**Aalborg Universitet**

# NEUROPLASTIC RESPONSES AFTER PROLONGED EXPERIMENTAL PAIN AND MULTIFOCAL TRANSCRANIAL DIRECT CURRENT STIMULATION

**BY**
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DISSERTATION SUBMITTED 2023

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# CV

Luisina holds the title of Bioengineer from the Faculty of Engineering at the University of Entre Rios, Argentina. Her early research experience is focused on evaluation of physical stress on cardiovascular and respiratory outcomes on healthy individuals. She was enrolled at Aalborg University as a Marie Skłodowska-Curie FRESCO PhD fellow to continue her research path on a broader multimodal assessment of neurophysiological responses during prolonged pain. Her PhD was supervised by Prof. Thomas Graven-Nielsen and co-supervised by Assistant Prof. Ana Maria Zamorano at the Center for Neuroplasticity and Pain (CNAP) at the Department of Health Science and Technology. During the PhD period, she has taken part in supervision of students' projects, revision of manuscripts in peer-reviewed journals and three research collaborations. Luisina's PhD project was supported by the Horizon 2020 research and innovation programme (Grant no. 754465) and the Danish National Research Foundation (DNRF 121). Her main research interests are trait and state-like markers of cognitive function and pain sensitivity.

# ENGLISH SUMMARY

Chronic pain occurs in approximately 20% of the adult population worldwide having a direct impact on the physical, mental, and social well-being. Given that the evaluation and management of pain disorders is challenging when co-morbidities are at play, experimental models of prolonged pain are instrumental in studying the physiology of nociception and analgesia as well as understanding the time course and degree of sensitization during pain progression, exploring its potential link to pain-free states, and assessing the therapeutic value of novel interventions.

One of the most frequently investigated noninvasive brain stimulation (NIBS) methods to modulate pain-induced plasticity is transcranial direct current stimulation (tDCS). Classical single site tDCS of the motor cortex (M1) shows, however, mild therapeutical effects on pain relief. Since brain regions do not operate isolated but interact with other regions through various excitatory and inhibitory projections, an arising research venue targets brain networks through multifocal tDCS. Multifocal tDCS stimulates multiple and distant areas over the scalp simultaneously to potentially promote advantageous plasticity for substantial recovery. Specifically, following multifocal tDCS of the motor network (network tDCS), a higher modulatory effect on corticomotor output has been reported in comparison to a classical single-site tDCS montage. The neurophysiological and psychophysical effects of this montage are, however, not fully studied.

The primary objective of this PhD project is, therefore, to investigate and characterize the time course of neuroplastic changes through event-related evoked potentials and resting-state EEG of the human brain after multifocal tDCS of the motor network while experimental pain progresses over the course of 24 hours.

To provoke prolonged pain, patches of topical capsaicin were applied on two consecutive days (studies I, II and III). To probe the temporal dynamics and nature of psychophysical and neurophysiological responses during pain, conditioned pain modulation (study I) and motor evoked potentials by single-pulse transcranial magnetic stimulation (study I), cortical event-related evoked potentials (ERPs) to noxious stimulation (study II) and resting-state EEG activity (study III) were assessed before and during pain. To evaluate the modulatory effect of multifocal tDCS, two daily sessions of network tDCS were applied (study I, II and III). The motor network was selected as cortical target because it has been stated that the motor cortex has a role in the modulation of the descending inhibitory pathways.

The findings of the first study indicate that prolonged pain reduces conditioned pain modulation expression and corticomotor output while active network tDCS normalizes such responses, as compared to sham. The results of the second and third studies show that prolonged pain downregulates the amplitude of ERPs and the frequency of alpha oscillations, respectively, whereas active network tDCS increases such cortical reorganization, compared to sham stimulation.

In conclusion, the results of this PhD work display that network tDCS can modulate the consequences of prolonged pain on neurophysiological outcomes and the descending pain inhibitory function otherwise reduced after 24-hour experimental pain.

# DANSK RESUME

Kroniske smerter forekommer hos cirka 20 % af den voksne befolkning på verdensplan, hvilket har en direkte indvirkning på deres fysiske, mentale og sociale velbefindende. Evalueringen og håndteringen af smerteforstyrrelser er udfordrende, især når komorbiditeter er involveret. Eksperimentelle modeller af langvarig smerte er afgørende for at studere fysiologien af nociception og analgesi samt for at forstå tidsforløbet og graden af sensibilisering under smerteprogression og dets sammenhæng med smertefri tilstande.

En af de mest undersøgte metoder til ikke-invasiv hjernestimulering (NIBS), med henblik på at modulere smerteinduceret plasticitet, er transkraniel jævnstrømsstimulering (tDCS). Klassisk enkeltplacering af tDCS på den motoriske cortex (M1) viser dog kun milde terapeutiske effekter på smertelindring. Da hjerneregioner ikke fungerer isoleret, men interagerer med andre regioner gennem forskellige excitatoriske og hæmmende projektioner, fokuserer ny forskning på hjernenetværk gennem multifokal tDCS. Multifokal tDCS stimulerer flere og fjernere områder over hovedbunden samtidigt for potentielt at fremme fordelagtig plasticitet og væsentlig genopretning. Specifikt er der rapporteret højere modulatorisk effekt på kortikomotorisk output efter multifokal tDCS af motornetværket (netværk-tDCS) sammenlignet med klassiske tDCS-monteringer. Dog er de neurofysiologiske og psykofysiske virkninger af denne montering ikke fuldt ud undersøgt.

Hovedformålet med dette PhD-projekt er derfor at studere og karakterisere tidsforløbet af kortikal reorganisering og psykofysiske responser efter multifokal tDCS af det motoriske netværk, mens eksperimentel smerte skrider frem i løbet af 24 timer.

For at fremkalde langvarig smerte blev der påført capsaicin-plastre topisk over en periode på to dage (undersøgelse I, II og III). For at undersøge den tidsmæssige dynamik og karakteren af psykofysiske og neurofysiologiske reaktioner under smerte, blev tilstandssmertemodulation (studie I), motorisk fremkaldte potentialer ved transkraniel enkeltpulsstimulering (studie I), sensorisk fremkaldte potentialer ved skadelig stimulation (studie II) og alfa-svingninger (undersøgelse III) vurderet før og under smerte. For at evaluere den modulerende effekt af multifokal tDCS blev der udført to daglige sessioner med netværk-tDCS (undersøgelse I, II og III). Det motoriske netværk blev valgt som kortikalt mål, fordi det er blevet påvist, at den motoriske cortex spiller en rolle i moduleringen af de nedadgående hæmmende veje.

Resultaterne fra den første undersøgelse indikerer, at langvarig smerte reducerer betinget smertemodulation og kortikomotorisk output, mens aktiv netværk-tDCS normaliserer sådanne reaktioner sammenlignet med sham-behandling. Resultaterne fra den anden og tredje undersøgelse viser, at langvarig smerte hæmmer amplituden af sensorisk fremkaldte potentialer og frekvensen af alfa-oscillationer, mens aktiv netværk-tDCS øger en sådan kortikal reorganisering sammenlignet med sham-behandling.

Konklusionen af dette PhD-projekt viser, at netværk-tDCS kan modulere konsekvenserne af langvarig smerte på neurofysiologiske udfald og den faldende smertehæmmende funktion, som ellers er reduceret efter 24 timers eksperimentel smerte.

# ACKNOWLEDGEMENTS

It is a pleasure to give a huge thank you to the people that have contributed to this PhD journey with technical input, invaluable support, and time. Your support has made this journey not only possible but also unforgettable. To Prof. Thomas Graven-Nielsen, thank you for the opportunity to be immersed in groundbreaking projects and cutting-edge technology, and for developing both the research and researchers with an insightful and constructive perspective. To Assistant Prof. Anna Zamorano, thanks for guiding me through the labyrinthine maze of academia and scientific research.

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joy through it all – and for venturing forth as the daring first volunteer in the uncharted territories of my long pilot studies too.

# PREFACE

This PhD thesis summarizes my research work completed at the Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University, Denmark, in the period from April 2018 to March 2021.

The aim of the thesis was to expand the understanding of the temporal dynamics of experimentally induced long-lasting pain through psychometric, psychophysical, and neurophysiological assessments and to characterize the influence of multifocal tDCS while such experimental pain developed over the course of 24 hours. The psychometric assessments evaluate pain affect, catastrophizing thinking and sleep quality, and the psychophysical assessments evaluate a paradigm of conditioned pain modulation. The neurophysiological assessments evaluate evoked responses through motor (MEPs) and cortical event-related (ERPs) evoked potentials as well as resting-state responses through electroencephalography (EEG).

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# TABLE OF ABREVIATIONS

ACC: Anterior cingulate cortex

CPM: Conditioned pain modulation

EEG: Electroencephalography

EMG: Electromyography

ERPs: Event-related evoked potentials

FDI: First dorsal interosseus (muscle)

ICI: Intracortical inhibition

M1: Primary motor cortex

MCS: Motor Cortex Stimulation (Invasive)

MEPs: Motor-evoked potentials

NGF: Nerve-growth factor

NRS: Numerical rating scale

PAF: Peak alpha frequency

PPD: Pressure pain detection thresholds

PPT: Pressure pain tolerance thresholds

S1: primary sensory cortex

S2: secondary sensory cortex

tDCS: Transcranial direct current stimulation

TMS: Transcranial magnetic stimulation

TSP: Temporal summation of pain

VAS: Visual analogue scale

Some concepts used throughout the dissertation:

**classical tDCS:** conventional tDCS using two big-size electrodes: one electrode applied on the target cortical region and the second electrode usually placed on the orbitofrontal cortex. The area of each electrode is generally 25 or 35 cm$^2$.

**multifocal tDCS:** tDCS using multiple electrodes in widespread areas of the brain. The area of each electrode is approximately 3.14 cm$^2$, but it is dependent of the brand of the tDCS equipment.

**focal tDCS (ring configuration):** This tDCS paradigm uses 1 central anode/cathode and 4 surrounding return electrodes. This paradigm is also known as high definition tDCS (HD-tDCS). The area of each electrode is approximately 3.14 cm$^2$, but it is dependent of the brand of the tDCS equipment.

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# CHAPTER 1.  INTRODUCTION

## *1.1 STIMULATION OF THE MOTOR CORTEX*

Throughout history different forms of non-invasive brain stimulation (NIBS) have been studied in healthy and clinical populations. One of the most applied NIBS paradigms is transcranial direct current stimulation (tDCS), which has been progressively evaluated from simple forms of tDCS powered by rudimentary components to sophisticated and patient-friendly devices[1] using two big-sized electrodes.

The primary motor cortex (M1) has garnered significant attention and research interest as a cortical target for pain modulation since the 1950s, when invasive stimulation of the motor cortex (MCS) was found to induce antinociception in animal models[2]. Later, based on that evidence, methods of invasive motor cortex stimulation in humans were developed, leading ulteriorly to motor cortex stimulation using epidural electrodes. Initial evidence showed epidural MCS has driven long-lasting pain relief in approximately 66% of neuropathic patients[2].

The early 1990s underscored the effectiveness and utility of repetitive transcranial magnetic stimulation (rTMS). Research showed that responsiveness to rTMS of the M1 is a fair predictor of epidural MCS-driven analgesia. At the end of 1990s, work using tDCS indicated analgesic results in clinical populations with a far less invasiveness and lesser discomfort. Over time, to reduce the poor spatial focality of the existing tDCS devices at the time, a multifocal paradigm of tDCS was designed to deliver direct currents in several brain areas with focused stimulation using small-sized electrodes and computer-assisted modelling combined with neuroimaging.

The content of section 1.1.1 will summarize the state of the art of tDCS and will cover the main differences and similitudes of classical tDCS and multifocal tDCS with a focus on the M1.

### 1.1.1.  TRANSCRANIAL DIRECT CURRENT STIMULATION: FROM CLASSICAL TO MULTIFOCAL PARADIGMS

Classical tDCS delivers a low constant current through relatively big electrodes (e.g. 35 cm$^2$) placed over specific areas of the scalp. Classical tDCS applied over the M1 exerts changes in cortical and corticospinal

excitability, functional connectivity indexes[3–5], metabolite levels, and at some extent, psychophysical responses[6,7].

tDCS has the qualities to be tolerable[8–12], portable, and can be operated by the patient itself[13,14]. Passive sham protocols are considerably standardized consisting of ramping up and ramping down periods during the first 30 and the last 30 seconds of the stimulation period. The current intensity during the rest of the stimulation period (after the ramping up and before the ramping down period) is 0. It is also established that double-blinding and cross-over studies are feasible[15–17].

tDCS produces local effects in the brain regions directly under the stimulation electrodes[18] and widespread effects in distant brain areas through functional connections[19,20]. The immediate effects[21] of tDCS consist of shifting, through non synaptic mechanisms, the resting membrane potential of cortical neurons towards either depolarization or hyperpolarization. The after-effects occur beyond the duration of the tDCS session and relies partly on synaptic mechanisms. The synaptic mechanisms depend on pre-synaptic and post-synaptic activity, akin to long-term potentiation and long-term depression-like plasticity.

A plethora of studies evaluated the potential antinociceptive effects of M1 tDCS in healthy pain-free populations. It is important to mention the mixed or inconsistent findings in the scientific literature. While some publications report an improvement of pain sensitivity measures such as pain thresholds[22,23], motor execution[24,25] and descending inhibitory function[26,27], other work obtained inconclusive results[23,28,29]. For example, classical M1 tDCS[30] and focal M1 tDCS (ring configuration)[16,31] did not modulate mechanical, pressure or thermal pain sensitivity in healthy individuals.

Randomized clinical trials applying several M1 tDCS sessions in chronic pain populations show certain degrees of antinociception e.g. in fibromyalgia[32–34] and neuropathic pain[35] patients, improved conditioned pain modulation (CPM) in osteoarthritis and post-surgical pain[36], as well as improved motor performance, reaction times and corticomotor output in Parkinson patients[37]. No clear differences though are observed in other multi-session trials from different research groups in neuropathic patients (with e.g. unilateral neuropathic pain and stable medication), as compared with the control stimulation (classical sham tDCS or focal sham tDCS)[13,38–40].

A reason behind conflicting findings in healthy[23] and clinical[13] cohorts may be due to underpowered studies i.e. small sample sizes. In healthy

individuals, evidence also suggests the absence of tDCS-driven modulation due to a lack of sensitized pathways[16,41]. In clinical cases, another reason behind conflicting findings is the heterogeneity in clinical profiles and comorbidities, challenging the interpretation of the outcomes.

Recent tDCS paradigms named multifocal tDCS were designed using small-sized electrodes displaying two main attributes: on one side, multifocal tDCS delivers focal stimulation by replacing big-sized 35 $cm^2$ rectangular electrodes for 3.14 $cm^2$ circular electrodes; on the other side, researchers may be able to enhance the modulatory capacity through the application of multifocal tDCS in functionally associated brain areas rather than targeting solely one single region. For instance, bilateral anodal stimulation of the left and right M1 has improved corticomotor excitability and motor performance while classical tDCS only modulated the former[42]. Also, stimulation of the left M1 and regions of its resting state motor network has increased corticomotor excitability beyond classical tDCS paradigms and has extended this facilitation for a longer period of time[43]. This research venue is clearly new though further attention and comprehensive studies are needed to understand what the exact effects are.

## 1.2 EXPERIMENTAL MODELS OF PAIN

Experimental models of pain have the capacity to model certain features of the pain experience to study its neural mechanisms[44], psychophysical responses, and even the effectiveness of therapeutical interventions[45–47]. Short-lasting experimental pain lasts from seconds to tens of minutes and can be provoked, for instance, through hypertonic saline injections[48–50], heat stimulation[51], intradermal capsaicin[52–56], and high frequency stimulation[57,58], which primarily excite nociceptors and affect pain sensitivity e.g. primary and secondary allodynia and hyperalgesia, as well as trigger neurophysiological changes e.g. cortical and corticomotor excitability changes.

Experimental and clinical observations emphasize the need for longer-lasting pain models though to understand the influence of the variable *time* on the pain system. Long-lasting pain models generally last for hours or days and can be provoked through e.g. injections of nerve-growth factor (NGF), exercise-induced delayed onset muscle soreness (DOMS), and prolonged exposition to topical capsaicin, which may resemble the temporal profile of certain clinical conditions such as neuropathic disorders[59].

It has been established that NGF injections and/or DOMS on a specific muscle elicit movement-evoked pain, deep-tissue pressure primary and widespread hyperalgesia[60], reduced muscle strength and altered cortical and corticomotor output[61–63].

Administration of topical capsaicin displays ongoing pain and secondary hyperalgesia and allodynia[6], attributes shared with neuropathic disorders[45,46,64], altered descending inhibition as indexed through CPM[65] and aberrant functional connectivity[66,67]. Relative to clinical settings, results of a meta-analysis of pharmacological efficacy in experimental pain models indicated that capsaicin may be a promising predictor to clinical analgesia[59] e.g. in neuropathic pain[47].

Taken altogether, the temporal factor of experimental pain may be determinant to study the physiology of nociception and analgesia in the transition from acute to prolonged pain.

## *1.3 METHODS FOR PROBING PAIN NEUROPLASTICITY*

Pain is a multidimensional and complex experience affecting the sensory-discriminatory, cognitive-attentional, and affective levels. It can be, therefore, evaluated in multiple edges. As a result of acute and chronic pain, a range of peripheral and central mechanisms are modulated inducing psychophysical, psychometric, and neural adaptive and maladaptive responses. Psychophysical responses can be studied for instance through batteries of quantitative sensory testing, temporal summation of pain (TSP) and CPM paradigms while psychometric responses can be measured using questionnaires exploring pain affect, catastrophizing thinking, anxiety and depression, general quality of life and sleep quality. Neurophysiological adaptations can be studied through changes in metabolite concentration (through e.g. magnetic resonance spectroscopy), cortical (e.g. electroencephalography - EEG) and subcortical activity and functional connectivity (e.g. functional magnetic resonance imaging - fMRI), transsynaptic corticomotor excitability (resting motor thresholds, I waves, intracortical inhibition – ICI, intracortical facilitation – ICF, amplitude of motor evoked potentials - MEPs), sensory and noxious reflexes (e.g. electromyography - EMG), to name a few.

In line, and as mentioned in section 1.2, topical capsaicin produces on-going long-lasting pain[66], mechanical secondary hyperalgesia[68], reduced CPM[65], altered intracortical inhibition[69], reduced MEPs[69], as well as reduced PAF[70].

Recent work additionally investigates the combined analysis of MEPs and PAF[71] as biomarkers of pain sensitivity in experimental prolonged and clinical cases.

This type of altered responses are found in patients with chronic neuropathic profiles with e.g. impaired CPM[72,73], abnormal glutamatergic levels in the anterior cingulate cortex (ACC)[74,75], altered resting-state functional connectivity in the default-mode networks[76], reduced peak alpha frequency (PAF)[77] and, as compared with non-neuropathic patients and healthy controls, reduced ICI[78] and MEPs[73,79].

## *1.4 AIMS OF THE PRESENT THESIS*

The four aims of this PhD project are enumerated as follows and are represented in Figure 1-1:

    A.  To evaluate the sensory and affective responses during prolonged experimental pain

    B.  To evaluate the direction and temporal dynamics of event-related responses during prolonged experimental pain

    C.  To evaluate the direction and temporal dynamics of resting-state cortical responses

    D.  To evaluate whether tDCS of the motor network can modulate the sensory, affective, and neurophysiological (event-related and resting-state cortical) alterations during prolonged experimental pain.

The framework to achieve these aims were:

1) **Provoking pain-related neuroplasticity:** Topical capsaicin was applied for 24 hours to provoke prolonged experimental pain (chapters 3, 4 and 5).

2) **Probing pain-related neuroplasticity:**
   Self-reported pain was evaluated through numerical pain scales, psychophysical assessments and psychometric questionnaires were administered to probe sensory and affective changes following prolonged pain (chapter 3).
   Motor-evoked potentials and cortical evoked potentials to noxious stimuli were performed to probe event-related changes following prolonged pain (chapter 4).
   Resting-state EEG activity (chapter 5) was analyzed using frequency analysis to probe resting-state cortical alterations.

3) **Modulating pain-related neuroplasticity**: To evaluate the modulatory effects of tDCS, 2 daily sessions of tDCS of the motor network were applied and their effects assessed on sensory-affective (chapter 3), event-related (chapter 4), and resting cortical (chapter 5) components.

Throughout the thesis, the publications involved in the PhD work are referred to as:

Study I: **Gregoret L.**, Zamorano AM., Graven-Nielsen T.  (2021) Effects of multifocal transcranial direct current stimulation targeting the motor network during prolonged experimental pain. European Journal of Pain, 25(6) 1241-1253. DOI: 10.1002/ejp.1743

Study II: **Gregoret L.**, Zamorano AM., Graven-Nielsen T (2023) Multifocal tDCS targeting the motor network modulates event-related cortical responses during prolonged pain. Journal of Pain, 24(2) 226-236. DOI: 10.1016/j.jpain.2022.09.010

Study III: **Gregoret L.**, Zamorano AM., Graven-Nielsen T (submitted) Electroencephalographic peak alpha frequency is reduced during 24-hour experimental pain and normalized after multifocal tDCS
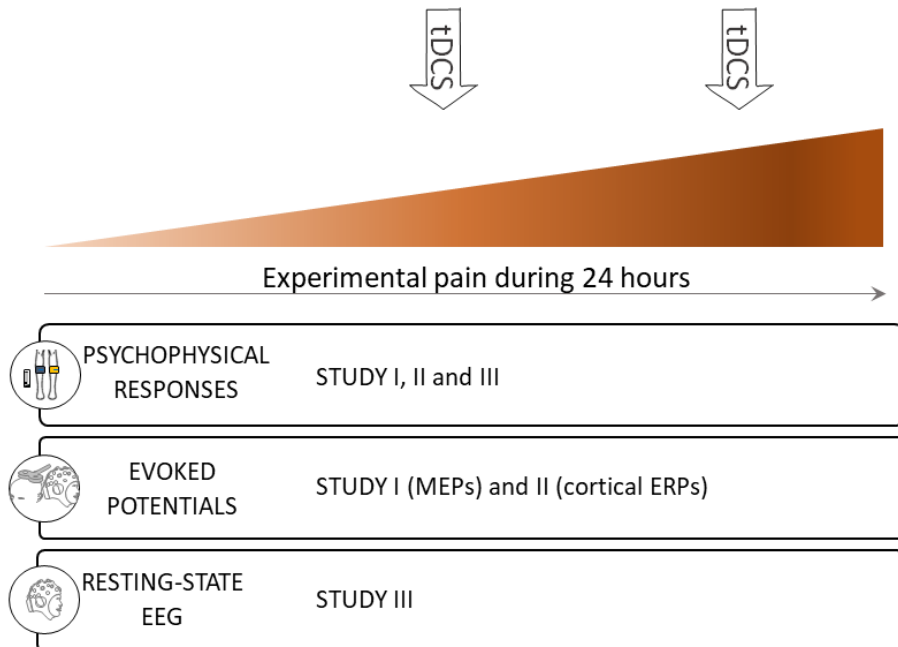


**Figure 1-1: Schematic of the general content of the present thesis.** Prolonged pain was elicited through the exposition to topical capsaicin for 24 hours (Studies I, II and III). Multimodal assessment of prolonged experimental pain consisted of psychophysical measures (Study I, II and III), motor evoked potentials (MEPs) (Study I), cortical event-related evoked potentials (cortical ERPs) to noxious stimulation (study II) and resting-state

electroencephalography (EEG) (study III). The impact of 2 (two) daily sessions of transcranial direct current stimulation (tDCS) was evaluated at the end of Day 2 (Study I, II and III).

Study I and Study II are based on one experiment with 38 healthy individuals and Study III is based on another experiment with 44 healthy individuals.

**Research questions**

A) Can topical capsaicin for 24 hours induce prolonged pain? (Chapter 3) Study I and III
B) Can topical capsaicin for 24 hours modify descending inhibitory function? (Chapter 3) Study I
C) Can topical capsaicin for 24 hours modify neurophysiological responses? (Chapter 4 and 5) Study I, II and III
D) Can network tDCS modulate pain perception during prolonged pain? (Chapter 3) Study I and III
E) Can network tDCS modulate descending inhibitory function during prolonged pain? (Chapter 3) Study I
F) Can network tDCS counteract pain-related neurophysiological responses (MEPs, N2P2 amplitudes, alpha oscillations)? (Chapter 4 and 5) Study I, II and III
G) Are there associations between pain-free PAF at baseline and pain intensity during prolonged experimental capsaicin pain? (Chapter 5) Study III

The general hypothesis was that prolonged pain would alter mood, catastrophizing thinking, and sleep quality, increase pain sensitivity as well as would reduce corticomotor excitability and electroencephalographic alpha oscillations. Conversely, it was hypothesized that network tDCS would counteract corticomotor excitability and electroencephalographic alpha oscillations.

# CHAPTER 2. TRANSCRANIAL DIRECT CURRENT STIMULATION OF THE MOTOR CORTEX

## *2.1 THE ROLE OF THE MOTOR CORTEX IN tDCS-DRIVEN PAIN MODULATION: Possible mechanisms*

The M1 is a brain region that plays a central role in movement preparation and execution, motor imagery, and learning new motor skills. It is expanded in individuals with refined motor abilities[80], and altered in those experiencing pain[62,81,82]. The M1 has anatomical or functional connections to bilateral primary sensory cortices (S1)[83], to its contralateral M1[43,83], frontoparietal[43,84] and cingulate cortices[85,86], thalamus[86] and cerebellum[83], among others. When it comes to motor cortex stimulation through tDCS, the mechanisms of tDCS-driven analgesia are unresolved. Some of the putative mechanisms are[87,88] corticothalamic inhibition, indirect inhibition of primary sensory processing, endo-opioidergic changes, descending pain inhibition and placebo-like effects (Figure 2-1).

Neuroimaging analyses in healthy individuals show that classical anodal M1 tDCS activates the ipsilateral thalamus[4,89,90], including the ventroposterolateral thalamic nucleus[90], which receives input from the spinothalamic tract involved in the transmission of nociceptive inputs, along with thermal[91], mechanical and proprioceptive inputs[92] and projects to the S1. That evidence may support the notion of top-down modulation through corticothalamic inhibition of nociceptive afferent volleys by stimulation of the M1 through tDCS. Recent fMRI-based connectivity analysis indicated classical anodal M1 tDCS increased corticothalamic coupling, in contrast to both cathodal and sham M1 tDCS[93].

Another putative tDCS mechanism relies on corticocortical pathways between S1 and M1[94]. Anatomical connections between the M1 and inhibitory neurons within the S1 were observed in rodents[95]. In that line, invasive stimulation of the M1 through MCS in animal models reduced sensory processing on the S1, as compared with stimulation of other cortical areas[88,96]. This clearly cannot be directly generalized to humans and to other stimulation paradigms such as tDCS and rTMS. In humans, this potential mechanism could be studied using cortical ERPs to innocuous stimulation or using other neuroimaging technologies through, for instance, source localization analysis. Tangentially associated to this putative mechanism,

active finger movements ipsilateral to noxious laser stimulation on the hand dorsum produced a reduction of activity on the contralateral S1 and secondary sensory (S2) cortices as well as a reduction of perceived pain, compared to the control condition[97]. These pieces of evidence may support the notion that modulation of M1 may contribute to pain reduction through inhibitory corticocortical projections to the S1, though clearly warrants further research.
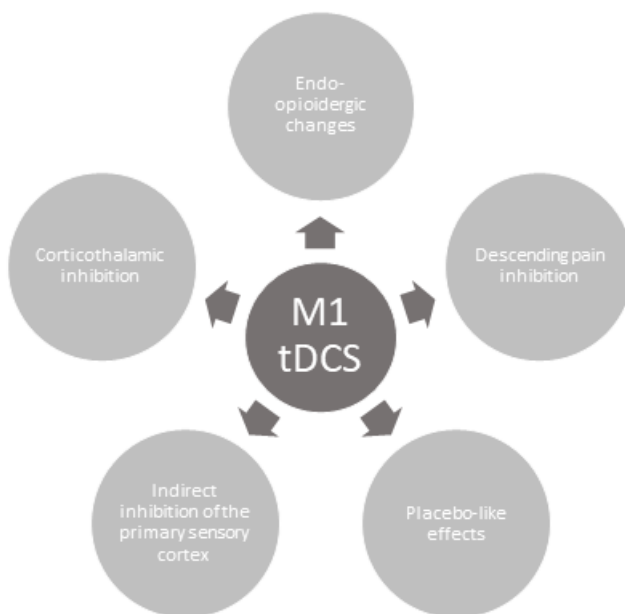


**Figure 2-1:** Putative mechanisms of M1 tDCS induced analgesia/antinociception.

Endogenous opioids are neuromodulators that bind to opioid receptors and, as a result of modifying the properties of their neural targets, they affect the release of their neurotransmitters. Endogenous opioids have then the capacity to modulate pain if that effect happens in neural targets involved in pain processing. Although the M1 has a relatively scarce degree of endogenous opioids, evidence shows M1 tDCS activates remote brain areas rich in µ-opioids. Following anodal M1 tDCS, there was increased activity, for example, in putamen[4,93] and cingulate cortex[68]. As well, a clinical observation using µ-opioid receptor radiotracers in PET scans suggested that anodal M1 tDCS reduces the binding potential levels of opioid receptors in neural structures involved in pain processing e.g. posterior thalamic nuclei, nucleus accumbens, as well as insular and cingulate cortices[98]. That type of finding should be confirmed in a full-powered study and possibly also using opioid antagonists e.g. naloxone, to understand if the lower binding

potential levels are related to the release of endogenous opioids. Opioid-driven analgesia is often reflected by reduced hyperalgesia and allodynia[99,100]. In line, some M1 tDCS studies have improved pain sensitivity[87] as indexed through increased detection[6] and pain thresholds. Although speculative, in rodents, administration of opioid antagonists lowered sensory processing on the S1 following invasive M1 stimulation[96], hinting that an M1-driven antinociceptive effect is connected to the endo-opioidergic system.

Connected to the endogenous opioidergic system and to the descending pain modulatory network, fMRI-based analysis has shown that M1 tDCS modulates BOLD responses in cortical and brainstem regions involved in descending inhibitory pathways e.g. ACC, and PAG[19], frequently affected in chronic pain patients. Improved CPM expression following M1 classical tDCS and focal M1 tDCS (ring configuration)[27,36,101,102] has been reported in several publications over the years in healthy and clinical cohorts. Recent work reported improved CPM effect after focal M1 tDCS (ring configuration), as compared with sham tDCS, while focal DLPFC tDCS (ring configuration) did not significantly modulate this outcome[103]. Stimulation of the M1 through tDCS, in other words, may trigger a top-down activation of descending pain inhibition towards the spinal cord and modulation of transmission of painful inputs.

Finally, a putative mechanism for tDCS analgesia is related to placebo-like or Hawthorne-like effects, wherein subjects or patients in the control tDCS intervention, often times, sham tDCS, experience analgesic effects. Sham tDCS aims to emulate the somatosensory sensations i.e., paresthesia induced by the electrical stimulation on the scalp without delivering significant electrical currents. To achieve that, passive sham tDCS protocols deliver electrical stimulation for a small-time window at the beginning (onset) and at the end (offset) of the stimulation period. This means that in these sham protocols, the intensity of the electrical current is null after the onset and before the offset. The placebo-like effect is multifactorial, including expectations[104] and beliefs[39]. Research shows the influence of tDCS-related expectations leading to favorable outcomes after tDCS. Positive framing before tDCS interventions has led to higher cognitive performance as compared with a negative framing group, both receiving the same tDCS protocol[104]. Samartin-Veiga and colleagues recently observed that 15 sessions of both sham and active tDCS improved clinical pain, fatigue, sleep quality and indexes of quality of life in fibromyalgia patients[38,39]. This placebo-like effect was observed after M1 tDCS but also after tDCS of other cortical targets such as the operculoinsular and prefrontal cortices[38,39]. Documenting subjects' expectations and beliefs as well as the general information provided to subjects prior to NIBS treatment may contribute to

understand causal effects of stimulation and reduce inter-subject variability[104].

It also remains elusive if the above possible mechanisms are complementary rather than exclusive. Some of the challenges are due to confounding factors e.g., comorbidities, limitations of existing neuroimaging technologies (e.g. low granularity, low spatial or temporal resolution), and inter-subject and intra-subject variability. Unlike stimulation paradigms such as MCS and M1 rTMS, classical tDCS of the left primary motor cortex does not exhibit level A efficacy in pain modulation on clinical disorders when considering sham-controlled and double-blinded studies[29,39]. Further research is needed to understand the influence of stimulation parameters e.g. current intensity, stimulation duration, and the number of stimulated areas (bipolar, focal (ring configuration), multifocal[94]), to potentially induce antinociception in chronic and experimental pain. A summary of multifocal tDCS studies targeting the M1 is listed in Appendix A and multifocal tDCS studies targeting solely other cortical targets in Appendix B.

## 2.2 CLASSICAL AND MULTIFOCAL tDCS

The scientific literature describes several tDCS paradigms with different electrode montages. Figure 2-2 illustrates the electrode montage and induced normal electrical field of A) classical anodal M1 tDCS, B) focal M1 tDCS (ring configuration) and C) multifocal tDCS of the resting-state motor network (network tDCS).

As mentioned in section 1.1.1., classical or traditional tDCS uses a bipolar electrode configuration. Classical M1 tDCS applies the target electrode on the M1 (usually the C3 position) and the return electrode usually on the orbitofrontal cortex (e.g. Fp2 position).

Focal M1 tDCS (ring configuration) also targets the M1 but in a different manner. It uses a target electrode in the C3 position and return electrodes in four surrounding positions. The inter-electrode distance varies across publications.

Multifocal tDCS applies constant current stimulation in several focused areas of the scalp. Unlike classical tDCS with rectangular electrodes (i.e. 5 x 5 or 7 x 5 cm$^2$) that delivers non-focal stimulation as well as higher current densities at the electrodes' edges[12,105,106], multifocal tDCS with smaller circular electrodes (approx. 2 cm of diameter) minimizes those challenges and offers the possibility to apply tDCS at multiple cortical sites simultaneously. Focal and multifocal tDCS, according to previous evidence, amplify and prolong excitation or inhibition, beyond classical tDCS paradigms[43,101,107–109].

Specifically, the network-tDCS montage applies anodal currents on the left (C1, C3) and right (C2, C4 and T8) M1 and cathodal currents on the prefrontal (Fz) and parietal (P3 and P4) cortices.
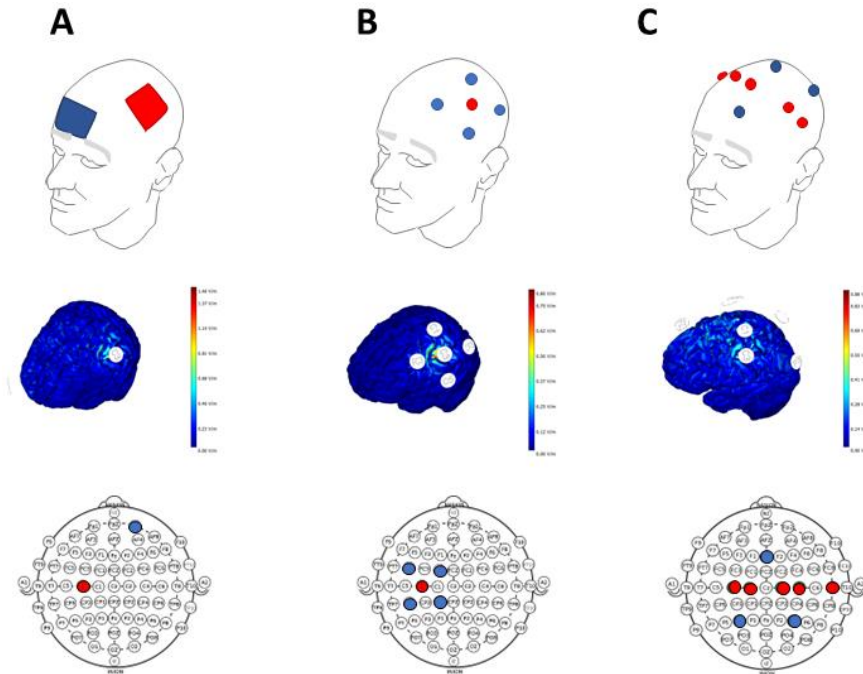


**Figure 2-2: Schematic of three different tDCS protocols** with their electrode montage and 3D brain plot of the normal component of the modelled electric fields (NIC2 software, Neuroelectrics, Spain). Anodal currents are shown in red and cathodal currents in blue. A) Classical anodal M1 tDCS B) focal M1 tDCS (ring configuration) C) tDCS of the resting-state motor network (network tDCS).

The network-tDCS paradigm was selected and investigated in the present PhD work (Figure 2-2-C).

### 2.2.1. MULTIFOCAL TDCS OF THE MOTOR NETWORK

Following the notion that the different areas of the brain do not work isolated but rather operate through neuronal projections in various networks, the resting-state motor network paradigm (network tDCS) was developed (Figure 2-2C) through resting-state functional connectivity magnetic resonance imaging using a seed-based analysis with the left motor cortex as

the target area while subjects were at rest[43] as first step. As a result of such analysis, relative to a region of interest on the left M1 cortical representation, the tDCS montage was designed assigning positive currents to the positively correlated brain areas and negative currents to the negatively correlated ones described elsewhere[43]. The current values of anodes are C1=872 µA, C3=1135 µA, C2=888 µA, C4=922 µA, T8=183 µA, and cathodes FZ=-1843 µA, P3=-1121 µA and P4=-1036 µA[43].

The goodness of fit using the normal component of modelled electric fields of classical M1 tDCS, network tDCS and a control tDCS paradigm (called network-mismatched tDCS) indicated that the network tDCS paradigm had a higher match with the motor network as indexed through weighted cross correlations[43].

The administration of 10-min network tDCS elicited higher corticomotor output in healthy individuals, as compared with classical M1 tDCS and the control tDCS paradigm at different time points as well significantly prolonged this excitation 60 minutes after while the control stimulations returned to baseline values[43]. This happened even though the normal and total electric fields induced on the left M1 were lower using the active network tDCS approach than sham network tDCS and classical M1 tDCS. Plus, evidence shows that the sensitivity of neural networks to weak electric fields e.g. induced by low currents, is higher than that of single neural units[110], suggesting that stimulation of functionally associated regions may drive higher modulation. It has been established that classical tDCS protocols stimulating the left motor cortex with higher total output currents do not produce significantly higher corticomotor output[111]. In other words, higher stimulation intensities do not seem to be a determining factor to higher modulation of corticomotor excitability.

# CHAPTER 3. NETWORK tDCS EFFECTS ON PSYCHOPHYSICAL RESPONSES

The assessment of psychophysical and psychometric responses in the present PhD work comprises subjective pain scores (Study I, II and III), conditioned pain modulation (Study I) as well as psychometric questionnaires (Study I and III) such as the Positive and Negative Schedule (PANAS) to assess pain affect, the Pain Catastrophizing Scale (PCS) to assess catastrophizing thinking, and the Pittsburgh Sleep Quality Index (PSQI) to assess sleep quality.
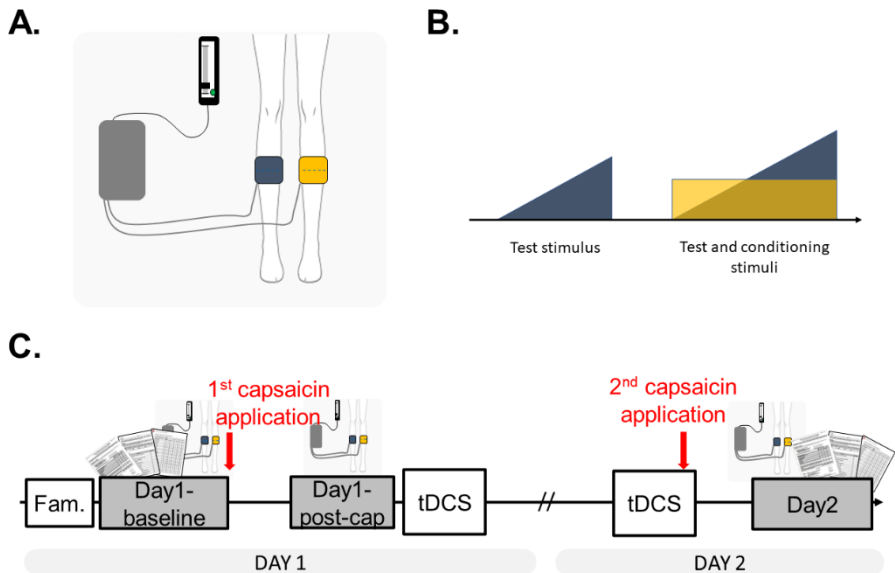


**Figure 3-1: Psychophysical and psychometric assessments in the present PhD work. A)** User-independent cuff-pressure algometry was applied on the calves to evaluate a CPM task (image modified from Gregoret et al., 2020). **B)** The CPM paradigm consisted of the test stimulus applied on the right calf (**blue** ramp) and the conditioning stimulus (**yellow** rectangle) on the left calf. The conditioning stimulus was applied at a constant pressure equal to 70% of the pain tolerance threshold of the left leg (previously measured) while the test stimulus ramped up starting from 0 kPa until the respondent could not tolerate the pressure any longer, pressing a button to

stop the stimulation. **C)** Diagram of study I. After familiarizing every respondent with the general workflow of the experiment procedure (Fam.), each respondent completed a CPM task and filled out the psychometric questionnaires (Day1-baseline). After the 1st capsaicin application, subjects completed again the CPM task (Day1-post-cap). To assess the effects of 2 sessions of tDCS and a 2nd capsaicin application, subjects filled out the psychometric questionnaires and completed the CPM task again. Subjects reported pain scores on a regular basis during the two-day experiment (except sleeping hours).

## *3.1 PAIN, CPM EXPRESSION AND PSYCHOMETRIC RESPONSES DURING PROLONGED PAIN*

### 3.1.1. SUBJECTIVE PAIN SCORES ARE EXACERBATED OVER TIME

The pain model in the three PhD studies (Study I, II and III) consisted of 2 daily applications of topical capsaicin patches (8%, 4 x 4 cm$^2$) on two different skin areas of the dorsum of the right hand[113,114]. Fixed at 0 (no pain) and 10 (worst pain imaginable)[115], the subjects reported subjective pain scores using a verbal numerical rating scale (NRS) during in-lab hours, and using the visual analogue scale (VAS) during off-lab hours via pain diaries. To evaluate the temporal profile of this pain model, pain was reported regularly during in-lab hours (every 20 minutes in study I and II, and every 10 minutes in study III). As a result of prolonged exposition to topical capsaicin, current and averaged pain scores significantly increased over time (Figure 3-2), as compared with the pain onset stage (Day2 vs Day1-post-cap1).

Pain scores in Study I, II and III were comparable to 1-hour[65,66] and 24-hour topical capsaicin[66] studies. The increased capsaicin pain observed in the present PhD work relies on increased peripheral nociceptive input, via the transient receptor potential vanilloid 1 (TRPV1) channels[59] in polymodal small-diameter unmyelinated C and to, a lesser extent, myelinated Aδ fibers[116]. Such activation triggers a cascade of reactions[117] and the onset of centrally induced pain mechanisms e.g. secondary allodynia[6,59] and secondary hyperalgesia[66,67] and decreased functional connectivity on hubs of the default-mode network[66,67]. Prolonged capsaicin pain also disrupts homeostatic plasticity as indexed through MEPs changes[118]. This homeostatic effect also happens in experimental muscle pain[119] and in chronic low back pain patients[120], as compared with healthy pain-free individuals.

Unlike previous research[65,66], which applied one single capsaicin patch for 24 hours, the present PhD work applied in total 2 capsaicin patches (on two different skin areas of the hand dorsum) per respondent in the same amount of time: the first patch on Day1 and the second patch on Day2 to ensure prolonged capsaicin pain given that Bement and colleagues reported reduced current pain scores after 24 hours using a 4 x 4 cm$^2$ patch.
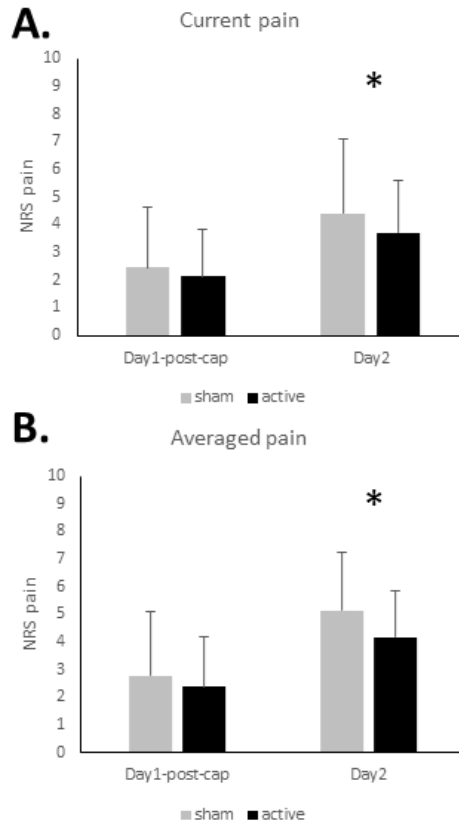


**Figure 3-2: A) Current and B) averaged pain scores combining all PhD studies.** at Day1-post-cap and Day2. Current and averaged pain increased at Day2, compared with the initial pain stage (Day1-post-cap) in both active and sham groups. Significantly higher than Day1-post-cap (*, p<0.001).

## 3.1.2. CPM AND PSYCHOMETRIC RESPONSES ARE AFFECTED DURING PROLONGED PAIN

The diffuse noxious inhibitory control (DNIC) system is a regulatory mechanism that inhibits nociceptive signals from the brainstem to the dorsal horn of the spinal cord in response to the activation of nociceptive pathways[121,122]. Descending pain inhibition can be assessed through CPM paradigms under the concept of "pain-inhibits-pain" often seen in healthy populations. Efficient CPM exerts a top-down pain reduction of a painful afferent input as a consequence of simultaneously or sequentially administering another painful afferent input heterotopically.

The CPM paradigm in study I consisted of cuff-pressure algometry (Figure 3-1A), applied on the right and left calves to deliver the test and conditioning stimulus, respectively. The conditioning stimulus was applied at 70% of the pain tolerance threshold (PTT) on the left leg (previously measured) while the test stimulus ramped up (Figure 3-1B) starting from 0 kPa until the respondent could not tolerate the pressure any longer, point at which they were asked to press a button to stop the stimulation. During that CPM task, subjects rated their perceived pain on the right calf using a digital VAS (0 no pain, 10 worst pain imaginable). The CPM-effect was finally calculated as the subtraction of the conditioned and unconditioned values at pressure pain detection thresholds (PDT) and PTT.

The analysis revealed that CPM-effect significantly reduced during the initial stage of pain at PDT (Figure 3-3A), in line with previous work[65]. CPM-effect at PTT only became significant after the 24-hour period (Figure 3-3B) though a tendency for reduced CPM happened at the initial stage of pain, as compared to baseline CPM levels. These results are supported by the exacerbated catastrophizing and sleep quality levels after 24-hour pain in study I and also in prior work, wherein CPM expression is reduced by sleep deprivation[123,124] and negatively correlated to pain catastrophizing levels[125–127] and pain duration[72,128].

Deficient descending pain modulation quantified by CPM tasks has been extensively described in chronic pain conditions[73,129–132] and correlated with disrupted periaqueductal gray (PAG)-to-dorsal pons connectivity when compared with pain-free subjects[129]. At a cortical level, reduced CPM efficacy is also linked to an engagement of cingulate and prefrontal cortices[133], which could be a reason behind inhibited CPM expression[134].
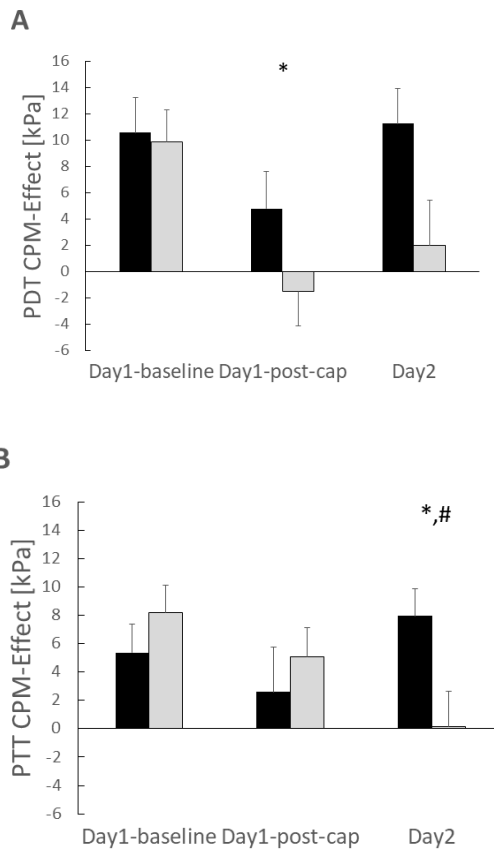
**Figure 3-3: CPM findings** at **A**) pressure pain detection (PDT), and **B)** at pressure pain tolerance (PTT) thresholds in the active (**black**) and sham (gray) groups. Significantly lower in the sham group compared to Day1-baseline (*, $p < 0.05$). Significantly lower compared to the active group (#, $p < 0.05$).

Specifically, during capsaicin-heat pain, connectivity reduced in hubs of the default-mode network as well as the descending pain modulatory system[68]. Given that higher CPM effect was linked to higher functional connectivity between the M1 and the PAG[135], modulation of such cortical activity may therefore represent an strategy for reverting pain neuroplasticity and potentially shape the descending pain modulatory system[136,137].

## 3.2 PAIN, CPM, AND PSYCHOMETRIC RESPONSES AFTER tDCS

### 3.2.1. SUBJECTIVE PAIN SCORES ARE NOT SIGNIFICANTLY MODULATED BY TDCS

The subjective current and averaged pain scores (Study I and III) increased over time when considering the pain onset stage (Day1-post-cap in Study I and Day1-post-cap1 in Study III) and Day2), indicating a consistent painful response after both active and sham network tDCS (Figure 3-2) and no apparent influence of active network tDCS on pain modulation during prolonged pain. When combining subjects from all PhD studies, there is not a statistically significant analgesic effect of network tDCS either (see appendix C section i), defying the benefit of network tDCS as a method for analgesia.

In line, prior work shows that a single session of classical M1 tDCS does not induce analgesic effects in heat pain[30] and capsaicin-heat pain[19] either. Further evidence shows that a small number of sessions (nr of sessions≤3) does not attenuate pain scores neither in chronic pain[29,40] after classical M1 tDCS, nor in experimental muscle soreness after multifocal M1-DLPFC tDCS[7]. It could be argued that a small number of tDCS sessions does not impact the pain system at the perception level even in sensitized individuals with long-lasting experimental and clinical pain. Challenging that hypothesis though, studies delivering multiple sessions of classical and multifocal tDCS of either M1 tDCS[13], DLPFC tDCS[138] and tDCS of the operculoinsular cortex[138] did not induce pain relief as compared to sham stimulation. Attributed to placebo effects[138], the sham stimulation also induced pain relief[13,138] and improved quality of life[38].

Interestingly, in that same study, the 15 sessions of either active tDCS of the M1, the DLPFC and the operculoinsular cortex enhanced mood at a larger extent than sham[138]. In Study I, two sessions of active network tDCS kept pain catastrophising thinking comparable to baseline pain-free levels but not mood. On the same outcomes, the results of Study III showed non-significant effects underlining the inter-subject variability of the pain model and of network tDCS.

## 3.2.2 CPM AND PSYCHOMETRIC RESPONSES ARE MODULATED BY TDCS

The results of enhanced CPM following a facilitatory tDCS protocol[27,36,101,103] have been confirmed and expanded in Study I, where CPM at PTT was improved following active network tDCS, as compared to sham. A neural basis behind these results may be linked to M1 excitability changes ulteriorly affecting supraspinal pathways involved in CPM such as the periaqueductal grey matter (PAG)[129] and rostral ventral medulla[27,68], driving descending inhibitory volleys through the spinal cord. The analysis of Study I revealed that positive and negative affect remained significantly affected by capsaicin pain in both active and sham groups, while catastrophism and sleep quality significantly changed only in the sham group, as compared to baseline levels. The analysis of study III revealed positive affect exacerbated in both active and sham groups, but the remaining psychometric measures remained below statistical significance even though the sample size in both studies was comparable. Those results possibly display that network tDCS contributes to relieve certain aspects of the pain experience such as catastrophizing thinking and sleep quality but not reliably or at least it is dependent of a subject's individual responsiveness to tDCS, to CPM[27], and/or the pain model.

In humans, Reidler and colleagues showed the first evidence of improved descending inhibition as measured with a CPM task following classical M1 tDCS[27]. The facilitated CPM expression was later replicated by two research groups following focal M1 tDCS (ring configuration) in healthy pain-free individuals using a similar CPM paradigm[26,103]. Challenging these results though, focal M1 tDCS (ring configuration), DLPFC tDCS (ring configuration) and M1-DLPFC tDCS (ring configuration) did not significantly affect CPM in a healthy pain-free population[7] and under experimentally induced muscle soreness[7]. Specifically during capsaicin pain, anodal M1 tDCS increases functional connectivity between the M1 and the PAG[19], compared to cathodal tDCS and shows only a tendency compared with sham. Taken altogether, these pieces of evidence warrant further investigation of inter-subject variability in the response to tDCS in different experimental pain models.

In clinical cohorts, M1 tDCS improved CPM[27,36,101,139] in osteoarthritis[102], and post-surgical pain[36], reducing in the latter cohort analgesics consumption after surgery compared with sham. Active M1 tDCS facilitated CPM effect compared with sham tDCS[139,140], while hyperalgesia was induced through

the administration of a µ-opioid receptor agonist, suggesting that patients who develop opioid-related hyperalgesia may be a relevant target population for further tDCS studies and potentially benefit from M1 tDCS.

It is important to note that active network tDCS did not significantly modulate CPM at PDT at Day2 (Figure 3-2A). A reason for such results may be related at least partly to placebo effects since tDCS blinding analysis shows a low guessing rate of the stimulation type (active, sham). Plus, the increased pain scores at Day2 may discard the possibility of increased CPM due to lower pain sensitivity or habituation to capsaicin effect.

## 3.3 MAIN FINDINGS OF THE CHAPTER

The main findings adding to the current knowledge are:

1. Current and averaged pain increased over time when considering the pain onset stage (Day1-post-cap in study I and Day1-post-cap1 in study III) and the 24-hour stage (Day2).
2. CPM-effect was reduced at PDT at Day1-post-cap, as compared with Day1-baseline. CPM-effect was reduced at PTT at Day2 in the sham group, as compared with Day1-baseline.
3. Affect was modulated but not consistently across Study I and III. Positive affect quantified by the PANAS questionnaire is the only one that was consistently modulated (and reduced) after 24-hours of pain in Study I and III.
4. Active network tDCS modulated CPM-effect at PTT but not at PDT.
5. Both active and sham network tDCS showed a tendency to increased CPM at pain detection levels on day 2.

# CHAPTER 4. NETWORK tDCS EFFECTS ON EVENT RELATED POTENTIALS

Event-related potentials are generally elicited through the repeated delivery of electrical, contact-heat, magnetic, auditory, or laser pulses, among other modalities. As a result of such stimulation, the nervous system integrates responses of varying amplitudes and latencies[141–145]. In the present work, event-related responses were studied based on motor-evoked potentials (MEPs – Study I) using single-pulse transcranial magnetic stimulation and electromyography (EMG – Figure 4-1A) and based on cortical event-related potentials (ERPs – Study II) to noxious electrical stimulation (Figure 4-1B) using electroencephalography (EEG).
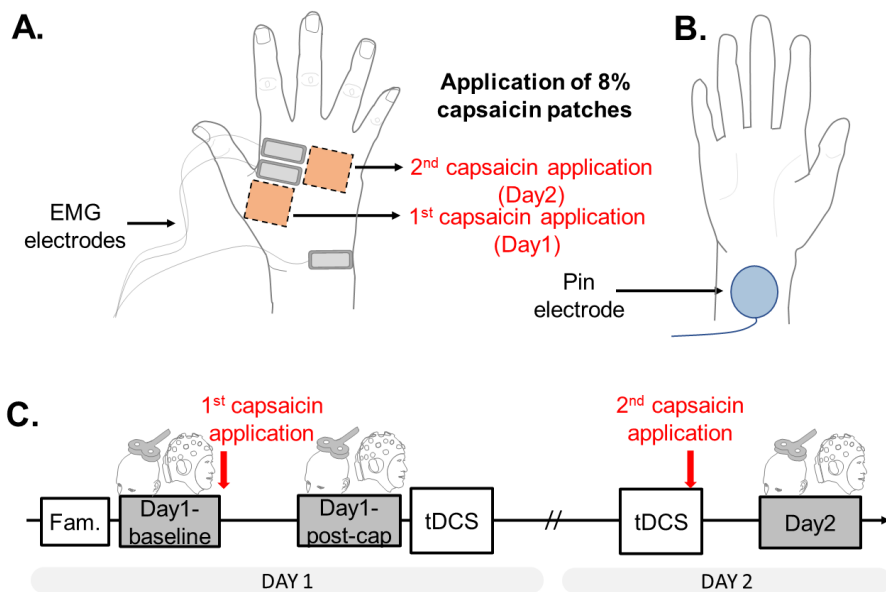


**Figure 4-1:** Electrode placement for **A)** MEPs (study I) on the dorsal part were collected placing bipolar electrodes on the belly of the first dorsal interosseus (FDI) muscle and a reference electrode on the ipsilateral ulnar styloid process, and **B)** cortical ERPs (Study II) on the distal volar side of the right arm (modified from Gregoret et al., 2021). **C)** Experimental diagram of

study I and II. After familiarization with the lab procedures (Fam.), MEPs and cortical ERPs responses were collected at baseline (Day1-baseline), after 25 minutes of capsaicin application on Day 1 (Day1-post-cap), and after receiving a second capsaicin patch as well as two daily sessions of either active or sham network tDCS (Day2).

# 4.1 MOTOR EVOKED POTENTIALS

The interplay between pain and corticomotor responses can be studied non-invasively using for instance single and double-pulse TMS. To infer corticomotor excitability indexed by the amplitude of MEPs, TMS is generally applied on the cortical representation of the primary motor cortex of a target muscle. Single-pulse TMS delivers monophasic pulses that, when above resting thresholds, elicit action potentials across corticospinal output projections[145], which are evaluated through EMG placing i.e. bipolar electrodes on the belly of the target muscle and a reference electrode typically on a relevant non-muscular–point. Given that TMS-induced MEPs over the first dorsal interosseus (FDI) muscle have acceptable intra-subject reliability in pain-free[147] and painful states[148], study I investigated corticomotor excitability selecting that hand muscle as target (Figure 4-1A).

### 4.1.1. MODULATION OF MEPS AFTER PROLONGED EXPERIMENTAL PAIN

In study I, the amplitude of MEPs did not significantly change 25 minutes after a first application of capsaicin, compared with pain-free baseline values. Martel and colleagues also found no significant changes in the TMS input-output recruitment curve 40 minutes after the application of low concentration topical capsaicin on the forearm[149]. Previous research described though reduced MEPs after 25 minutes of low concentration topical capsaicin application[69,150] in a similar sample size. Pain intensity does not seem to justify the conflicting MEPs findings since pain scores were comparable in the publications that found and the publications that did not find significant MEP reduction. Three possible explanations for these results are linked to capsaicin concentration values (8% in study I and 1-3% in previous studies), to different targeted muscles (hand vs forearm muscles) and to the experiment design. Concerning the latter, a facilitatory impact on MEPs amplitude[151] was observed when electrical phasic peripheral stimulation is delivered prior to (but not after) the administration of single-pulse TMS, suggesting that experiments should be prudently designed to

prevent carry-over effects from stimulation of those modalities. Even though MEPs were collected systematically at the beginning of each assessment in Study I i.e., before recording cortical ERPs, two full assessments were done in the same day and approximately 60 minutes apart from each other. Consequently, that could explain the lack of MEPs changes during the initial stage of pain.

After 24 hours with topical capsaicin, MEPs significantly decreased in the sham network tDCS group compared with pain-free baseline values and compared with Day2 values in the active network tDCS group (Figure 4-2). Although the underlying explanation of corticospinal inhibition during pain is still under debate, it has been suggested to be a transitory adaptive response to protect the body part under pain from further harm[152–154] and to promote tissue recovery. Greater MEPs reduction has been connected to lower levels of kinesiophobia[155] and lower pain severity during short-lasting pain[156]. In line, during short-lasting capsaicin pain, lower kinesiophobia scores were linked to greater reduction in the slopes of TMS-related recruitment curves[157]. Opposed to short-lasting pain, corticomotor inhibition is connected to higher pain severity during long-lasting experimental pain[156,158]. Based on those pieces of evidence, the time course of MEPs changes may indicate adaptive (short term – to promote recovery) and maladaptive (long term - maladaptive motor strategies, kinesiophobia, higher pain) responses. It is important to note there were no significant associations between MEPs depression and pain scores in Study I and other studies[159]. Thus, the degree of such associations is unclear, warranting further evaluation.

On another note, pain-related corticomotor consequences may also be limb-specific or muscle-specific. Hypertonic saline injected to the m. FDI decreased MEPs while identical doses injected to the extensor carpi radialis brevis muscle (using the same cortical hotspot) led to no significant excitability changes in the same cohort[48]. Hypertonic saline doses also elicited opposing MEPs responses between the upper and lower limbs[154] in an otherwise healthy population. These pieces of evidence suggest that different muscles in the upper and lower limbs hold different motor strategies possibly related to survival or self-preservation behavior. Certainly further research is needed to evaluate whether and in, which degree self-preservation strategies (i.e. kinesiophobia) are involved in the development of chronic pain[157] or if they serve other purposes.

Combining the results of MEPs in study II and CPM in study I, no significant associations were observed between CPM effect and MEPs changes neither during prolonged pain nor after active tDCS (see Appendix C section ii). These results challenge the notion that higher corticomotor excitability is associated to higher hypoalgesia. Opposed to that, prior research has reported elevated MEPs along with greater CPM efficiency (negative values) in a healthy pain-free population[160]. The amplitude and duration of MEPs significantly correlated with CPM efficiency when CPM was administered using the cold pressor test and heat pain as conditioning and testing stimuli, respectively. The main differences with this PhD work are the presence of ongoing pain (capsaicin tonic pain vs pain-free individuals) and the CPM paradigm (cuff pressure algometry vs cold pressor test and heat pain).

## 4.1.2. FACILITATION OF MEPS AFTER NETWORK TDCS

While capsaicin pain progressed over 24 hours, study I indicates that active network tDCS facilitates corticomotor excitability compared to pain-free baseline values and to the sham group as well (Figure 4-2). In accordance with the present results, healthy pain-free individuals have exhibited facilitated MEPs after network tDCS[43]. These MEPs findings are in accordance with previous M1 tDCS work showing MEPs facilitation after 30 min, 60 min, and in some cases, 120 min[161] after the session is over, depending on the stimulation parameters (intensity, duration) and electrode montage[162].

Anodal M1 tDCS[163–169] and multifocal tDCS paradigms targeting the M1 bilaterally (anodal currents on both left and right M1)[42,165] have also facilitated MEPs responses in pain-free states. It has been established that the aftereffects of classical anodal M1 tDCS include elevated MEPs along with elevated slopes of TMS I-O recruitment curves and I-waves, which are dependent, at least partly, on receptor efficacy and synaptic (possibly also non-synaptic) plasticity[164]. Generally, the synaptic mechanisms depend on pre-synaptic and post-synaptic activity. In the primary motor cortex (M1), these after-effects involve gamma aminobutyric (GABA) and glutamatergic calcium-dependent neurotransmitters through N-methyl-D-aspartate (NMDA) receptors[108,161,163,164,170–173].

Other studies though show statistically insignificant MEPs changes[174] after active tDCS paradigms. Such opposing outcomes potentially underline intra-subject and inter-subject variability to the response of both, single-pulse TMS and tDCS[175,176] and differences in stimulation parameters, e.g.

stimulation duration, intensity, and sham paradigms across studies. Whether these mechanisms are different when using network tDCS approaches, it remains unknown and exceeds the scope of this PhD work.

Relative to cathodal and sham tDCS, anodal M1 tDCS lowered BOLD responses of the S1, otherwise elevated by capsaicin-heat pain. Anodal M1 tDCS has additionally facilitated functional connectivity between the M1 and PAG[177], as compared with cathodal M1 tDCS. When anodal M1 tDCS was applied in clinical cohorts, MEPs were facilitated in neuropathic[29,178] and migraine patients [109]. Under topical capsaicin pain, stimulation of the M1 using (inhibitory) low frequency rTMS has resulted in elevated pain scores[85], denoting possibly the pain modulatory capacity and state-dependency of the M1. Later work using the same pain model and (facilitatory) high frequency DLPFC rTMS has resulted in reduced pain scores[69,179] along with increased MEPs and reduced ICI values. Combining these results, it appears to be that excitation of the M1 is to some extent linked to pain reduction while inhibition contributes to exacerbation of pain and lower connectivity between the M1 and regions of the descending pain modulatory network.

Anodal-left and cathodal-right M1 tDCS (a type of bilateral tDCS paradigm) did not significantly modulate corticomotor output neither in healthy individuals nor in stroke patients[180]. A later study using a similar bilateral tDCS paradigm also obtained no significant MEPs changes using single-pulse TMS and no GABA and glutamate changes as measured with magnetic resonance spectroscopy[181]. Plus, tDCS studies applying higher currents on the M1 do not enhance MEPs more significantly either[111]. These pieces of evidence may support the notion that anodal currents over both the left and right M1 contribute to significantly higher MEPs than other paradigms (bilateral anodal(left) and cathodal(right) M1 tDCS, classical single-site M1 tDCS).

On the whole, although repeated movements do restore corticomotor output in pain-free states (initially reduced by inhibitory cathodal M1 tDCS), this effect does not seem to occur when in pain[182], possibly due to a lack of resolution of pain-related neurobiological mechanisms e.g. altered homeostatic plasticity and functional connectivity. Such MEPs results underline the role of tDCS in the counteraction of pain-related changes.
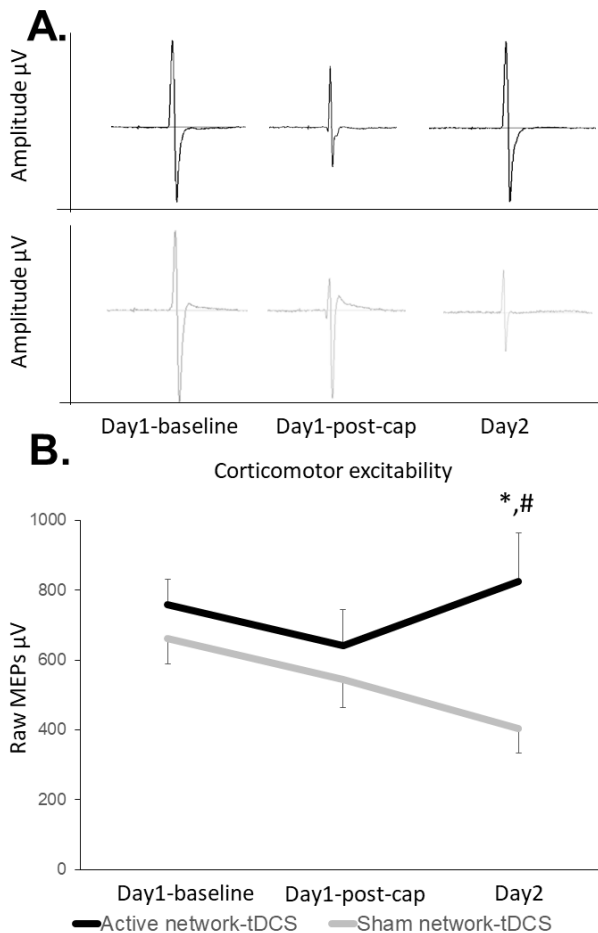
**Figure 4-2: MEPs findings. A)** Temporal profile and morphology of the averaged MEPs response of a representative subject at Day1-baseline, Day1-post-cap and Day2 (modified from Gregoret et al., 2019)). **B)** Corticomotor excitability at baseline before pain induction (Day1-baseline), after 25 minutes of capsaicin application on Day 1 (Day1-post-cap) and after the second capsaicin application as well as the two daily tDCS sessions on Day2 (Day2). Significantly reduced in the sham group compared with Day1-baseline (*, $p<0.05$). Significantly reduced compared with the active group (#, $p<0.05$). (modified from Gregoret et al., 2021)).

## *4.2 CORTICAL EVENT-RELATED POTENTIALS TO NOXIOUS STIMULATION*

The aim of Study II was to understand how the central nervous system processes and integrates repeated noxious stimuli on an already sensitized system due to 24-hour experimentally induced prolonged pain and following network tDCS.

The main outcome was the amplitude of N2P2 components of cortical ERPs at the $C_Z$ position recorded using a 32-channel EEG system following the 10-10 international EEG configuration. To investigate the direction and magnitude of change of these cortical evoked responses, electrical pulses were delivered on the distal volar forearm (Figure 4-1B) by an electrical stimulator at two times the baseline electrical pain thresholds (EPTs) at varying interstimulus intervals fluctuating randomly from 8 to 12 seconds, while experimental pain developed on the hand dorsum. The explanation for such electrode placement (distal volar forearm) is to evaluate facilitated central mechanisms due to capsaicin exposure rather than solely peripheral mechanisms, and at the same time apply it on a skin area suitable for pin electrode placement.

### 4.2.1. REDUCTION OF CORTICAL ERPS DURING PROLONGED PAIN

N2-P2 components of cortical ERPs are biphasic deflections, which arise as a result of, among others, salient afferent stimulation and are mainly detected in the cingulate and the operculoinsular cortices[91,113,184]. The 2 groups of subjects in Study II reduced N2P2 amplitudes 50 minutes following the first capsaicin application (Figure 4-3), supporting previous results of N2P2 depression after topical capsaicin administration[144]. Following the course of 24 hours, N2P2 responses remained reduced in the sham group, denoting the sustained suppression of these cortical responses under prolonged exposition to capsaicin. The N2P2 reduction could be due the contribution of spinothalamic tract (STT) inhibition[185], to a CPM-like effect, to habituation or to salience or attentional shifts from the electrical pulses towards topical capsaicin pain. Reduced N2-P2 amplitudes were observed when delivering laser pulses in the area of capsaicin-induced secondary hyperalgesia[185,186]. Such effect were attributed to STT inhibition[185] in view that laser-related pain scores were not altered in the presence of facilitated central mechanisms. Study II applied instead electrical pulses on a widespread hyperalgesic area, underlying also facilitated central

mechanisms. Given that pain scores due to electrical pulses did not differ before and after capsaicin pain, STT inhibition may also be accountable for this depression.

A CPM-like effect, on the other hand, is unlikely. Even though the application of a CPM paradigm with noxious withdrawal reflex as conditioning stimulus and auditory evoked potentials[187] as test stimulus did result in reduced N2P2 auditory responses, it is important to note that a significant effect of descending pain inhibitory volleys in Study II is dubious because electrical pain thresholds and pain scores due to the electrical pulses remain remained statistically not different and those stimulations were homotopically applied. Additionally, a lack of CPM-like effects have been previously reported when delivering a battery of QST under prolonged capsaicin pain[65].

Habituation to electrical stimulation has also been connected to reduced cortical responses when the stimulation frequency of the stimulation pulses was constant[109,188,189]. As previously mentioned though, the applied stimulation frequency in Study II fluctuated randomly between a range of 0.08 Hz to 0.125 Hz (8 to 12 seconds of interstimulus interval) minimizing a habituation effect.

N2-P2 ERPs are also subject to modulation by attentional shifts[187,190–192] and may therefore be a salience proxy of a given afferent input[189,193] rather than solely nociceptive input. The administration of nociceptive laser and non-nociceptive electrical stimulation on the right hand dorsum as well as the delivery of auditory and visual stimuli elicits N2-P2 responses[113,194], indicating that N2-P2 cortical ERPs are non-specific to pain but strongly linked to the salience appraisal across all modalities[193]. To support this, an exploratory analysis of Study II shows that N2 amplitudes (and not P2 amplitudes) significantly reduced at 50 minutes after capsaicin application (see Appendix C section iii) in both active and sham groups. Future studies are warranted to explore attentional reorientation[187], salience, and unpleasantness in both early stages (Day1-post-cap in Figure 4-1) and in prolonged stages (Day2 in Figure 4-1) of pain through e.g. attentional scales. Simultaneous sensory and painful stimulation, in fact, "compete for representation" within the upper nervous system[113,191,192,195,196].

Finally, the direction of change of cortical ERPs responses seems to be dependent of the type of pain model (deep, intradermal, or superficial) and distinct levels of pain severity and pain duration. While topical capsaicin

reduces N2P2 amplitudes[144], intradermal capsaicin, by contrast, increases N2-P2 amplitudes when the stimulation pulses are applied on the area of secondary hyperalgesia[56]. Opposing directions of change are also observed in other cases e.g. suppressed S1 processing (early ERPs) in other models like hypertonic saline solution[151,197] and elevated S1 activity under experimental muscle[62] and clinical pain[141].
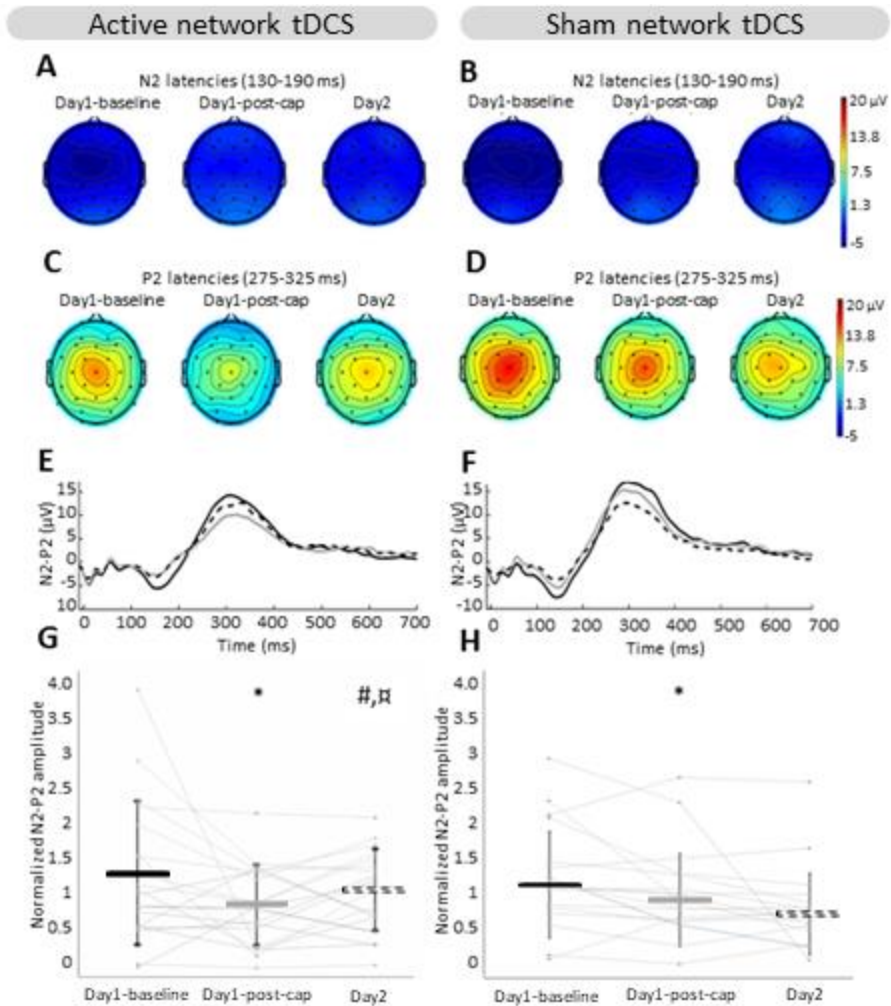


**Figure 4-3: Cortical ERPs findings.** Scalp topographies at Day1-baseline, Day1-post-cap, and Day 2 in the active and sham groups of **A-B)** N2, and **C-D)** P2 responses. Grand average (above) of the N2P2 component of ERPs to noxious stimulation after **E)** active and **F)** sham network tDCS (modified from Gregoret et al., 2023). Mean (±SD) of N2P2 peak-to-peak amplitudes in

the **G)** active and **H)** sham groups. Significantly reduced compared with Day1-baseline levels (*, p<0.05). Significantly increased compared with Day1-post-cap (#, p<0.05). Significantly increased compared with the sham group (¤, p<0.05). *Reprinted from Journal of Pain®. Gregoret, L; Zamorano, A; Graven-Nielsen, T. "Multifocal tDCS targeting the motor network modulates event-related cortical responses during prolonged pain" Vol 24, 2, p. 226-236. Copyright 2023 with permission from Elsevier.*

## 4.2.2. CORTICAL ERPS ARE FACILITATED AFTER NETWORK TDCS

Unlike corticomotor output, the impact of tDCS on cortical ERPs remains less explored. This means there is a scarce number of scientific publications evaluating the influence of tDCS on these cortical outcomes. The findings of Study II reveal that network tDCS antagonizes N2-P2 inhibition during prolonged pain, as compared with sham levels and with values before receiving tDCS (Figure 4-3). Csifcsak and colleagues obtained reduced N2P2 responses after cathodal M1 tDCS but no changes after anodal M1 tDCS in healthy pain-free individuals[198], whereas in a different study also in healthy pain-free individuals, N2-P2 amplitudes elevated after anodal M1 tDCS[199]. It is important to observe the significant rise in that study occurred as compared with baseline (rather than when compared with sham)[199]. In view of that evidence in pain-free individuals, the results of this PhD work may be related at least partly to the input specificity of LTP-like changes after anodal direct current stimulation observed in *invitro* studies[200]. This implies that pathways with facilitated ongoing activity (at context-related synapses) will undergo further modulation[200] as opposed to pathways with low ongoing activity. Direct current stimulation may promote, in other words, synaptic plasticity in neural pathways already experiencing certain degree of neural plasticity. To support that, tDCS[109] and rTMS[201] of the M1 modulated N2P2 responses in chronic patients while N2P2 results in healthy pain-free individuals are less conclusive in both rTMS[202] and tDCS[174,198].

Interestingly, under capsaicin pain, inhibitory low frequency rTMS reduced N2P2 amplitudes compared with sham rTMS[85], showing an opposite direction of change as compared with facilitatory NIBS paradigms, including network tDCS. Other network-based tDCS paradigms targeting simultaneously the premotor area, supplementary motor area, and the M1 enhanced modulation of electrophysiological responses when compared with focal tDCS (ring configuration) targeting solely the M1[203].

As discussed in chapter 3, network tDCS did not modulate pain at the perception level since pain scores due to electrical pulses remained comparable in both active and sham groups. Opposed to that, under capsaicin pain, low frequency rTMS led to N2P2 reduction accompanied by higher pain scores[85]. Those results possibly hint different modulatory capacities of these 2 NIBS (tDCS vs rTMS).

During thermal pain stimulation, anodal M1 tDCS has reduced regional cerebral blood flow (rCBF) in the anterior insula[30], a region associated with salience detection[113] and considered, alongside the dorsal ACC, one of the primary nodes of the salience network[204]. Decision-making related to salience is also associated to this subregion (anterior insula) though. An exploratory analysis of Study II indicates that tDCS significantly modulated the P2 but not the N2 amplitudes (see Appendix C section iii) during prolonged capsaicin pain. Whereas the N2 components of evoked potentials is commonly associated to salience detection, the P2 components are attributed to perceptual processing but both influenced by attention[191].

## *4.3 MAIN FINDINGS OF THE CHAPTER*

The main findings adding to the current knowledge are:

1. Reduced N2-P2 and N2 amplitudes after 50 minutes of capsaicin pain.
2. Reduced N2-P2, N2 and MEPs responses after ~24-hour pain in the sham group only.
3. Capsaicin pain produces a reduction of cortical (N2P2) and corticospinal (MEPs) responses, but it seems they occur at different times.
4. Active network tDCS facilitates cortical and corticospinal responses, compared with sham stimulation.
5. Specifically, active network tDCS significantly facilitates N2-P2 and P2 amplitudes, as well as MEPs.

# CHAPTER 5. NETWORK tDCS EFFECTS ON RESTING-STATE EEG

This chapter shows the findings of the frequency analysis of resting-state EEG activity after active and sham network tDCS. The main EEG outcomes were the peak alpha frequency (PAF) and alpha power in the 8-12 Hz range. PAF was estimated using the center of gravity[205–207] (CoG) formula and alpha power was quantified with fieldtrip routines[208]. The experiment design of Study III is illustrated in Figure 5-1 representing the EEG assessments before pain induction (Day1-baseline), 25 minutes and ~90 minutes after the first capsaicin application (Day1-cap1 and Day1-cap2, respectively) and on Day 2 after receiving the second tDCS session. In every assessment, resting-state EEG was collected for 4 minutes with eyes closed while subjects sat comfortably on a chair.
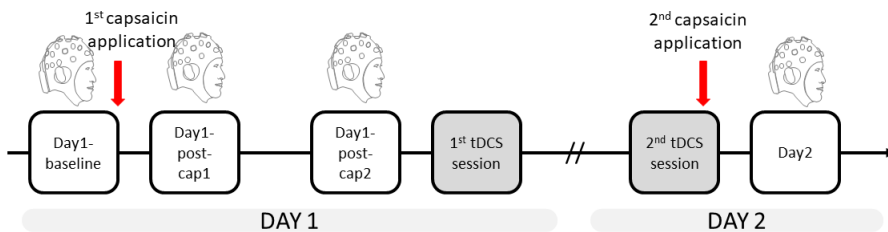


**Figure 5-1: Experimental design of Study III.** EEG activity was recorded at the beginning of Day1 (Day1-baseline), 25 minutes (Day1-post-cap1) and ~90 minutes (Day1-post-cap2) after the first capsaicin patch application, and 25 minutes after the second capsaicin patch application on Day 2 (Day2). Each topical capsaicin patch was applied at the end of Day1-baseline assessments and 5 minutes before the end of 2nd tDCS session at Day2. A session of network tDCS (active, sham) took place at the end of Day1 and at the beginning of Day2.

## 5.1 NINETY-MINUTE AND 24-HOUR EXPERIMENTAL PAIN MODULATES THE FREQUENCY OF ALPHA OSCILLATIONS

Study III adds to the evidence of decreased PAF, in this case, after 90 minutes (Figure 5-2 – in both active and sham groups) and after 24-hour pain (sham group only). Past research has reported decreased PAF during short-lasting heat pain for approximately 5 minutes[209] and capsaicin-heat pain after approximately 20 minutes of incubation[210,211]. Longer-lasting pain models through NGF injections and exercise-induced DOMS reduced PAF

(centroparietal and parietooccipital) after 6 days of pain induction while performing eccentric muscle contractions of the wrist, underlining to an extent the role of ongoing pain on PAF modulation. Importantly, when subjects were at rest, there were not significant PAF changes, which could be credited to the absence of ongoing pain in that pain model.

Reduced PAF also arose during both sensory (warm) and aversive (auditory) stimulation[209]. Such results suggest that PAF is modulated at sensory, attentional, and affective (unpleasantness) levels. Capsaicin-heat pain has reliably reduced PAF (after approximately 20 minutes of incubation), linking it to reduced alpha power in the 10-12 Hz range[206]. The sensitivity to and the presence of pain were attributed in that study to such PAF and alpha power reduction, respectively. It is important to note that alpha power reduced on the ipsilateral side to pain stimulation and contralateral side to the hand that rated pain perception, which pose methodological differences with Study III (pain was rated in a verbal NRS *after* EEG recordings, alpha power was assessed in central electrodes i.e., $C_Z$, C3 and C4). PAF reductions have also been observed in other manipulations i.e., PAF is not exclusively modulated under a painful experience. PAF has decreased during cognitive tasks in both healthy pain-free individuals[212] and traumatic brain injury patients[213].
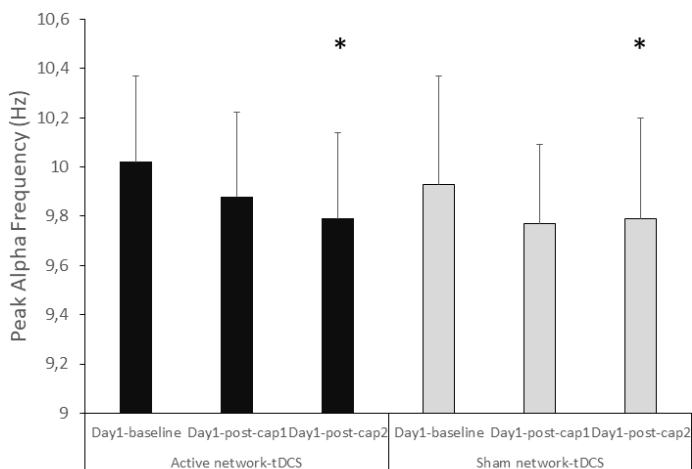


**Figure 5-2: PAF findings during pain.** Mean PAF (±SD) during pain development at Day1-baseline, Day1-post-cap1, and Day1-post-cap2 in the active and sham network tDCS groups. Significantly reduced compared with Day1-baseline (*, $p < 0.05$).

PAF modulation is therefore context-dependent and also possibly dependent of both, the presence of ongoing pain and the duration of pain[214]. In line, PAF in study III decreased after 90 minutes but not after 25 minutes of capsaicin application under this sample size. Previous research indicates that PAF is not reduced in healthy individuals following circa 20 minutes of capsaicin-heat pain[205] as well as following 4 days of muscle soreness[215] (whereas 6 days do reduce PAF). Given that the signal processing method and sample sizes of the current PhD study are comparable to them, it could be presumed these differences rely in part on the temporal dimension of pain. PAF of a clinical population has indeed correlated to each patient's pain duration[214]. Despite the fact that the mechanisms driving PAF reduction during experimental pain is under debate, PAF studies in neuropathic cohorts regard PAF slowing (as opposed to healthy controls) to corticothalamic dysrhythmia[77]. While alpha power modulation appears to be paradoxical in clinical and experimental pain (alpha power is bolstered in clinical pain and lessened in experimentally induced pain), PAF appears to be consistently reduced in both experimental and clinical tests. Although based on conjecture, prior[77,209] and current evidence suggest that PAF reduction is related to sensory input[209] and pain presence instead of to maladaptive/adaptive neuroplasticity.

## 5.1.1. SLOWER PAF DURING PAN-FREE STATES IS LINKED TO HIGHER PAIN SCORES DURING CAPSAICIN PAIN

The present work expands the role of baseline PAF during pain-free states to higher future pain severity. Study III showed a negative correlation between PAF at Day1-baseline and averaged pain in off-lab hours. In line, individuals with slower PAF have been connected to higher self-reported pain as measured through VAS scales during experimentally induced capsaicin-heat pain[70,205] and muscle soreness at rest[71,210,211]. This same response pattern has been shown in neuropathic patients[77]. Even though the EEG analysis in the present work focused in the central area (C3, CZ, C4), evidence shows this effect occurs in multiple regions[77] or in the whole scalp[216].

## 5.1.2. TWENTY-FOUR HOUR PAIN DID NOT SIGNIFICANTLY MODULATE ALPHA POWER

The lack of significant alpha power modulation in Study III supports prior research under phasic heat pain, tonic capsaicin-heat pain and NGF-induced muscle pain at rest[70,205,207,211]. Clinical research also shows no significant alpha power differences between patient populations and healthy pain-free controls, despite the fact that PAF is lower in the former group

(patients)[214]. This is supported by studies from other research groups, wherein no significant alpha modulation of neither local nor global metrics occurred under chronic low back pain, compared with healthy controls[217]. Conversely, tonic heat pain did reduce alpha power while administered for approximately 5 minutes[209]. The primary distinctions between that recent study and this PhD study are the nature of experimental tonic pain (heat pain vs capsaicin pain), intensity, duration, and CoG extraction method (multiple ICs vs one single IC). Source-localized analysis as well as local and connectivity metrics indicate reduced alpha oscillations on the contralateral sensorimotor cortex under also tonic heat pain on the left and right hands[51,218]. Alpha power reduction was also reported during the cold pressor test for approximately 1.5 minutes[219] using a different extraction method (unrelated to components of ICA). These pieces of evidence emphasize that alpha modulation may be dependent to the type of pain (short-lasting and long-lasting experimental pain and clinical pain) and/or the extraction method.

Connecting the work of the previous and this thesis chapter, past research has linked EEG alpha activity and MEPs amplitudes. In short, it was established that the higher the alpha power, the lower the MEPs amplitudes in pain-free healthy individuals. The opposite also applies i.e. the lower the alpha power, the higher the MEPs amplitude. For example, power spectral density analysis using a low-density EEG system and MEPs induced by single-pulse TMS at the cortical motor representation of the abductor pollicis brevis muscle of the right hand negatively correlated alpha power before TMS pulses to the amplitudes of subsequent MEPs[220]. Later, a different research group, using source localization algorithms aimed to characterize if this MEPs-alpha power association was confined to a specific cortical region (local oscillations) or a widespread brain response in the alpha range (global oscillations). The link between alpha power before TMS and MEPs amplitude was significant only when evaluating alpha power on the sensorimotor cortex[221], hinting a local effect. This is also supported by EEG studies investigating the impact of motor tasks on alpha oscillations. For instance, local alpha activity at central electrodes is modulated when performing finger motor tasks [222–225].

More recent research using EEG-based source localization analysis through low resolution brain electromagnetic tomography (LORETA) algorithms and TMS reveal that alpha activity increased over the left DLPFC after approximately 40 minutes of 1% capsaicin application[149], as compared with pain-free states. As compared to the remaining subjects, M1 activity increased in the beta range as well as M1-precuneus connectivity but only amongst subjects with decreased recruitment curve slopes. The

differential responses observed in that study signal that pain influences the motor system, but that effect varies on an individual basis.

## 5.2 NETWORK tDCS FACILITATES PAF DURING PROLONGED EXPERIMENTAL PAIN

After two sessions, active network tDCS improved central PAF values when compared with sham network tDCS and pre-tDCS levels (Figure 5-3), but alpha power was not significantly affected. It has long been assumed that alpha activity directs sensory processing in a rhythmic manner, with states of alpha oscillation desynchronization associated with higher neural processing and synchronization with its downregulation[224,226]. PAF enhancement in the central region (C3, Cz, C4) may thus be linked to reduced sensory processing[227] and increased idling[228,229]. One session of classical M1 tDCS increased PAF in healthy individuals, which could be attributed to endogenous opioid release because of elevated pressure pain thresholds (PPTs) and lowered negative affect[230]. PAF is also increased following intense but not low intensity physical activity, which could rely partly on exercise-induced opioid discharge[231,232]. PAF was found to be higher after classical DLPFC tDCS in patients with neurodevelopmental disorders[233], possibly due to increased alpha coherence between the temporal and prefrontal regions. Given the lack of significant pain modulation by active network tDCS in the current study, the rise in central PAF could be attributed to a direct increase in cortical excitability through non-synaptic mechanisms following active tDCS. This relies on the fact that the network tDCS montage delivers anodal currents over the central electrodes (C1, C3, C2, C4, and T8), changing the resting membrane potential of the nerve cells (dendrites and axons) under the stimulation electrodes towards depolarization (inward flow). Generally, the change of resting membrane potential depends on the intensity and polarity of the electrical current (inward or outward flow)[29,170,234,235], on the specific cortical target (somatodendritic compartments, interneurons and axon endings), the spatial orientation of those targets relative to the induced electric field, and possibly also, to disease-related neuroplasticity[94].

As opposed to the mentioned studies, it was reported that M1 tDCS influences and elevates PAF on distant regions of interest, such as the occipital cortex[230]. That presumption may be, thus, debatable. According to 3D computational modelling and functional connectivity analysis using magnetic resonance imaging, network tDCS creates a lower electric field and current density on the M1 than other montages such as classical M1 tDCS and network-mismatch tDCS[43]. Finally, when assessed using single-pulse TMS, network tDCS elevated corticomotor output outperforming the

mentioned paradigms, signaling that tDCS effects are at least partly mediated by cortical and spinal excitability changes.

Classical M1 tDCS causes referred excitability changes on hubs of the salience and descending pain modulatory networks, which are involved in pain integration and regulation[4,19,167,234,236] but does not seem to affect self-reported pain perception[30]. The current PAF results were significant when subjects with an average pain of at least 2 on a 0-10 NRS scale were considered[205], highlighting the importance of models that evoke moderate prolonged pain. In line with that, as already mentioned in a previous chapter, M1 tDCS attenuated the integration of pain during a TSP paradigm when administering pulses at suprathreshold but not at threshold painful levels[41]. Likewise, tDCS over the M1 and DLPFC has reduced the perceived pain during a TSP paradigm with cuff-pressure algometry[7] when experiencing muscle soreness but not in a pain-free population, signaling that tDCS may act upon affected nociceptive systems with facilitated central mechanisms [19,177].
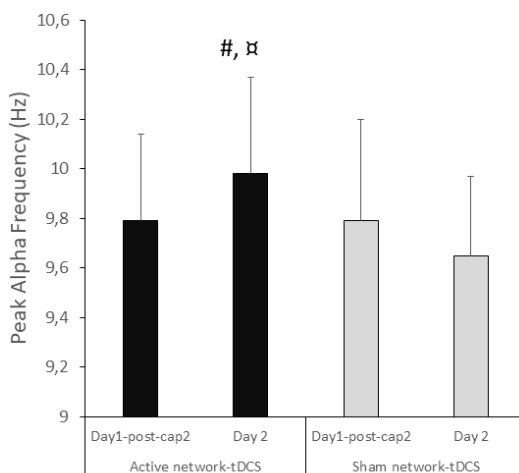


**Figure 5-3: Peak alpha frequency (PAF) findings after tDCS.** PAF increased at Day2 in the active network tDCS group, compared with Day1-post-cap2 and with sham. Significantly increased compared with Day1-post-cap block 2 (*, p<0.008). Significantly higher compared with sham network tDCS group (#, p<0.02).

The absence of significant alpha power changes after network tDCS adds to the existing literature. In healthy individuals, tDCS transmitted over the M1 influenced alpha power as compared with cathodal tDCS but not compared

with sham[237], whereas tDCS applied over the DLPFC elevated delta and theta power but not alpha power[229]. When compared with sham stimulation, five daily sessions of classical anodal M1 tDCS lowered both alpha power in the frontoparietal cortex and pain perception in fibromyalgia patients[238]. In healthy individuals, alpha power changed following one session of anodal tDCS applied over the DLPFC[239], the posterior parietal and the occipital cortices[237,240]. A posterior study detected reduced global alpha power, compared with sham, immediately after both anodal and cathodal M1 tDCS, indicating that tDCS effects on alpha power may be independent of the stimulation polarity[241].

## *5.3 MAIN FINDINGS OF THE CHAPTER*

The main findings adding to the current knowledge are:

1. After 90 minutes of capsaicin application (called Day1-post-cap2 in Figure 5-1), both groups (active and sham) reduced PAF, compared with baseline levels.
2. Two sessions of active network tDCS significantly increased PAF, as compared with PAF values after 90-minutes of capsaicin pain on Day1 and as compared with the sham group on Day2.
3. Alpha power showed no significant main effects nor interactions across the 2-day experiment.

# CHAPTER 6. CONCLUSION & LIMITATIONS

The main goals of this PhD thesis were to evaluate the time course of subjective pain and a set of psychophysical as well as psychometric responses (study I, II and III), event-related potentials (study I and II) and resting-state EEG activity (study III) after undergoing network tDCS during 24-hour experimental pain (Figure 6-1).
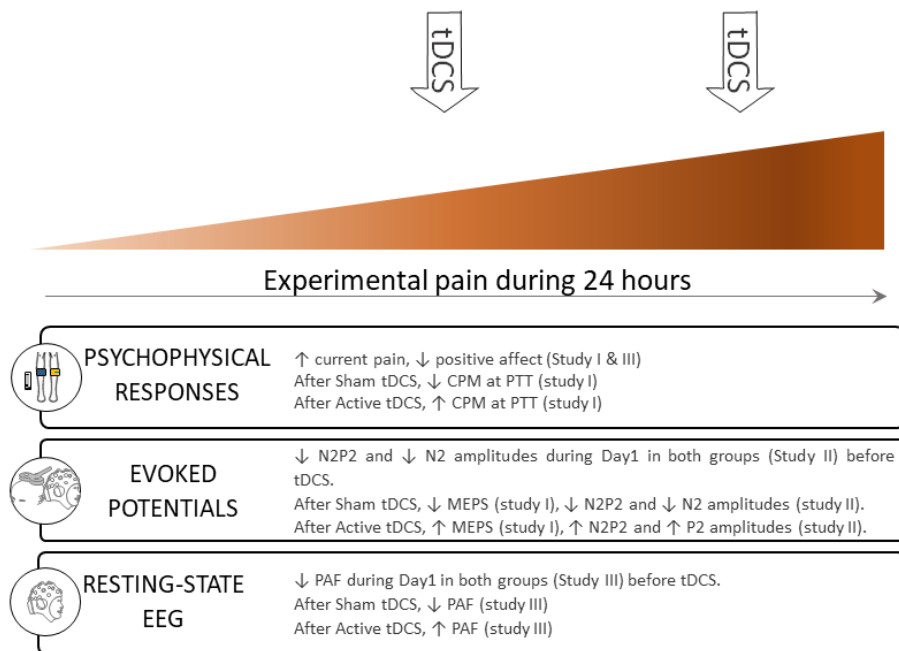


**Figure 6-1: Schematic with main findings of Studies I, II and III.**

## *6.1 CONCLUSION*

The results of this PhD project provide insights and expand the understanding of the time course and direction of change during prolonged pain states and after a facilitatory tDCS protocol using neurophysiological, psychophysical, and psychometric measures.

The proposed capsaicin pain model induced prolonged pain for approximately 24 hours. Positive affect was exacerbated consistently (studies I and III), whereas negative affect, pain catastrophizing and sleep quality were influenced but inconsistent (Study I vs Study III). CPM effect was significantly reduced at PDT after approximately 60 minutes of first patch application in both groups (active and sham), compared with baseline. CPM effect at PTT was inhibited only after 24-hour pain in the sham group while the active tDCS group of subjects showed facilitated CPM expression. N2P2 amplitudes of ERPs showed inhibited responses after approximately 50 minutes of first patch application (Study II). PAF were significantly reduced after 90 minutes in both active and sham groups (Study III).

After 24-hour pain, active network tDCS induced long-lasting neurophysiological changes i.e. facilitated corticomotor excitability, amplitude of N2P2 components of ERPs as well as PAF, while inhibition of those parameters occurred during the same window of time after sham network tDCS. Adding to previous research, this tDCS paradigm did not modulate pain perception in Study I, II and III, emphasizing the limitations it poses for analgesic applications.

The work shown in this PhD thesis explored the feasibility of applying a comprehensive set of neurophysiological, psychophysical, and psychometric measures aimed at characterizing prolonged pain states from different edges and angles. This type of studies can contribute to minimize the impact of confounding factors and the many variables involved in pain studies, and can open the door to multimodal triangulation of results e.g. how resting motor thresholds are connected to tDCS-driven modulation of sensory evoked potentials or how CPM baseline levels are connected to tDCS-driven modulation of affect, to name a few.

## 6.2 LIMITATIONS

Even though, the reliability of the 24-hour capsaicin pain model is warranted, it has been established that corticomotor excitability is stable in half-an-hour periods[242] and shows acceptable reliability during short-lasting and long-lasting periods [242–245]. Pain scores[246], CPM[247], N2P2 cortical ERPs[248] and EEG frequency[249] metrics also showed acceptable reliability. Taken together, these findings indicate that the electrophysiological and psychophysical measures used in this study are not sensitive to time effects.

This PhD work lacked a control group of healthy pain-free individuals to understand if the neurophysiological and psychophysical changes are due to a time effect. Since classical M1 tDCS was not included either, the conduction of experiments including a group receiving classical M1 tDCS would help to understand if the observed effects are linked to the motor network rather than the simulation of the left motor cortex.

The conclusions of this PhD work show that tDCS can modulate neurophysiological responses (studies I, II and III) and based on study I, psychophysical responses during pain. It cannot be ruled out though that this modulation may not be directly associated to pain intensity albeit to shifts of salience or unpleasantness. Future work should consider then a thorough experiment design, wherein salience as well as unpleasantness are investigated for 24 hours and after tDCS sessions.

## 6.2. IMPLICATIONS

The implications for future studies in this field are:

Evaluate neurophysiological (MEPs, cortical ERPs, PAF and alpha power) and psychophysical (sensory and pain thresholds, CPM, TSP) responses once experimental capsaicin pain subsides or when suppressing capsaicin pain by cooling down the capsaicin patch area.

Chronic pain and prolonged experimental pain involve maladaptive and adaptive neuroplaticity, respectively. Network tDCS studies in clinical populations may throw light into the time-course as well as the direction and magnitude of changes in those systems.

These evaluations should be tested with the so-called "active" sham or network-mismatched tDCS, where 4mA of total output current are delivered throughout the 20-min sham stimulation.

Electrode placement for the network tDCS paradigm is based on neuroimaging scans of a small number of subjects (reported by Fischer et al 2017) and adapted using the international 10-10 EEG system. Anatomical differences should be accounted for in the future to achieve optimal electrode positions for every respondent.

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# APPENDICES

# Appendix A. Overview of studies investigating the effects of tDCS with M1 montages on electrophysiological and psychophysical outcomes

A summary of studies targeting the M1 though multifocal tDCS, focal tDCS, and (from 2015 and onwards) classical tDCS are listed in this appendix with the following highlights: the specific tDCS montage (target area), the number of tDCS sessions, the total delivered current, the duration of each session and the type of sham protocol ("active" sham, passive sham).

| Authors | Year | tDCS Montage | Protocol | Main findings |
|---------|------|--------------|----------|---------------|
| Zaki Hanna et al (Bulletin Neuro) | 2023 | Sequential bilateral M1 tDCS | 5 sessions. 2 mA. 40 min. Passive sham | ↓ pain scores and ↓ range of movement after active tDCS in post-surgical neuropathic patients, as compared with baseline. |
| Andre-Obadia et al (Neurotherapeutics) | 2023 | Classical anodal M1 tDCS, hf M1 rTMS | 5 sessions. 2 mA. 20 min. Passive sham. | Similar % of responders in both rTMS and tDCS. ↓ pain scores (29% reduction) in responders to anodal M1 tDCS in drug resistant neuropathic pain. |
| Baik et al (Life) | 2023 | Classical anodal right M1 tDCS | 10 sessions. 2 mA. 20 min. Passive sham. | No significant improvement in pain, depression, and quality of life in patients with central post-stroke pain, compared to sham. Compared to baseline, the active tDCS group improved depression and quality of life. tDCS effects depend on the lesion location. |
| Kold et al (Eur JPain) | 2023 | M1-DLPFC tDCS | 3 sessions. 4 mA. 20 min. | ↓ Temporal summation of pain in the active tDCS group in individuals sensitized with experimentally induced muscle |

| | | | Passive sham. | soreness. |
|---|---|---|---|---|
| Gregoret et al (JPain) | 2023 | Multifocal tDCS of the motor network | 2 sessions. 20 min. Passive sham | ↑ N2P2 amplitudes compared with sham during pain. Pain sensitivity was not significantly changed compared with sham. |
| Jiang et al (Neuroscience letters) | 2022 | Focal M1 tDCS (ring configuration), focal DLPFC tDCS (ring configuration), sham tDCS | 1 session. 2 mA. 20 min. Passive sham | ↑CPM after M1 tDCS. No significant CPM changes after DLPFC tDCS and no significant changes in pain sensitivity after M1 and DLPFC tDCS tDCS. |
| Ianonne et al (Neuroscience research) | 2022 | Focal Right M1 tDCS (ring config), classical M1 tDCS, sham | 1 session. 2 mA. 25 min. Passive sham | ↑motor learning after M1 tDCS, compared with sham. |
| Calzolari et al (BioRXiv) | 2022 | M1 classical tDCS, cerebellum classical tDCS, sham | 1 session. 2 mA. 20 min. Passive sham. | M1 tDCS induces distant changes on the cortex, thalamus, and cerebellum. |
| Beumer et al (Brain Science) | 2022 | classical and focal M1 tDCS | - | Data driven workflow using neuroimaging (MRI and EEG) and tDCS (customized tDCS protocols) to identify 3 steps: tissue segmentation, source localization and stimulation optimization. |
| Samartin-Veiga et al (Pain) | 2022 | left M1 tDCS, Left operculo-insular tDCS, left DLPFC tDCS | 15 sessions. 2 mA. 20 min. passive sham. | ↓ anxiety and ↓ depression after M1, operculo-insular, and DLPFC tDCS, compared with sham. |

| | | | | |
|---|---|---|---|---|
| Samartin-Veiga et al (Qual. Life Res.) | 2022 | left M1 tDCS, Operculo-insular tDCS, DLPFC tDCS, passive sham | 15 sessions. 20 min. | Quality of Life (QOL) was not significantly different compared with sham. |
| Swissa et al (Scientific reports) | 2022 | S1 multifocal tDCS, M1 multifocal tDCS, sham tDCS | 1 mA. 15 min. Passive sham | ↓ Reaction time and endpoint error in men. ↓ Reaching movement time in women |
| Van der Cruijsen et al (Frontiers Human Neuro) | 2022 | Multifocal tDCS, classic M1 tDCS, sham tDCS | 1 session. 4 mA. 12 min. Passive sham. | Neither classic M1 tDCS nor multifocal tDCS increased MEPs (baseline normalized) in healthy pain free individuals compared with baseline or sham at any time point. |
| Kold et al (JPain) | 2022 | Focal M1-DLPFC tDCS (ring configuration), sham tDCS | 3 sessions. 4 mA. 20 min. Passive sham. | ↑ pressure pain thresholds in both active and sham, compared with baseline levels |
| DaSilva et al (Front Pain Res) | 2022 | Bilateral M1 tDCS | 2 mA.- | Comparison of lab-based and home-based montages for bilateral M1 tDCS. |
| Machado et al[250] (Scientific reports) | 2021 | Focal M1 tDCS (ring configuration), M1 classical tDCS | 1 session. 2.4 mA. 20 min. Active sham | Endurance athletes did not have significant changes in exercise performance and psychophysiological responses neither after classical tDCS nor focal tDCS, compared with active sham. |
| Gregoret et al (EurJpain) | 2021 | tDCS of the motor network | 2 sessions. 4 mA. 20 min. Passive | ↑ MEPs (log transformed) and CPM (PPT) compared with sham during pain while pain sensitivity was not significantly changed. |

| | | | sham | |
|---|---|---|---|---|
| Masina et al (Scientific reports) | 2021 | Focal Right M1 tDCS (Ring config.), classical right M1 tDCS. | 1 session each. 2 mA. 20 min. Passive sham. | ↓ alpha power after right M1 tDCS with lower alpha power at baseline. |
| Kold et al (Pain) | 2021 | Focal M1 tDCS, DLPFC tDCS, M1-DLPFC tDCS (ring config.). | 3 sessions each. 20 min. Passive sham | No significant changes in pain sensitivity in any active tDCS paradigm, compared with sham in healthy pain-free individuals. |
| Handiru et al (IEEE EMBC) | 2021 | Focal M1 tDCS (ring configuration) | 1 session. 2mA. 20 min. | Focal M1 tDCS was applied in chronic stroke patients. Stroke-related lesions were included automatically into the model. |
| Maezawa et al (Brain stimulation) | 2020 | Multifocal bilateral anodal M1 tDCS (4 mA), classical M1 tDCS (2 mA) | 1 session. 20 min. Passive sham | ↑ MEPs after classic M1 tDCS and bilateral anodal M1 tDCS, compared with sham. ↑ muscle force after bilateral anodal M1 tDCS compared with sham. They propose that current density (mA/cm2) is key in increasing MEPs and psychophysical responses. |
| Meeker et al (Front Human Neur) | 2019 | Anodal and cathodal M1 classical tDCS | 1 session. 1 mA 20 min. Passive sham. | Under capsaicin-heat pain, anodal M1 tDCS normalized neurophysiological responses on the descending pain modulatory network. |
| Chen et al (IEEE EMBS) | 2019 | tDCS of M1 and premotor cortex, supplementary motor area (SMA), focal | 1 session. 2 mA. 10 min. | ↑ corticomuscular coherence after tDCS of M1-premotor-SMA, as compared with focal M1 tDCS (ring configuration) |

| | | M1 tDCS (ring configuration) | | |
|---|---|---|---|---|
| Naegel et al[86] (Journal of Headache and Pain) | 2018 | Classical anodal, cathodal, and sham M1 tDCS | 1 session. 20 min. 1.5 mA. Passive sham | ↑ activation of multiple brain areas in the anodal M1 tDCS, compared to cathodal tDCS, not compared to sham. No significant pain sensitivity changes. |
| Fischer et al (Neuroimage) | 2017 | tDCS of the motor network (4 mA), classic M1 tDCS (2 mA), *active* sham network-mismatch tDCS (4 mA) | 1 session each, 12 min. "active" sham. | ↑ MEPs (baseline normalized) compared with classic M1 tDCS and network-mismatch tDCS in healthy individuals |
| Thibaut et al (Neuroscience letters) | 2017 | Classical anodal M1 tDCS, tPCS | 1 session. 2 mA. 20 min. Passive sham. | ↑ high beta power over the temporal and parietal regions after M1 tDCS, as compared with sham. No significant effect on pain sensitivity. |
| Attal et al (Pain) | 2016 | M1 classical tDCS. M1 rTMS. | 3 sessions. 2 mA. 30 min. Passive sham | No significant pain reduction in the active tDCS group, as compared with sham. ↓ pain after 3 sessions of M1 rTMS sessions in neuropathic patients. |
| Flood et al (JPain) | 2016 | Focal M1 tDCS (ring configuration) | 1 session. 2 mA. 10 min. passive sham. | ↑ CPM in healthy pain-free men after active focal M1 tDCS. |
| Donnel et al (Brain stim) | 2015 | multifocal M1 tDCS 2x2 | 5 sessions. 2 mA. 20 min. Passive sham. | ↓ pain and improved motor evaluation after active multifocal M1 tDCS (2x2), as compared with sham. Positive affect reduced in both active and sham groups. |

| | | | | |
|---|---|---|---|---|
| Regina et al (Frontiers in Behavioural Neurosc) | 2015 | Classical M1 tDCS with melatonine | 1 session. 2 mA. 20 min. passive sham. | Significant MEP changes in the melatonine+anodal tDCS, as compared with melatonine-sham tDCS and placebo-sham tDCS. No significant changes in serum BDNF levels in any condition. |
| DaSilva et al (Frontiers Behaav. Neuros) | 2015 | Focal (2x2 and ring config.) M1 tDCS and M1 classical tDCS, bilateral DLPFC tDCS, occipital cortex tDCS | 1 session. 1-2 mA. | Compared to focal M1 tDCS (ring config), focal 2x2 M1 tCDS produced more focused cortical effects. Negligible subcortical effects in both focal tDCS paradigms. |
| Roy et al | 2014 | Focal M1 tDCS (ring config.) | 1 session. 20 min. Passive sham. | ↑ ERD during motor imagery during and after active tDCS session |
| Kuo et al (Brain stimulation) | 2013 | Focal anodal and cathodal M1 tDCS (ring config.) and classical M1 tDCS | 1 session 2 mA. 10 min. Passive sham. | ↑ MEPs after both focal M1 tDCS and classical M1 tDCS. MEPs facilitation lasted for 2 h after focal anodal M1 tDCS. |
| Villamar et al (JPain) | 2013 | Focal M1 tDCS (ring configuration), sham tDCS | 1 session. 2 mA. 20 min. Passive sham. | ↓ pain after anodal and cathodal focal M1 tDCS, compared to sham. ↑ mechanical detection thresholds after anodal tDCS, compared to sham. |
| Borckardt et al (JPain) | 2012 | Focal M1 tDCS (ring configuration) | 1 session. 2 mA. 20 min. Passive sham | No significant changes in HPT and MPT after active M1 tDCS.<br><br>↓ thermal wind-up pain, WDT, cold sensory thresholds in the active group, as compared with sham. |

# Appendix B. Overview of studies investigating the effects of multifocal tDCS with cortical targets other than the M1.

| | | | | |
|---|---|---|---|---|
| Xiong et al (Pain medicine) | 2023 | Focal anodal and cathodal ACC tDCS | Passive sham. | Focal cathodal ACC tDCS ↑ heat and pressure pain thresholds, compared to sham. |
| Steyaert et al (PLOS ONE) | 2022 | Left DLPFC tDCS (multichannel tDCS), passive sham | 2-3 mA, 20 min. Passive sham | ↓ reduced secondary hyperalgesia area (induced by high frequency stimulation) after anodal DLPFC tDCS, compared with sham |
| Zhou et al (Front. Hum. Neurosci) | 2022 | Network-based tDCS (dorsal attention and default mode networks) | Passive sham. | ↓ gait variability. No significant changes in gait speed or other average gait metrics. |
| Ziegler et al (Progress in Brain R.) | 2021 | tDCS of the inferior frontal gyrus (IFG) | 5 sessions. 0.25-0.5 mA. Passive sham | 0.5 mA IFG tDCS reduced omission errors and produced a lower P3 reduction, as compared to 0.25 IFG tDCS. |
| Abellaneda-Perez et al (Front Aging Neuros) | 2021 | Multifocal frontoparietal tDCS, and another montage fronto-posteromedial tDCS, sham tDCS | 1 session. 4 mA. Passive sham. | Multifocal tDCS targeting the frontoparietal regions modulated functional coupling, compared to sham. |

| | | | | |
|---|---|---|---|---|
| Lema et al (Scientific reports) | 2021 | Classical DLPFC tDCS, DLPFC tRNS, sham | Passive sham. | No statistical changes after anodal tDCS. tRNS significantly increased attention and performance during the Attention Network test (ANT) |
| Sehatpour et al (Brain stimulation) | 2021 | Classical and focal tDCS of visual cortex. | 1 session. 2 mA. (duration not indicated). Passive sham. | ↑ reaction times after focal tDCS, compared to sham. Compared to classical tDCS, ↑ coherence between motor and SMA nodes after focal tDCS. |
| Wang et al (IEEE EMBC) | 2019 | Focal DLPFC tDCS (ring config.) | 1 session. 2 mA. 30 min. Passive sham. | Focal DLPFC tDCS reduced alpha and beta power, as compared with sham. Working memory improved when combining training and focal tDCS. |
| Donaldson et al (J Neurophysiology) | 2019 | rTPJ tDCS (ring config.), sham | 1 session. 2 mA. 20 min. | anodal rTPJ tDCS modulates task performance, compared to sham. Cathodal rTPJ tDCS showed a tendency to reduced P300 ERPs. |
| To et al[251] (Scientific reports) | 2018 | dACC tDCS (ring configuration on Fz and Fp1, Fp2, F7 and F8) | 1 session. 1 mA. 20 min. Passive sham. | ↑ Beta power in the dACC after anodal dACC tDCS and ↑ theta power in the dACC and rACC after cathodal dACC tDCS. |
| Hill et al (Brain stimulation) | 2018 | Focal DLPFC tDCS (ring configuration), DLPFC+PC tDCS, sham tDCS | 1 session. 1.5 mA. 15 min. Passive sham. | ↑P60 TMS-evoked responses after both DLPFC and focal DLPFC+PC tDCS. Also relative to baseline, focal DLPFC+PC tDCS ↓N100 responses and ↑theta and gamma power. Working |

| | | | | |
|---|---|---|---|---|
| | | | | memory was not modulated. |
| Hill et al (Neuroimage) | 2017 | tDCS and classical tDCS of the DLPFC, sham | 1 session. 1 mA. 20 min. Passive sham. | P60 TMS-evoked responses after both focal tDCS and classical DLPFC tDCS. Widespread changes after focal DLPFC tDCS. |
| Hogeveen et al (Brain stimulation) | 2016 | Focal tDCS (ring config.) of the inferior frontal cortex (IFC), classic tDCS of IFC | 1 session. 1 mA. 20 min. active sham (active occipital tDCS). | ↑ performance after a stop-signal task in both focal tDCS and classical tDCS of the IFC, as compared to active sham (active occipital tDCS). |

# Appendix C. Statistical analyses

Section i) Statistical analysis on current pain, and averaged pain as well as psychometric questionnaires combining participants of all the present PhD studies.

| | F value | P value (unadjusted) | $n_p^2$ | Interpretation |
|---|---|---|---|---|
| **Two-way ANOVA of current pain (at Day1-post-cap1 and Day2)** | | | | |
| Main effect of TIME | 30.879 | 0.00 | 0.309 | Compared with Day1-post-cap, current capsaicin pain ↑ at Day2 in both active and sham groups |
| Main effect of GROUP | 1.630 | 0.206 | 0.023 | - |
| TIMExGROUP interaction | 0.315 | 0.576 | 0.005 | - |
| **Two-way ANOVA of averaged pain (at Day1-post-cap1 and Day2)** | | | | |
| Main effect of TIME | 71.078 | 0.00 | 0.507 | Compared with Day1-post-cap, averaged capsaicin pain ↑ at Day2 in both active and sham groups |
| Main effect of GROUP | 2.746 | 0.102 | 0.038 | - |
| TIMExGROUP interaction | 1,343 | 0.251 | 0.019 | - |

Section ii) Pearson correlations of pooled data using CPM and MEPs in Study I before receiving tDCS

| | R | P value (unadjusted) | Interpretation |
|---|---|---|---|
| CPM-PDT and MEPs at Day1-baseline | -0.52 | 0.378 | No significant association |
| CPM-PTT and MEPs at Day1-baseline | -0.120 | 0.237 | No significant association |
| CPM-PDT at Day1-baseline and ΔMEPs at Day1-baseline and Day1-post-cap | 0.020 | 0.453 | No significant association |
| CPM-PTT at Day1-baseline and ΔMEPs (Day1-post-cap – Day1-baseline) | 0.010 | 0.476 | No significant association |

Section iii) Analysis of N2 and P2 amplitudes of cortical ERPs of study II

| | F value | P value (unadjusted) | $n_p^2$ | Interpretation |
|---|---|---|---|---|
| **Two-way ANOVA of normalized N2 amplitudes (at Day1-baseline and Day1-post-cap) during pain** | | | | |
| Main effect of TIME | 6.67 | **0.014** | 0.164 | Significant reduction of N2 amplitudes at Day1-post-cap |
| Main effect of GROUP | 0.56 | 0.460 | 0.016 | - |
| TIMExGROUP interaction | 0.00 | 0.988 | 0.000 | - |
| **Two-way ANOVA of normalized N2 amplitudes (at Day1-post-cap and at** | | | | |

| Day2) after tDCS | | | | |
|---|---|---|---|---|
| Main effect of TIME | 0.44 | 0.509 | 0.013 | - |
| Main effect of GROUP | 0.22 | 0.640 | 0.007 | - |
| TIMExGROUP interaction | 1.18 | 0.285 | 0.034 | - |
| **Two-way ANOVA of normalized P2 amplitudes (at Day1-baseline and Day1-post-cap) during pain** | | | | |
| Main effect of TIME | 3.52 | 0.069 | 0.94 | - |
| Main effect of GROUP | 0.33 | 0.069 | 0.094 | - |
| TIMExGROUP interaction | 3.52 | 0.069 | 0.094 | - |
| **Two-way ANOVA of normalized P2 amplitudes (at Day1-post-cap and at Day2) after tDCS** | | | | |
| Main effect of TIME | 0.08 | 0.776 | 0.002 | - |
| Main effect of GROUP | 1.93 | 0.173 | 0.054 | - |
| TIMExGROUP interaction | 7.74 | **0.009** | 0.185 | Post hoc analysis shows the active group increased P2 amplitudes at Day2, compared to the sham group (p<0.05) and to Day1-post-cap (p<0.05) |