utilized conventional tDCS montages, while Wan et al. and Jiang et al. utilized HD-tDCS montages[201,208,209,227,228]. These studies demonstrating non-significant results strengthen the reliability of the results of *Study I*.

4.2. MODULATING CENTRAL PAIN MECHANISMS IN HEALTHY, PAIN-FREE SUBJECTS USING HD-tDCS

In *Study III*, the hypothesis that HD-tDCS may produce its analgesic effects through modulation of endogenous pain modulation mechanisms was investigated[15,47,173,229]. Nociceptive signals are subject to extensive processing by facilitatory and inhibitory mechanisms from the moment they reach the dorsal horn[230,231]. The processing is commonly referred to as endogenous pain modulation[231]. Dysfunctional endogenous pain modulation is a crucial part of the pathophysiology of several chronic pain conditions and may be a central component in the transition from acute to chronic pain[18,21,230–235]. The increased pain sensitivity and hyperalgesia patients experience might be due to an imbalance between descending inhibitory mechanisms, i.e. conditioned pain modulation (CPM) and facilitatory mechanisms of pain, i.e. temporal summation of pain (TSP), which can be tested experimentally[234]. Evaluation of the pain modulatory systems is termed dynamic QST[84,222,236]. In *Study I*, the dynamic QST measures, TSP and CPM, were recorded in succession of the static QST (Fig. 6). The dynamic QST results of the healthy subjects without pain (*Study I*) who were administered either DLPFC+M1-tDCS or Sham-tDCS were compared in *Paper III*[237]. In the following sections, an overview of the dynamic QST assessments and results are presented.

The experimental pain necessary to assess the endogenous pain modulatory mechanisms can be induced in various ways, i.e. thermal pain (contact or water immersion), pressure pain (handheld pressure algometry), cutaneous mechanical pain (pinprick) etc.[21,25,222,234,236,238]. In the present work, the dynamic QST was assessed with user-independent cuff pressure algometry, which has shown excellent reliability[27,238–242]. An illustration of the cuff pressure algometry setup is shown in Fig. 13.

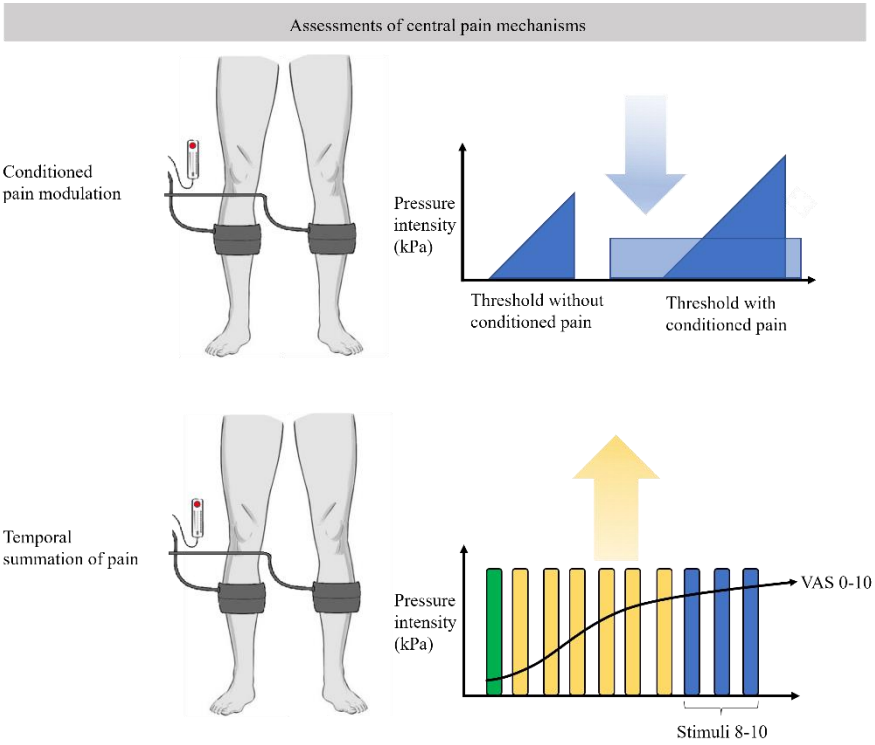


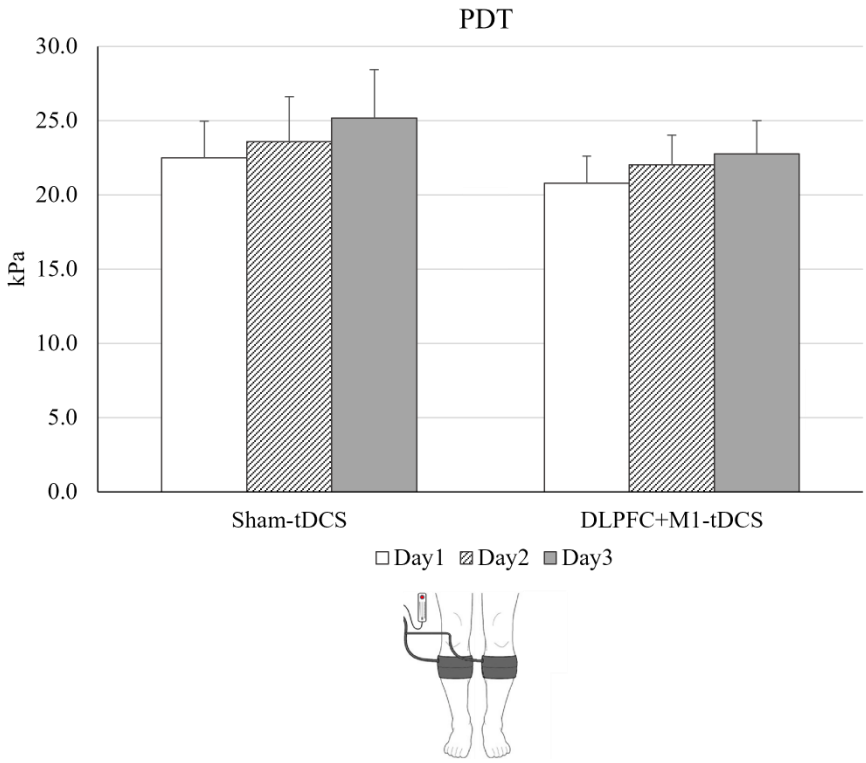
Figure 13. Dynamic quantitative sensory testing protocols. Conditioned pain modulation (CPM) and Temporal summation of pain (TSP) were assessed with a computer-controlled cuff pressure algometer.

A computer-controlled cuff pressure algometer (Nocitech, Aalborg, Denmark) with a 13 cm wide inflatable tourniquet cuff (VBM, Germany) was used to assess cuff-pressure pain detection thresholds (PDT), cuff-pressure pain tolerance thresholds (PTT) on both legs, as well as CPM and TSP on the right leg. The cuffs were placed 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. Subjects were instructed to rate the pressure-induced pain using a hand-held electronic 10 cm visual analogue scale (VAS, 0 cm meaning 'no pain' and 10 cm meaning 'worst pain imaginable')[237].

The cuff-pressure intensities used in the TSP and CPM assessments are established beforehand by assessing PDT and PTT. PDT was defined as the cuff pressure in the first instance where the VAS exceeded 1 cm[240]. The PTT was defined as the maximum pressure pain the subject could tolerate. Detailed descriptions of the cuff-pressure algometry assessments are presented in *Paper III*[237]. To explore the modulatory effects of both the HD-tDCS and the pain model on cuff-pressure sensitivity in the legs, PDT and PTT were included as secondary outcomes in *Study III*.

4.3. CUFF-PRESSURE PAIN DETECTION AND TOLERANCE

The HD-tDCS was hypothesized to attenuate the cuff-pressure sensitivity due to the analgesic effects M1-tDCS has previously been shown to produce in other pain modalities (i.e. thermal, electrical, mechanical)[84,243]. The cuff-induced pressure pain detection-and tolerance thresholds of the Sham-tDCS group and the DLPFC+M1-tDCS group are shown in Fig. 14.



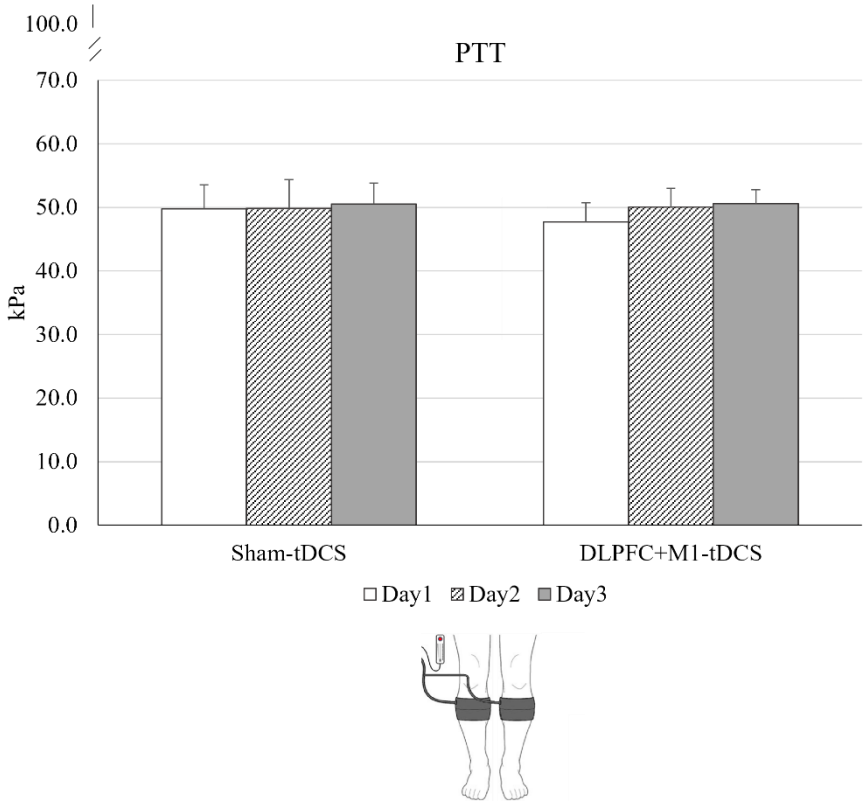


Figure 14. Mean (±SEM) cuff-pressure pain detection threshold (PDT) and cuff-pressure pain tolerance threshold (PTT). Data was recorded on three consecutive days, Day1 (before HD-tDCS), Day2 and Day3 (after HD-tDCS), in two groups from Study I (Sham-tDCS and DLPFC+M1-tDCS). tDCS, Transcranial direct current stimulation; DLPFC, dorsolateral pre-frontal cortex; M1, primary motor cortex; SEM, standard error of the mean.

There were no significant differences between the two groups in the PDT and PTT on any of the three days. This indicates that the active HD-tDCS did not significantly modulate the leg pressure sensitivity compared to Sham-tDCS. No previous studies have assessed the effects of tDCS on cuff-pressure sensitivity, however the potential pain modulating properties of tDCS are suggested to be non-specific to the modality of experimental pain[84]. Therefore the negative findings of the modulatory effect of the HD-tDCS can be considered conflicting with the body of studies that have reported positive effects of tDCS on pain thresholds[84,243] (section 4.1). However, the findings are in line with the results of the static QST in *Study I* (section 4.1), which similarly showed little-to no HD-tDCS modulation of somatosensory thresholds in healthy, pain-free subjects.

4.3.1. TEMPORAL SUMMATION OF PAIN

The up-stream mechanism of TSP refers to the progressive increase in neuronal output during a train of identical afferent nociceptive stimuli[21,27]. Facilitation of temporal summation is a result of endogenous upregulation of the central integrative mechanism and is considered a measure of increased central gain of pain[21,234]. Facilitated TSP has been demonstrated in various pain conditions, including osteoarthritis[27,244,245], post-operative pain[26,246], fibromyalgia[247], neuropathic pain[248] and more[21,24,249,250]. TSP is assessed by applying ten short-lasting stimuli at PTT level with 0.5 Hz frequency[234,240]. The participants continuously rated the cuff-induced pain using the electronic VAS (Fig. 13). For each cuff stimulus, a VAS score was extracted, and the TSP effect was defined as the average VAS score from stimuli 8-10 (blue bars in Fig. 13) subtracted from the first stimulus (green bar in Fig. 13)[24,237,240,251]. It was hypothesized that the active HD-tDCS would reduce the TSP effect[237]. The TSP effect results of the Sham-tDCS group and the DLPFC+M1-tDCS group are shown in Fig. 15.

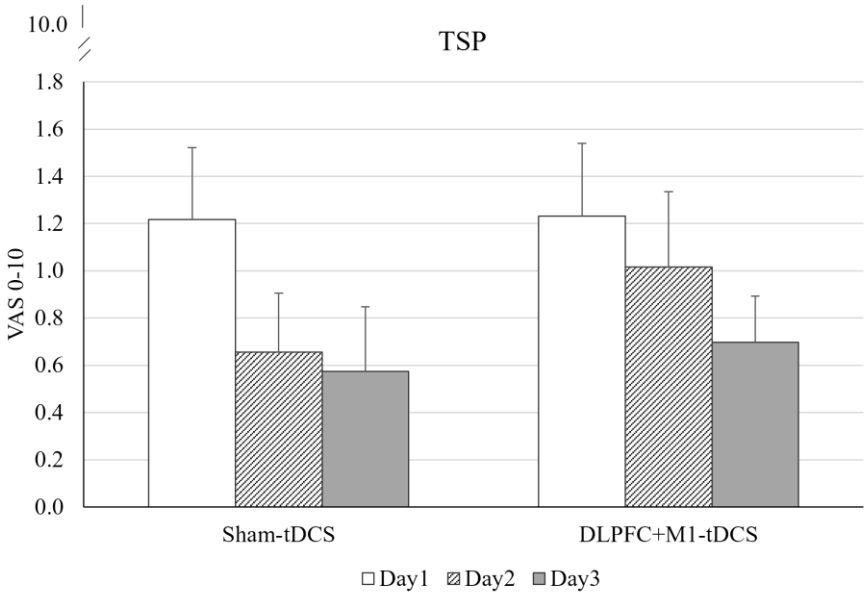


Figure 15. Mean (±SEM) temporal summation of pain (TSP) effect. TSP effect over 3 days, Day1 (before HD-tDCS), Day2 and Day3 (after HD-tDCS) in the two groups from Study I (Sham-tDCS and DLPFC+M1-tDCS). tDCS, Transcranial direct current stimulation; DLPFC, dorsolateral prefrontal cortex; M1, primary motor cortex; SEM, standard error of the mean.

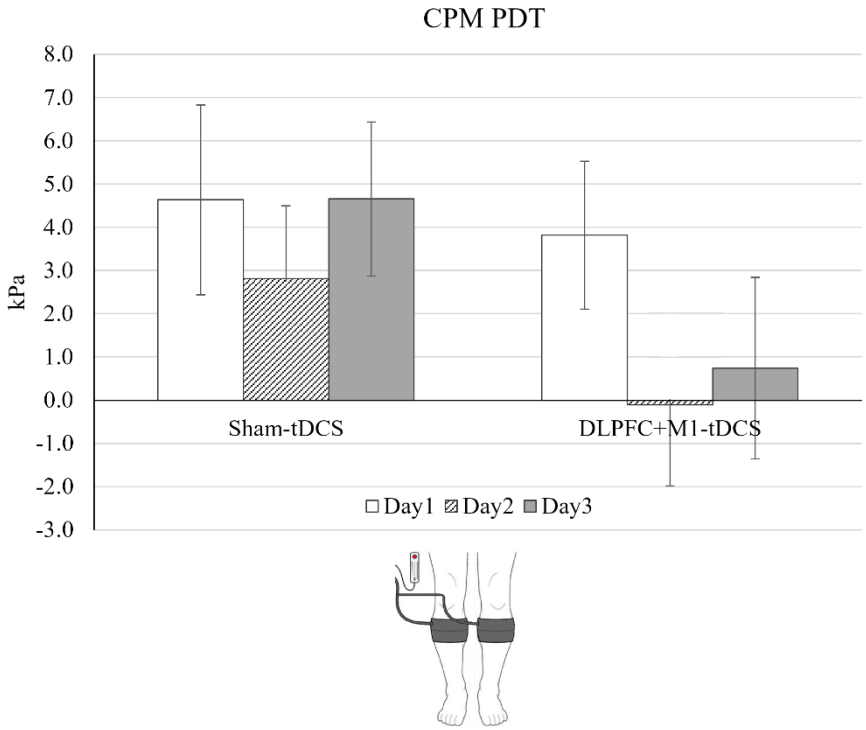
Both groups demonstrated positive TSP effects on all three days, with higher pain ratings at stimuli 8-10 than stimulus 1, showing that the TSP paradigm worked as intended. However, there were no significant differences in the TSP of the DLPFC+M1-tDCS group compared to the Sham-tDCS group. Multiple other studies have examined the effects of tDCS on the TSP mechanism, although with conflicting findings[92,120,153,168,209,252–254]. In line with the present findings, Jürgens et al. (2012), Gurdiel-Álvarez et al. (2021)[92] and Gregoret et al. (2021)[120] showed no significant tDCS modulation of TSP compared to sham-tDCS. On the other hand, Hughes et al. (2018[a])[252] and (2018[b])[253] demonstrated positive inhibition of TSP with M1-tDCS in healthy subjects. These studies did however utilize a different TSP paradigm by applying transcutaneous electrically evoked painful stimuli and only showed positive analgesic effects on pain evoked at 20 Hz[252,253]. The different methodologies may drive the conflicting findings.

The present findings indicate that healthy subjects are not susceptible to modulation of the TSP mechanism. This may be explained by a ceiling effect, entailing that the endogenous pain facilitatory mechanisms cannot be modulated to a level of higher functionality than the baseline TSP of a healthy system. This theory is supported by Giannoni-Luza et al. (2020), who argue that pain neurocircuitry dysfunction provides a more extensive range of modulation, resulting in tDCS being less efficient in healthy subjects than in subjects with perturbed nervous systems[84].

4.3.2. CONDITIONED PAIN MODULATION

In animals, diffuse noxious inhibitory control (DNIC) describe a type of descending inhibitory control system that is triggered by a noxious stimulus distant to the control response[255]. This down-stream capacity is driven by endogenous descending inhibitory control of dorsal horn neurons along the neuroaxis[21,230,232,234,256]. The human counterpart to DNIC is called CPM and requires a descending control also[255]. In layman's terms, the CPM mechanism entails that 'pain inhibits pain' or that the intensity of a painful stimulus is reduced by the application of a second painful stimulus[18,230,235,257]. However, the CPM mechanism is more complex than the DNIC system, and the mechanism is affected by mood, cognition, gender, affective and even psychosocial components[254,257–259]. Similar to TSP, dysfunctional CPM mechanisms have been demonstrated in various patient groups suffering from chronic pain, including osteoarthritis[244,260], patellofemoral pain[24], chronic headache[261], fibromyalgia[233,262,263], various types of visceral pains[249,264], and more[21,23,234,265]. As a result, the diffuse noxious inhibition control mechanisms are believed to be an important factor in the development from acute to chronic pain[234,266]. In the CPM test paradigm, a distant painful conditioning stimulus is used to affect a test stimulus[238,256]. In *Study I*, the CPM was assessed with a cuff-pressure paradigm (Fig. 13). The PDT and PTT were assessed on the right leg, while the left leg was simultaneously provoked with a painful tonic pressure stimulus at 70% intensity of the previously established PTT. The CPM effect was defined as the difference between the PDT (and PTT) with and without the

presence of the conditioning pain stimulus[238,239]. It was hypothesized that the CPM efficacy would be improved by the active HD-tDCS, as CPM is an endogenous antinociceptive mechanism that may drive the analgesic effect of the stimulation[84,237].



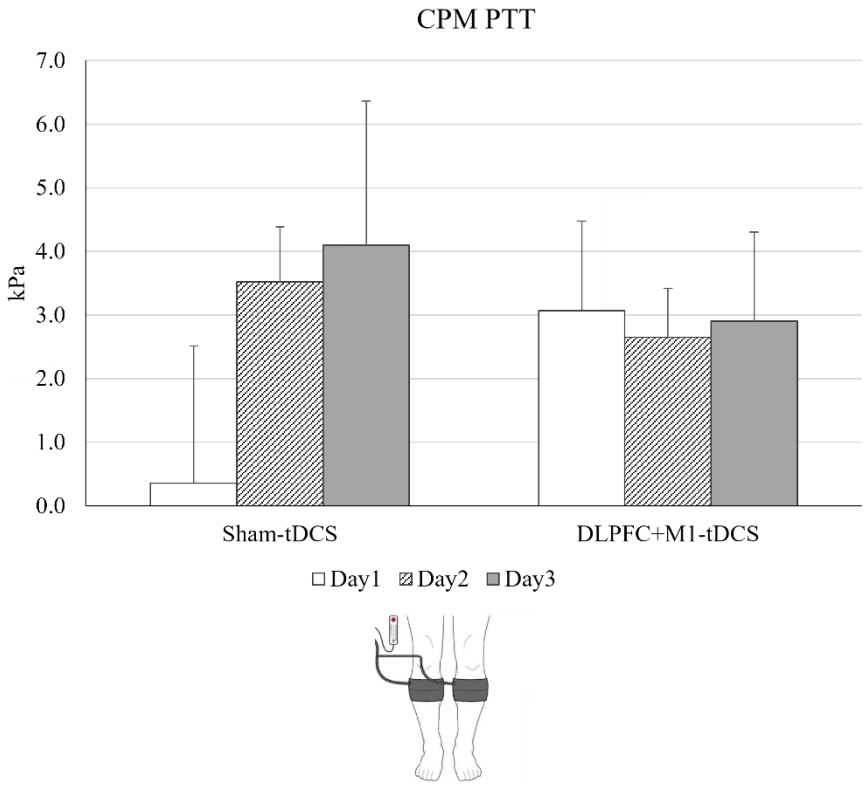


Figure 16. Mean (±SEM) conditioned pain modulation of cuff-pressure pain detection thresholds (CPM PDT) and conditioned pain modulation of cuff-pressure pain tolerance thresholds (CPM PTT). The CPM PDT and CPM PTT effects over 3 days, Day1 (before HD-tDCS), Day2 and Day3 (after HD-tDCS) in two groups from Study I (Sham-tDCS and DLPFC+M1-tDCS) tDCS, Transcranial direct current stimulation; DLPFC, dorsolateral prefrontal cortex; M1, primary motor cortex; SEM, standard error of the mean.

The analysis revealed that there were no significant differences in the CPM PDT or CPM PTT effect between the groups at any time. This indicates that the active HD-tDCS did not modulate the CPM effect significantly differently than sham-tDCS. The findings contradict the hypothesis that active HD-tDCS would enhance the CPM efficacy. A number of studies have shown positive modulatory effects of tDCS on the CPM mechanism in healthy subjects, including Reidler et al. (2012)[205] and Flood et al. (2016)[100] administering conventional M1-tDCS, and latest Wan et al. (2021)[201] and Jiang et al. (2022)[208], administering M1-HD-tDCS. However, Jürgens et al. (2012)[209] and Silva et al. (2015)[267] administering conventional M1-tDCS and Gregoret et al. (2021)[120] administering HD-tDCS of M1 all demonstrated findings in line with the present study, with no modulatory effect on the CPM mechanism in healthy subjects, which support the present findings. It is unclear what drives the discrepancies in the

findings of the different studies, but variations in CPM methodology and tDCS-protocols may play an important part. This underlines that transparent and rigorous methodology is imperative when investigating these elusive subjects.

4.4. SUMMARY AND DISCUSSION OF FINDINGS OF THE MODULATORY EFFECT OF HD-tDCS ON THE SOMATOSENSORY SYSTEM IN HEALTHY PAIN-FREE SUBJECTS.

The primary aim of *Study I* and *Study III* of healthy subjects were to investigate whether HD-tDCS could modulate the somatosensory system. In *Study I,* three active HD-tDCS configurations (DLPFC-tDCS, M1-tDCS or DLPFC+M1-tDCS) that have previously been suggested to modulate the somatosensory system were assessed compared to sham-tDCS. It was hypothesised that the potential modulation would be functionally reflected in increased pain and sensory thresholds in a healthy population. In *Study III,* the effects of DLPFC+M1-tDCS montage on the central pain mechanisms were assessed compared to Sham-tDCS in healthy, pain-free subjects. The findings of *Study I* demonstrated that none of the somatosensory modalities was modulated significantly differently by any of the active HD-tDCS montages (DLPFC, M1 and DLPFC+M1) compared to Sham-tDCS. However, unrelated to the HD-tDCS type, a general decrease in somatosensory sensitivity (PPT, MPT, TDT and thermal thresholds) was demonstrated over the course of the three study days, indicating habituation to the sensory testing or possibly a placebo effect. The findings of *Study III* on the healthy pain-free subjects demonstrated the HD-tDCS of DLPFC+M1 did not modulate either of the endogenous pain modulatory mechanisms (CPM and TSP) significantly compared to Sham-tDCS.

The analgesic effect of tDCS that is seen in chronic pain patients has been suggested to be driven by inhibition of pain-and somatosensory processing, potentially by motor cortex-driven inhibition of primary sensory cortex, or by activation of endogenous mu-opioid systems[47,48,171]. The non-significant results of *Study I* and III do not refute the existence of a possible clinical analgesic effect of tDCS, nor that tDCS may induce the suggested neurological changes. Instead, the findings indicate that regardless of the potential modulatory effect of the three HD-tDCS configurations, the stimulation does not produce identifiable functional changes in the somatosensory pain-and detection thresholds compared to sham-tDCS in healthy subjects.

These findings could be explained by a number of theories. One is that healthy subjects are less susceptible to neuromodulation of the somatosensory system due to a ceiling effect. This theory was described by Giannoni-Luza et al. (2020), who suggest that pain neurocircuitry dysfunction provides a more extensive range of potential modulation[84]. Alternatively, homeostatic plasticity, which is an endogenous mecha-

nism maintaining the neural activity within an optimal physiological range (equilibrium), may counter-act the exogenous modulation of tDCS. Modulation of homeostatic plasticity has been suggested to be a mechanism underlying the effect of brain stimulation and is a research area of increasing interest[46,268–270].

Another non-invasive brain stimulation method has also shown a pattern of being influenced by the state of the nervous system. Ciampi de Andrade et al. (2014) demonstrated that the effects of repetitive transcranial magnetic stimulation (rTMS) depends on the level of N-methyl-d-aspartate glutamate receptors, which is altered in various chronic pain conditions[271,272]. They argue that the analgesic effect of rTMS is driven by restoration of dysfunctional systems in pain patients. A theory that is supported by Moisset et al. (2016), who demonstrated that the rTMS-induced pain relief correlated with restoration of normal cortical excitability in chronic pain patients[273]. rTMS rarely increase the excitability above normal values, which may account for the lack of significant changes in healthy subjects[271]. In other words, there is no loss of function to restore in the healthy system.

Another theory that can explain the non-significant results is that HD-tDCS may be inferior at modulating functional somatosensory thresholds than conventional tDCS. The majority of comparable studies with positive findings utilized a conventional M1-tDCS montage with a single anode and cathode (section 6.2.1-6.2.5). The prospect of increasing the focality of the electrical field is intriguing and has been shown to better modulate the excitability of neurons than the non-specific conventional tDCS (section 5.3). However, there is no linear relationship between the intensity of the electrical field and the outcome of the stimulation (Section 5.6 & 5.8). Contrarily, the larger electrical field of conventional tDCS may drive the positive outcomes by stimulating more areas involved in the processing of somatosensation. More studies comparing the functional effects of HD-tDCS vs conventional tDCS are necessary to investigate this hypothesis. A final theory that can explain the demonstrated non-significant results is that tDCS produces no neurophysiological effects other than modulating the excitability of neurons. This theory was suggested by Horvath et al. (2015[a-b]) following two systemic reviews on the subject[274,275]. Although, a number of more recent systemic reviews and meta-analyses on the effects of tDCS also dispute this theory[15,37,84,138,166,276,277].

4.5. MAIN FINDINGS OF INVESTIGATING THE MODULATORY EFFECT OF TDCS ON SOMATOSENSORY PAIN AND DETECTION THRESHOLDS

- Neither HD-tDCS configurations targeting dorsolateral prefrontal cortex (DLPFC), primary motor cortex (M1), nor DLPFC+M1 simultaneously

modulated somatosensory pain and detection thresholds better than Sham-tDCS. (*Study I*).

- Unrelated to the tDCS group, the healthy subjects showed an increase in somatosensory thresholds over the three study days, indicating habituation to the sensory testing or possibly a placebo effect of the tDCS. (*Study I*).
- The HD-tDCS did not modulate the conditioned pain modulation or temporal summation of pain in the healthy, pain-free subjects compared to sham-tDCS

CHAPTER 5. MODULATION OF THE SOMATOSENSORY SYSTEM OF HEALTHY SUBJECTS WITH INDUCED PROLONGED PAIN USING HD-TDCS

It was hypothesised that the state of the subject's CNS might influence the response to the HD-tDCS, entailing that healthy subjects may respond differently than pain patients with an altered nociceptive system[21–25]. Some of the changes in the CNS occur rather shortly after the onset of prolonged pain, which affords the opportunity to investigate the phenomena under controlled experimental conditions[20]. In *Study II* it was aimed to assess the modulatory effects of HD-tDCS on the somatosensory system during the first days of prolonged pain and the initial pain-induced changes to the somatosensory system. To explore this the healthy subjects of *Study II* were administered an experimental prolonged pain model, aimed to induce persistent muscle soreness and concomitantly provoke perturbation of their nervous systems[189,190].

5.1. EXPERIMENTAL PROLONGED PAIN

All 40 subjects of *Study II* were administered prolonged pain provocation in the form of intramuscular injection of 5 μg (0.5 ml) nerve growth factor (NGF) into the right hand first dorsal interosseous muscle (FDI). A detailed description of the pain model is described in *Paper II*[278]. Intramuscular injection of NGF induces long-term sensitization and time-dependent hyperalgesia, indicating potential involvement of both central and peripheral pain mechanisms[189,279,280]. The NGF produces no immediate pain but can induce long-lasting hyperalgesia and increased mechanical sensitivity for up to 14 days[190,279]

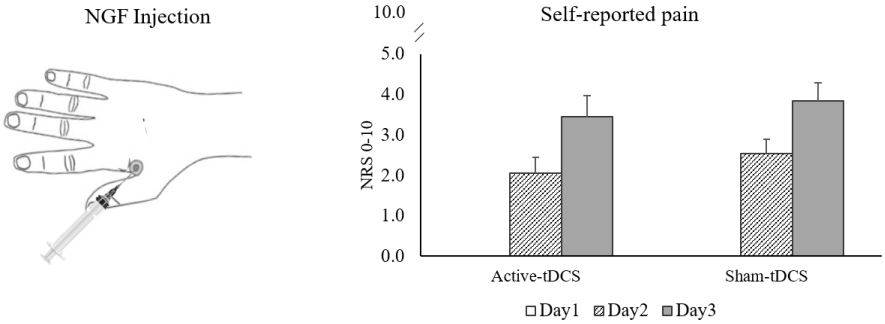


Figure 17. Experimental pain model and concomitant self-reported pain in Study II. The illustration on the left shows the administration of an intramuscular bolus injection of nerve growth factor (NGF) in the right hand first dorsal interosseous muscle. The bar chart on the right (Mean±SEM) shows the self-reported pain of the two groups on a numerical rating scale (0-10) on Day1, Day2 and Day3.

The pain model was assessed on a self-reported numerical rating scale from 0-10 (NRS 0-10), 0 being no pain at all and 10 being the worst pain imaginable. The pain model successfully induced pain in both the Active-HD-tDCS group receiving DLPFC+M1-tDCS and the Sham-tDCS groups that started with no pain (0.0) on Day1 and peaked on Day3 (*Paper II*). The pain intensity was not significantly different between the groups, indicating that the active HD-tDCS was not significantly more effective than Sham-tDCS in modulating the self-reported pain. The pain intensities are similar to what has been demonstrated in earlier studies[279–281].

5.2. MODULATING SOMATOSENSORY DETECTION-AND PAIN THRESHOLDS IN HEALTHY SUBJECTS WITH INDUCED PROLONGED PAIN

Similar to *Study I*, the effects of HD-tDCS on the detection and pain thresholds were assessed using static QST in *Study II*. However, in *Study II*, this was done before and after administering the experimental prolonged pain model (Fig. 6). As the NGF was administered in the FDI muscle, the sensory thresholds were assessed in this area. Illustrations of the static QST are presented in Fig. 18.

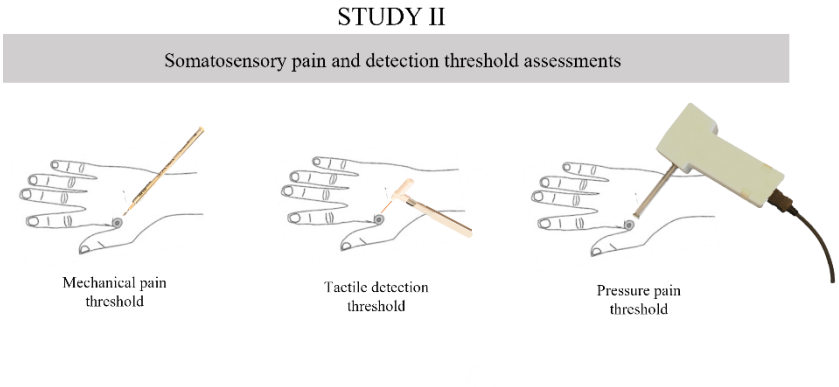


Figure 18. Static quantitative sensory testing in Study II. The somatosensory thresholds; mechanical pain threshold, tactile detection threshold and pressure pain threshold, were determined on the skin above the muscle belly of the first dorsal interosseous muscle.

Both the flexor carpi radialis muscle assessed in *Study I* and the FDI muscle assessed in *Study II* are modulatable with transcranial stimulation of the M1 at the C3 electrode using the EEG 10-10 system, so the change of assessment site was presumed not to affect the outcome[282–285]. Thermal and vibration detection assessments were not used in *Study II* as the intramuscular NGF produces deep tissue mechanical hyperalgesia, and those modalities were presumed not to be affected[280]. Additionally, the pressure pain thresholds (PPT) were assessed with a slower acceleration rate of pressure (20 kPa/s instead of 30 kPa/s) in *Study II*, as the smaller muscle was expected to be more sensitive to pressure and thus reach the threshold more quickly.

5.2.1. PRESSURE PAIN THRESHOLDS IN SUBJECTS WITH INDUCED PROLONGED PAIN

The PPTs of the two groups assessed in *Study II* are presented in Fig. 19.

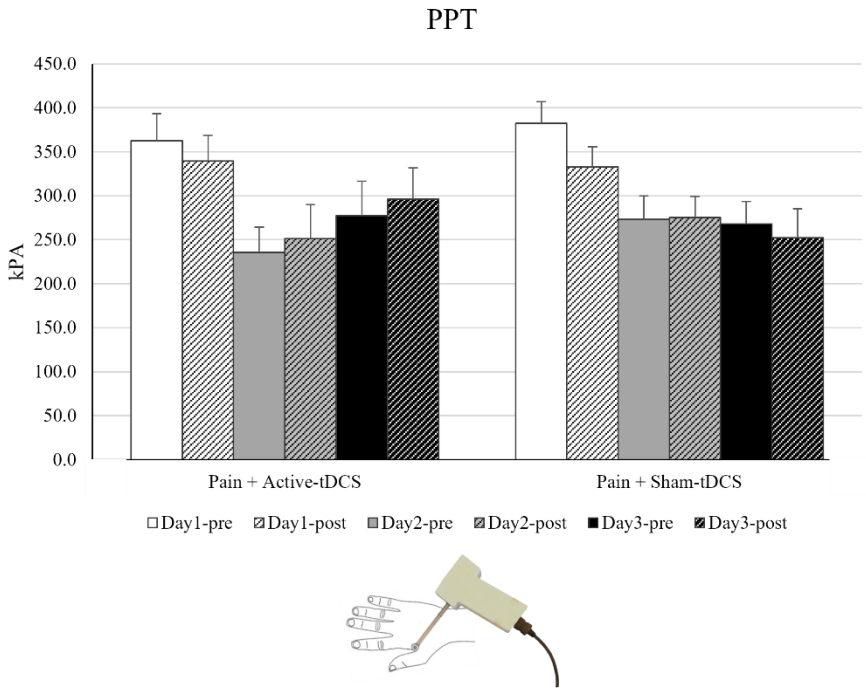


Figure 19. Mean (+SEM) pressure pain thresholds in subjects with experimental pain. Pressure pain thresholds (PPT), over 3 days before (nonshaded) and after (shaded) HD-tDCS, for the two groups in Study II. tDCS, Transcranial direct current stimulation; SEM, standard error of the mean.

The PPTs of both groups in *Study II* decreased concurrently with the establishment of hyperalgesia induced by the experimental prolonged pain. This is in line with previous studies assessing the hyperalgesic effect of the pain model[189,190,279,280]. There were no significant differences in the development of the PPTs over the course of the six assessments between the Pain + Active-tDCS group and the Pain + Sham-tDCS group. This indicates that the HD-tDCS was not significantly more effective than sham-stimulation at modulating the pressure pain sensitivity. This was the first study investigating the modulatory effect of HD-tDCS on PPTs following provocation of experimental prolonged muscle pain. Since the pain model was intended to mimic the initial symptoms of chronic musculoskeletal pain, a comparison with the effects of tDCS on the PPTs in chronic pain populations is relevant. Numerous clinical studies have demonstrated antinociceptive tDCS effects on the pressure sensitivity in chronic pain

conditions, including in fibromyalgia[97], painful diabetic neuropathy[286], knee osteoarthritis[287] HTLV-1 infection[288], painful hepatitis[289], upper limp neuropathic pain[153]. However, a few studies have reported null-findings; notably, a study in fibromyalgia patients by Mendonca (2011)[290], showed no effects of M1-tDCS on PPTs compared to Sham-tDCS but did demonstrate that the tDCS had positive effects on self-reported pain (VAS 0-10). The negative findings of Mendonca's (2011) study may be attributed to a rather small sample size (N=6 in each group), and the study also employed an unconventional tDCS-configuration with the cathode placed at an extracephalic position[290]. Despite isolated negative studies, tDCS appear to modulate the pressure sensitivity in pain patients and thus opposes the findings of *Study II*. Conclusively the healthy subjects with experimental pain provocation responded to tDCS more similarly to healthy subjects than chronic pain patients.

In *Study II*, pressure algometry was also used to assess self-reported pain (NRS 0-10) during pressure stimulus administered at baseline threshold level[278]. This assessment is described in detail in *Paper II* and was categorized as *pain intensity on a numerical rating scale at pressure pain threshold* (NRS@PPT, 0-10). It was hypothesized that pressure stimulus administered at baseline threshold level would elicit moderate to intense pain following NGF-provoked muscle sensitization on Day2 and Day3. The NRS@PPT of the Sham-tDCS group and the DLPFC+M1-tDCS group are shown in Fig. 20.

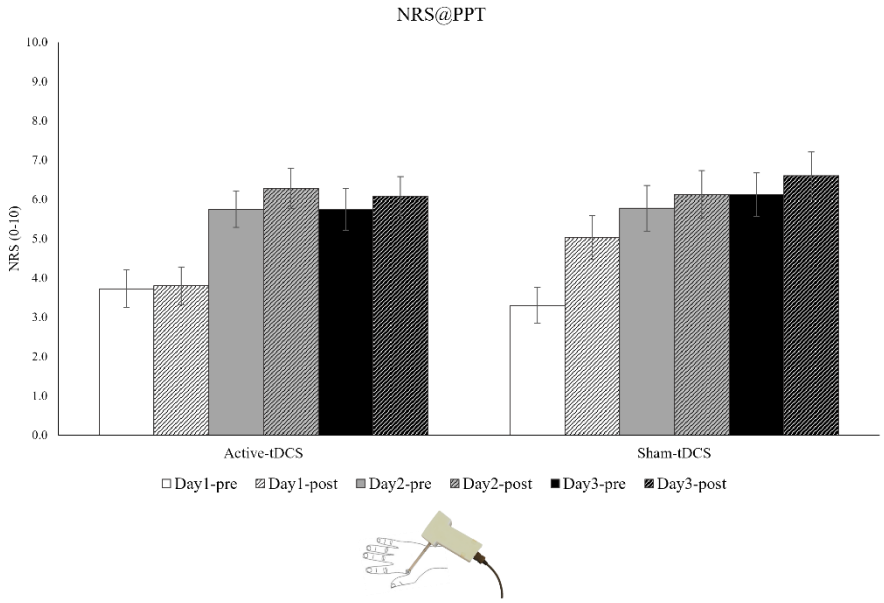


Figure 20. Mean (±SEM) pain intensity at pressure pain threshold in subjects with experimental pain. Pain intensity (numerical rating scale, 0-10) at pressure pain threshold (NRS@PPT), over 3 days before (nonshaded) and after (shaded) HD-tDCS, for the two groups in Study II. The PPT represent the threshold the participants identified the pressure as painful. tDCS, Transcranial direct current stimulation; SEM, standard error of the mean.

Interestingly the baseline NRS@PPT were at ~4 NRS@PPT in both groups. Administering a pressure stimulus at the pain threshold level would presumably only produce low-intensity pain, as the pain threshold is by definition the lowest stimulus intensity that elicits a sensation of pain[194]. The high baseline value may have resulted from the pressure stimulus being applied for an extended time, producing a wind-up effect like temporal summation of pain[233,291]. Despite the surprising baseline value, the experimental pain model successfully increased the pressure sensitivity in both groups to a level where pressure stimulus administered at the baseline threshold level induced pain of moderate to high intensity (~6 NRS@PPT). The NRS@PPT were not significantly different between groups at the end of the intervention (Day3-post), however the Active-tDCS group showed significantly lower NRS@PPT at Day1-post compared to the Sham-tDCS group, where the hyperalgesia appears to have already been established. This may indicate that the HD-tDCS either produced an immediate short-term analgesic effect or possibly delayed the establishment of hyperalgesia from Day1-post to Day2-pre. The assessment with pressure algometry administered at baseline threshold level is novel and is primarily useful due to the gradual change in muscle sensitivity provoked by the persistent pain model, which is why it was first introduced in *Study II*.

5.2.2. MECHANICAL PAIN THRESHOLDS IN SUBJECTS WITH INDUCED PERSISTING PAIN

The MPTs of the two groups assessed in *Study II* are presented in Fig. 21.

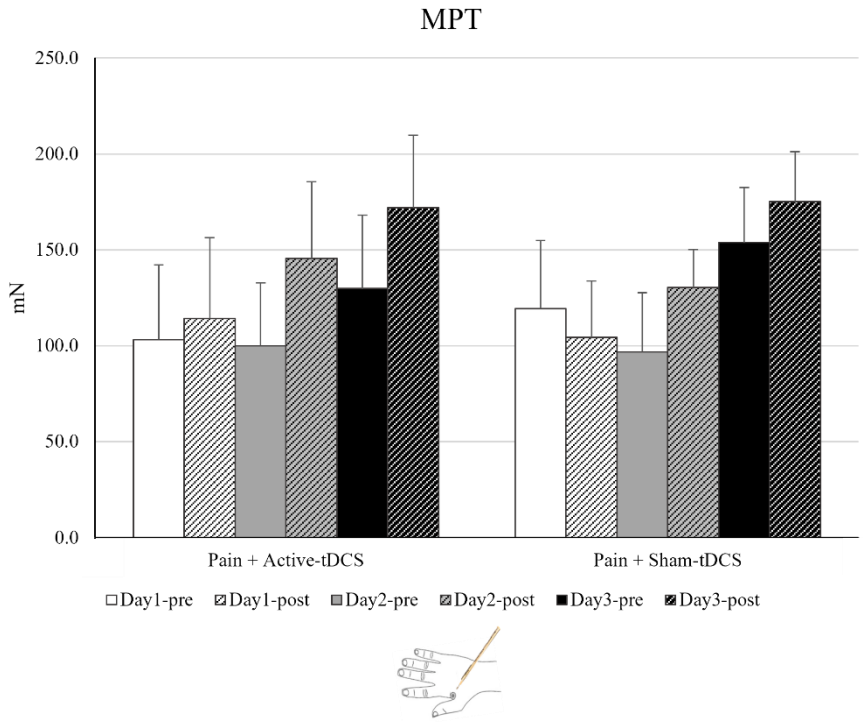


Figure 21. Mean (±SEM) mechanical pain threshold in subjects with experimental pain. Mechanical pain threshold (MPT), over 3 days before (nonshaded) and after (shaded) HD-tDCS, for the four groups in Study I. The MPT represent the threshold at which the participants identified the pressure and mechanical stimulation as painful. tDCS, Transcranial direct current stimulation; SEM, standard error of the mean.

There were no differences over time or between groups in the MPTs in *Study II*. This indicates that neither the experimental pain provocation that both groups received nor the HD-tDCS altered the mechanical pain sensitivity. The pain model was administered intramuscularly, so the lack of modulation of cutaneous mechanical sensitivity is not surprising. The finding is in line with Andersen et al. (2008), who similarly

found no effects of intramuscular NGF-injections on cutaneous mechanical sensitivity[279]. No previous studies have investigated the modulatory effect of HD-tDCS on MPTs following provocation of experimental prolonged muscle pain. Only a single study has investigated the effects of M1-tDCS on MPTs in a chronic pain condition[84]. Khedr et al. (2017) demonstrated an increase in MPTs in fibromyalgia patients after 10 sessions of 20 min, 2 mA anodal M1-tDCS with cathode placed extra-cephalic on the contralateral arm[292]. Conclusively intramuscular injection of NGF does not appear to produce cutaneous hypersensitivity, and the MPTs are not modulatable with HD-tDCS in either healthy subjects or subjects experiencing prolonged experimental pain.

5.2.3. TACTILE DETECTION THRESHOLDS IN SUBJECTS WITH INDUCED PROLONGED PAIN

The TDTs of the two groups assessed in *Study II* are presented in Fig. 22.

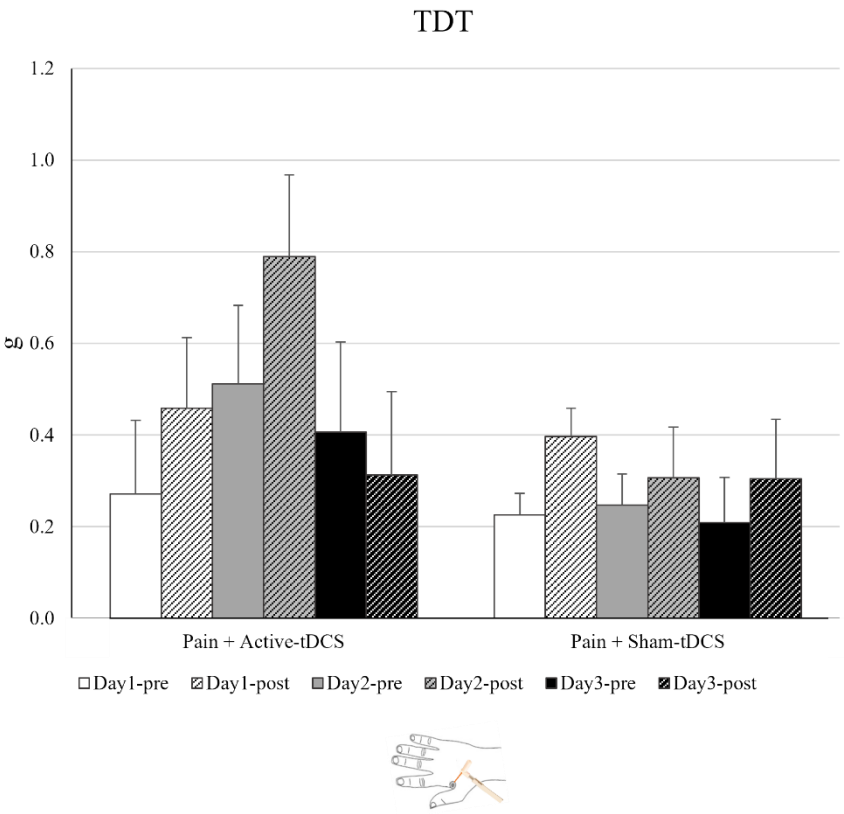


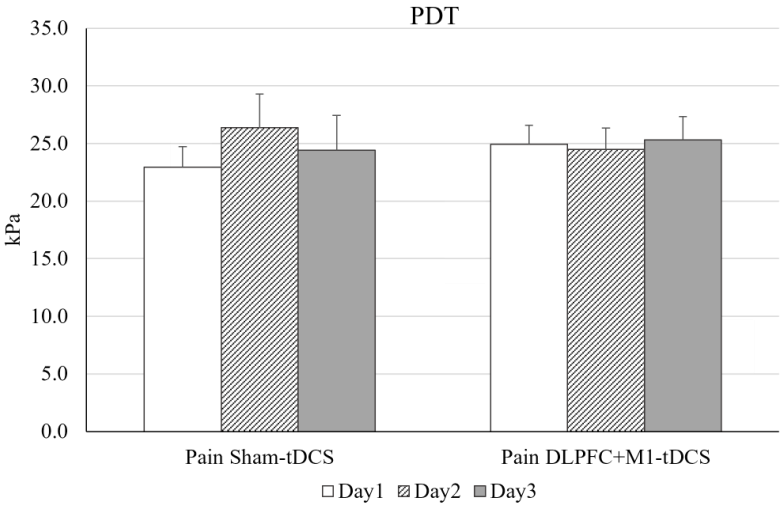
Figure 22. Mean (±SEM) tactile detection threshold in subjects with experimental pain. Tactile detection threshold (g) (TDT), over 3 days before (nonshaded) and after (shaded) HD-tDCS, for the two groups in Study II. TDT represents the pressure stimulus intensity needed for the participants to detect the touch of the Von Frey filament. tDCS, Transcranial direct current stimulation; SEM, standard error of the mean.

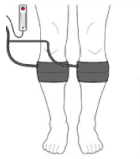
Similar to the MPT findings, there were no differences over time or between groups in the TDTs in *Study II*. This indicates that neither the experimental pain provocation nor the HD-tDCS modulated the tactile detection sensitivity. Assessing tactile detection sensitivity following intramuscular injection of NGF is novel, and the results hereof are not comparable to earlier experimental studies. In clinical studies, loss of function of TDT with hyposensitivity has been demonstrated in patients suffering from fibromyalgia, painful neuropathies, myofascial pain and chronic daily headache[293–295], indicating that the state of the nervous system influences the TDT. However, musculoskeletal pain conditions, which the NGF-model aims to mimic the early phase of, have not previously been shown to influence TDTs[198,296–298], so the lack of significant findings in *Study II* is not surprising. No previous studies have investigated the modulatory effect of tDCS on TDT in experimental or chronic pain conditions, so no frame of reference is established to compare the results to. Conclusively the findings of *Study I* and *Study II* suggest that neither anodal HD-tDCS nor intramuscular injection of NGF modulate the mechanical detection thresholds of tactile or vibration stimuli in healthy subjects.

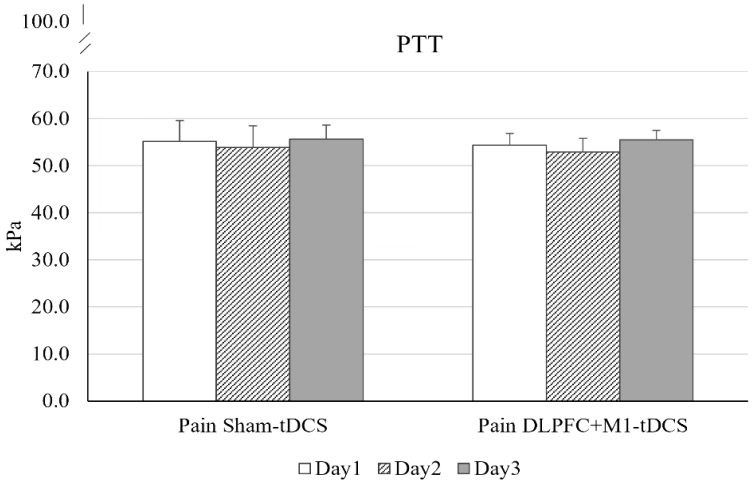
5.3. MODULATING CENTRAL PAIN MECHANISMS IN SUBJECTS WITH INDUCED PROLONGED PAIN USING HD-TDCS

Like in *Study I*, the dynamic QST measures TSP and CPM were recorded in succession to the static QST in *Study II* (Fig. 6). The dynamic QST results of the healthy subjects with experimental persistent pain (*Study II*), who were administered either DLPFC+M1-tDCS or Sham-tDCS were compared in *Paper III*[237]. The assessment protocols were identical to *Study I* (section 4.2), only differing by the subjects being administered the experimental prolonged pain model after the baseline assessments.

The intramuscular injection of NGF in the FDI was not hypothesized to modulate the cuff-pressure sensitivity due to the pain model being highly localized[189,190,279]. The HD-tDCS was hypothesized to attenuate the cuff-pressure sensitivity due to the analgesic effects M1-tDCS has previously been shown to produce in other pain modalities (i.e. thermal, electrical, mechanical)[84,243]. The PDTs and PTTs of the two groups in *Study II* are presented in Fig. 23.


PDT
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
kPa
Pain Sham-tDCS
Pain DLPFC+M1-tDCS
Day1
Day2
Day3




100.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
PTT
kPa
Pain Sham-tDCS
Pain DLPFC+M1-tDCS
Day1
Day2
Day3

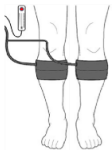


Figure 23. Mean (±SEM) cuff-pressure pain detection threshold (PDT) and cuff-pressure pain tolerance threshold (PTT) in subjects with experimental pain. Data was recorded on three consecutive days, Day1 (before HD-tDCS), Day2 and Day3 (after HD-tDCS). Two groups from Study I without pain (Sham-tDCS and DLPFC+M1-tDCS) and two groups from Study II with pain (Pain Sham-tDCS and Pain DLPFC+M1-tDCS). tDCS, Transcranial direct current stimulation; DLPFC, dorsolateral prefrontal cortex; M1, primary motor cortex; SEM, standard error of the mean.

There were no significant differences between the two groups in the PDT and PTT on any of the three days in *Study III*. This indicates that neither the experimental prolonged pain nor the active HD-tDCS were significantly more effective in modulating the leg-pressure sensitivity than no pain or Sham-tDCS. The lack of modulation by the pain model on leg-pressure sensitivity indicates that the pain model is localized and does not induce sensibility or hyperalgesia in the legs. No previous studies have assessed the effects of tDCS on cuff-pressure sensitivity, however, the potential pain modulating properties of tDCS are suggested to be non-specific to the modality of experimental pain[84]. Therefore the non-significant findings of the modulatory effect of the tDCS can be considered conflicting with the existing studies that report positive effects of tDCS on pain thresholds[84,243] (section 4.1). However, the findings are in line with the results of *Study I* and *Study II* (section 4.1), which similarly showed little- to no tDCS modulation of somatosensory thresholds in healthy subjects.

5.3.1. TEMPORAL SUMMATION OF PAIN

It was hypothesized that the prolonged muscle pain would increase the TSP in the group that received Sham-tDCS and that this would be attenuated in the HD-tDCS group.

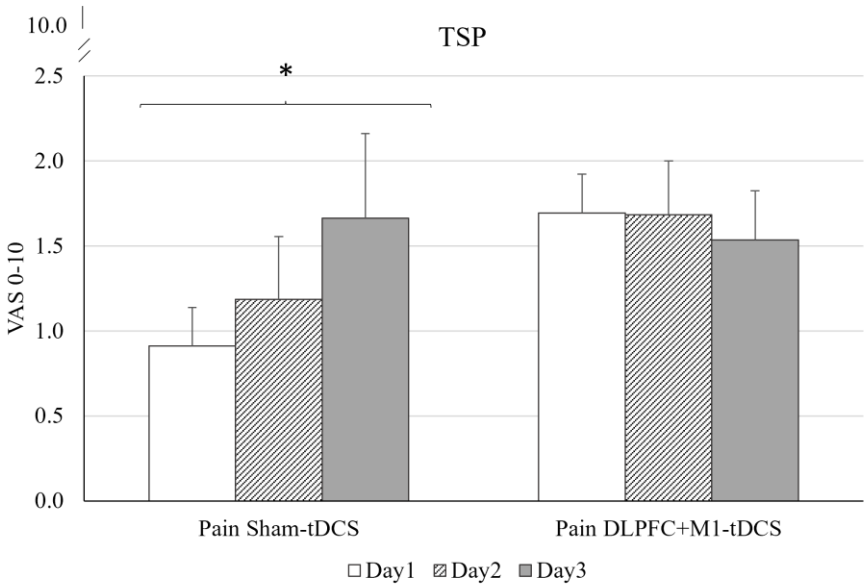


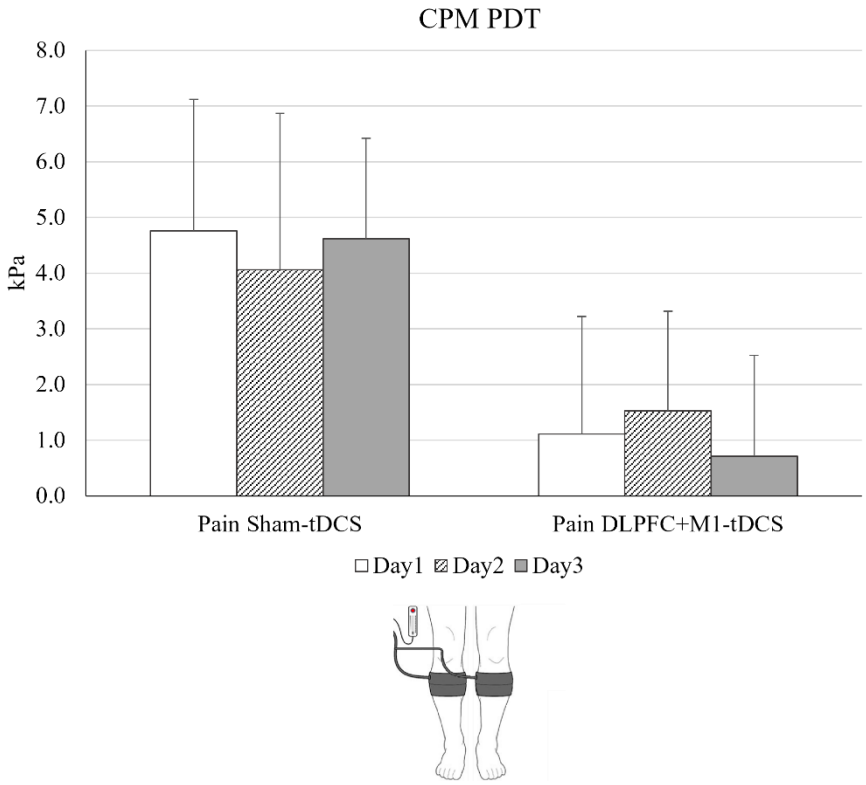
*Figure 24. Mean (±SEM) temporal summation of pain (TSP) effect in subjects with experimental pain. TSP effect over three days, Day1 (before HD-tDCS), Day2 and Day3 (after HD-tDCS) in the two groups from Study II (Pain Sham-tDCS and Pain DLPFC+M1-tDCS). tDCS, Transcranial direct current stimulation; DLPFC, dorsolateral prefrontal cortex; M1, primary motor cortex; SEM, standard error of the mean. (P ≤ 0.05 is indicated with * for group differences).*

Both groups demonstrated positive TSP effects on all three days, with higher pain ratings at stimulus 8-10 than stimulus 1, showing that the TSP paradigm worked as intentioned. The analysis of the development of the TSP effect in the four groups revealed that the TSP was facilitated in the Pain Sham-tDCS group after baseline compared to the Pain DLPFC+M1-tDCS group. The facilitated TSP is possibly due to an enhanced central integrative mechanism[18]. That the pain model in the FDI induced changes in the TSP away from the pain locus is an important and novel finding. This strengthens the translational utility of the model, as similar mechanisms have been demonstrated in chronic pain conditions, i.e. low back pain patients showing facilitated TSP when assessed on the lower legs with cuff-pressure stimulation[251]. The facilitation of TSP was not seen in the Pain DLPFC+M1-tDCS group, despite experiencing similar pain intensities in the hand (Fig. 24). This indicates that the HD-tDCS antagonized the changes from establishing or possibly inhibited the ascending pain signals resulting in an attenuated wind-up mechanism. However, this is contradicted by the lack of modulation of PDT and PTT. These findings are in line with Braulio et al. (2018), who demonstrated that administration of remifentanil in healthy subjects produces dysfunction in the TSP mechanism and that 20 min of conventional anodal M1-tDCS mitigates this perturbation[228]. Contrarily to this, McPhee et al. (2021)[254] and

Lewis et al. (2018)[153] demonstrated no modulation of TSP in patients suffering from lower back pain and neuropathic pain, respectively.

5.3.1. CONDITIONED PAIN MODULATION

In *Study II*, the CPM was assessed with the same cuff-pressure paradigm as in *Study I* (Fig. 13). It was hypothesized that the prolonged muscle pain would inhibit the CPM in the group that received Sham-tDCS. The hypothesis is based on the review by Goubert et al. (2015), which demonstrated that prolonged pain could inhibit the CPM mechanism[265]. It was further hypothesised that the active HD-tDCS would stunt this inhibition or possibly increase the CPM effect. The CPM effect of both PDT and PTT are shown in fig. 25.



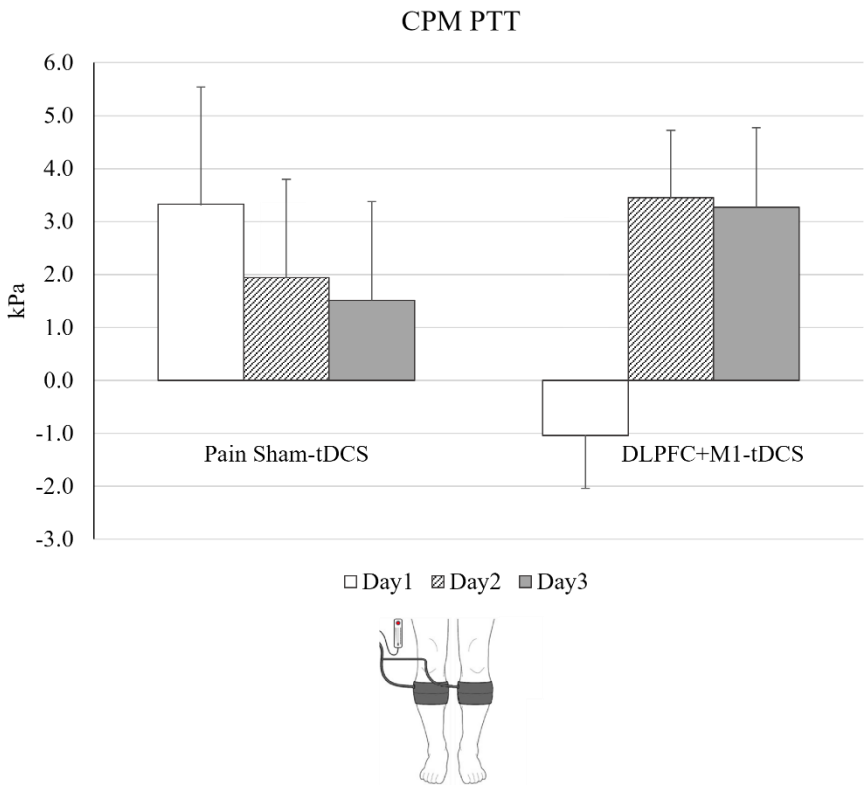


Figure 25. Mean (±SEM) conditioned pain modulation of cuff-pressure pain detection thresholds (CPM PDT) and conditioned pain modulation of cuff-pressure pain tolerance thresholds (CPM PTT) in subjects with experimental pain. The CPM PDT and CPM PTT effects over 3 days, Day1 (before HD-tDCS), Day2 and Day3 (after HD-tDCS). Two groups from Study II with pain (Pain Sham-tDCS and Pain DLPFC+M1-tDCS). tDCS, Transcranial direct current stimulation; DLPFC, dorsolateral prefrontal cortex; M1, primary motor cortex; SEM, standard error of the mean.

The analysis revealed that there were no significant differences in the CPM PDT or CPM PTT effect between the groups at any time. This indicates that neither the persistent pain model nor the active HD-tDCS modulated the CPM effects significantly differently than sham-tDCS and no-pain. Interestingly the CPM PTT of the *Pain DLPFC+M1-tDCS* group was negative at baseline, indicating highly variable or possibly dysfunctional CPM mechanisms[241,256] in this group before any intervention was administered. Large intersubject variability of CPM has previously been reported[208,299] and is not detrimental to the study design, as the baseline values were not significantly different between the groups. The findings contradict the hypothesis of the effect of the experimental persistent pain, which was assumed to inhibit the CPM. However, the findings are in line with a previous study, which demonstrated that a similar experimental pain provocation facilitated the TSP but did not modulate the

CPM[280]. This indicates that the down-stream CPM mechanism may be more resistant to exogenous provocation than the up-stream TSP mechanism.

The findings of *Study III* are conflicting with a number of clinical studies investigating the effect of tDCS on the CPM mechanism in chronic pain patients. Studies using either conventional tDCS or HD-tDCS of M1 have shown positive effects in both fibromyalgia[97] osteoarthritis[158,287] and post-operative pain[300]. Although, a few studies have demonstrated findings in line with the present study, with no modulatory effect of tDCS on the CPM mechanism[84,301]. The conflicting findings between the present study and the majority of clinical studies may be due to the duration of pain in the experimental setting. Previous studies have suggested that the decrease of CPM efficacy is related to the duration of pain[302] and may need to undergo chronification before showing dysfunction[303]. In other words, the relatively short duration (three days) of the experimental pain may not have been sufficient to induce changes in the CPM effect. Since there was no dysfunction to restore, homeostatic plasticity may have counteracted the possible modulatory effect on the CPM mechanism, keeping the antinociceptive mechanism in optimal functional range, as suggested in section 4.4.

5.4. SUMMARY AND DISCUSSION OF FINDINGS OF THE MODULATORY EFFECT OF HD-TDCS ON SOMATOSENSORY THRESHOLDS IN SUBJECTS WITH INDUCED PROLONGED PAIN.

In *Study II*, the intramuscular injection of NGF successfully induced pain and hyperalgesia, which was reflected in self-reported pain (NRS 0-10). The intensity of the self-reported pain was not significantly different between the active HD-tDCS group and the Sham-tDCS group. However, the active HD-tDCS group demonstrated delayed manifestation of deep-tissue hyperalgesia. The active HD-tDCS of DLPFC+M1 did not modulate the other somatosensory thresholds assessed with static QST significantly differently than Sham-tDCS. Taken together, these findings indicate that HD-tDCS does not modulate the somatosensory pain-and detection sensitivity differently than Sham-tDCS but may modulate properties of specific pain mechanisms in healthy subjects.

In the subjects with injected NGF in *Study III* the experimental pain provocation facilitated the endogenous up-stream facilitatory pain mechanism (TSP) but not the down-stream inhibitory mechanism (CPM). Similarly, the pain model did not affect the cuff-pressure pressure sensitivity of the legs. The facilitation of TSP may reflect sensitization of the pain system, similar to the symptoms of the early phases of chronic pain conditions[21,234]. The HD-tDCS of DLPFC+M1 did not modulate the endogenous down-stream inhibitory mechanisms (CPM) differently than sham-tDCS, in the subjects induced with experimental persistent pain. The active HD-tDCS of DLPFC+M1 did however inhibit the pain-driven facilitation of TSP in the subjects administered NGF, which the sham-tDCS did not. This finding was attributed to the active HD-

tDCS antagonizing the manifestation of the sensitization or potentially inhibiting the ascending pain signals, which decreased the wind-up mechanism.

The findings indicate that the efficacy of HD-tDCS might be linked with the presence of sensitized central pain mechanisms and exert its analgesic effects through modulation of these pain mechanisms. This is in line with the findings of *Study II*, which demonstrated that the tDCS delayed the manifestation of hyperalgesia but did not modulate the pain and detection thresholds of non-noxious stimuli. It is also in line with the suggested mechanism underlying the analgesic effect of rTMS; that the stimulation promotes restoration of dysfunctional systems to an optimal range, which explains why healthy subjects do not show a significant response[271,273].

As the experimental pain provocation did not functionally perturb the CPM mechanism, it is unknown whether a dysfunctional CPM mechanism may be modulated by tDCS similarly to TSP or if tDCS predominantly exerts its effects through modulation of up-stream mechanisms. Overall, these findings indicate that the tDCS intervention is highly brain-and nervous system-state dependent.

5.5. MAIN FINDINGS.

- The experimental persistent pain model of intramuscular injection of nerve growth factor (NGF) in the right hand first dorsal interosseous muscle successfully induced pain and hyperalgesia. (*Study II*)
- The pain model did not modulate the cuff-pressure sensitivity in the legs.
- The pain model did not perturb the endogenous down-stream inhibitory pain mechanism (conditioned pain modulation).
- The pain model facilitated the endogenous up-stream facilitatory pain mechanism (temporal summation of pain).
- HD-tDCS of dorsolateral prefrontal cortex (DLPFC) and primary motor cortex (M1) simultaneously did not modulate the cuff-pressure sensitivity in neither the healthy subjects nor subjects administered the pain model compared to sham-tDCS.
- The HD-tDCS did not modulate the conditioned pain modulation in the healthy subjects with prolonged pain compared to sham-tDCS.
- The HD-tDCS antagonized the manifestation of the maladaptive neuroplastic facilitation of temporal summation of pain or possibly produced an inhibition of the ascending pain signals, decreasing the wind-up mechanism.
- HD-tDCS of DLPFC+M1 did not modulate the somatosensory pain-and detection thresholds or pain intensity in subjects with experimental persistent pain, but may have delayed the manifestation of hyperalgesia. (*Study II*).
- Taken together, the findings suggest that the effects of HD-tDCS on the somatosensory system are dependent on the state of the nervous system.

CHAPTER 6. CONCLUSION

The present PhD project investigated the modulatory effects of various HD-tDCS configurations on the somatosensory system in healthy subjects. Results of *Study I* showed that HD-tDCS targeting M1, DLPFC and DLPFC+M1 did not modulate the somatosensory pain and detection thresholds more than sham-tDCS in healthy subjects. The results of *Study II* demonstrated that the experimental prolonged pain model could produce hyperalgesia and pain reflected in deep tissue pressure pain sensitivity but not in pain and detection sensitivity of cutaneous stimuli. Additionally, the study demonstrated that HD-tDCS did not modulate the sensory thresholds significantly differently than Sham-tDCS but did delay the hyperalgesia from establishing, indicating modulation of specific pain-related mechanisms. *Study III* demonstrated that the pain model perturbed the up-stream facilitatory pain mechanism, but not the down-stream antinociceptive pain mechanism or the cuff-pressure sensitivity at the legs. The HD-tDCS antagonized the pain-model induced maladaptive facilitation of the up-stream pain mechanism but did not modulate the antinociceptive mechanism or the cuff-pressure pain sensitivity, indicating that the efficacy of HD-tDCS appears to be dependent on the state of the brain and nervous system, and may be linked with the presence of sensitized pronociceptive pain mechanisms.

Together the studies presented in this dissertation have hopefully contributed to an improved understanding of the modulatory effects of HD-tDCS on the somatosensory system in healthy humans. Highlighting the potentials and shortcomings of the technology, as well as providing better insight into the functional somatosensory effects of the stimulation.

6.1. FUTURE PERSPECTIVES:

In spite of the increasing interest in the potential of non-invasive brain stimulation, too little research is being conducted in systematically controlled experimental settings. The research field of tDCS has, perhaps too rapidly, leapt to clinical studies of the rehabilitation effects without a solid foundation of the mechanistic and functional underpinnings of the modulatory effects[304]. Particularly the complex interactions of the properties of the electrical field, the neuroanatomical targets and the resulting functional changes are not at all fully uncovered[42,44,62]. Utilizing brain imaging techniques (fMRI-or TMS-navigated tDCS montages) in tDCS-research may improve the predictability of the tDCS effects and minimize non-responders[86,305,306]. Until these interactions are better understood, following consensus criteria of tDCS configurations is vital to ensure reproducibility and comparability between studies[16,37].

Additionally, more systematic studies of the differences in modulatory effects of HD-tDCS compared to its technological predecessor, conventional tDCS, are necessary to navigate the direction of the technological development.

Finally, as demonstrated in the present thesis, the effects of tDCS are brain-and nervous system state dependent, making experimental studies in healthy subjects challenging to translate to pathological conditions with perturbed nervous systems. The considerable variance in the baseline state of a research population may be detrimental to the scientific outcome of experimental studies, so highlighting intersubject variations in the population or ensuring homogenous population samples is important. Uncovering biomarkers that can help identify which subjects will benefit from tDCS would also be helpful in this regard. The present thesis has highlighted the potential of conducting controlled experimental studies with pain models that mimic the central and functional symptoms of the pain conditions. Utilization of these models may contribute to decreasing population heterogeneity in studies and enable better future studies.

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