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BY
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DISSERTATION SUBMITTED 2018

AALBORG UNIVERSITY
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PHD THESIS

by

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CV

In 2009, I graduated in Medicine and Surgery with a grade of 110/110 “cum laude” at the University of Siena (Italy), obtaining the qualification of Medical Doctor. From 2010 to 2015, I enrolled at the School of Specialization in Sport and Exercise Medicine, University of Florence, obtaining the qualification of Sports Physician. Since the beginning of my clinical training, I focused my learning on the assessment and the treatment of the disorders of the musculoskeletal system, on the use of the musculoskeletal ultrasound imaging and the ultrasound-guided interventions. In 2013, these skills produced an intensive collaboration with Italian Swimming Federation aimed at developing the application of rehabilitative ultrasound imaging in sport rehabilitation and injury prevention in elite athletes. From 2014, I worked as an occupational trainee at the Center of Clinical Research Excellence in Spine Pain, University of Queensland (Australia), where I have trained to use intramuscular electromyography of the trunk muscles and kinematic evaluation of the trunk movement. In October 2015, I have been enrolled as a PhD fellow in CNAP, Aalborg University (Denmark). My research project aimed at probing the nature and the time-course of cortical neuroplastic changes provoked by muscle pain across several days and at modulating the cortical pain neuroplasticity by repetitive transcranial magnetic stimulation. In parallel with my PhD project, I have contributed in developing two researches projects: Parabolic flight (Inter-Agency Partial Gravity Campaign, Bordeaux, 2018) and Bed-rest study (joint ESA/NASA Artificial Gravity Study, Cologne, 2019), in collaboration with European Space Agency (Space Medicine Office) and Northumbria University (Aerospace Medicine and Rehabilitation Laboratory). These projects aimed at developing countermeasures to maintain the human spine in healthy conditions during long space missions and on Lunar and Martial surfaces.

ENGLISH SUMMARY

Chronic musculoskeletal pain is one of the main causes of living with disability. Yet, one of the major problems in planning new therapeutic strategies is that the mechanisms causing pain are not completely clear. Recent pain researches have highlighted the role of nervous system in maintaining pain chronicity due to maladaptive neuroplasticity. However, it is still unclear how neuroplasticity is modified during the transition from acute to chronic pain and when neuroplastic changes appear. Therefore, the first aim of the present Ph.D. project was to investigate the nature and time-course of cortical neuroplasticity provoked by long-lasting muscle pain. In addition, interventions able to reverse pain neuroplasticity have been recently proposed to treat musculoskeletal pain. Consequently, the second aim of this project was to modulate the cortical excitability changes provoked by long-lasting muscle pain applying consecutive daily sessions of repetitive transcranial magnetic stimulation (rTMS) to the left dorsolateral prefrontal cortex (DLPFC).

To provoke long-lasting muscle pain, three pain models were used in healthy subjects: eccentric exercise-induced delayed-onset muscle soreness (DOMS) (Study I), muscle pain induced by repeated intramuscular injections of nerve growth factor (NGF) (Study II and III) and a combination of muscle pain provoked by NGF and eccentric exercise-induced DOMS (Study II).

To probe the nature and time-course of cortical excitability changes, motor evoked potentials induced by transcranial magnetic stimulation and somatosensory evoked potentials induced by electrical stimulation of a nerve were collected before and during the application of the three pain models (Study I, study II and III). These two neurophysiological measurements were selected because they are generated in specific sensorimotor cortical regions and their changes have been previously interpreted as sign of neuroplasticity.

Finally, to modulate pain neuroplasticity, daily sessions of 10Hz rTMS were applied to the left DLPFC during long-lasting muscle pain provoked by intramuscular injections of NGF (Study III). The left DLPFC was selected because this cortical region has been suggested to play a key role in pain perception and pain suppression.

The results of the first and second study suggested that muscle pain induced by DOMS, intramuscular injections NGF and the two models combined are able to provoke long-lasting muscle pain up to 20 days, muscle hyperalgesia and functional disability. Moreover, temporary cortical excitability changes were probed: While DOMS inhibited the corticomotor excitability, intramuscular injections of NGF facilitated it. Additionally, intramuscular injections of NGF impaired both frontal

and centro-parietal sensory cortical excitability while DOMS impaired only centro-parietal sensory cortical excitability. In conclusion, these findings suggest that eccentric exercise-induced DOMS and muscle pain induced by NGF provoked different cortical sensorimotor adaptations.

The results from the third study showed that consecutive daily sessions of 10Hz rTMS to the left DLPFC modulated the corticomotor and sensory cortical adaptations during muscle pain provoked by intramuscular injections NGF, as well as reduced hyperalgesia, pain intensity and functional disability.

In conclusion, the results of this Ph. D. project showed promising findings regarding the opportunity to provoke and to modulate pain-induced cortical neuroplasticity across several days as well as analgesic effects of daily sessions of 10 Hz left DLPFC rTMS.

DANSK RESUME

Kronisk muskuloskeletal smerte er den største årsag til funktionsnedsættelse på verdensplan. Alligevel er et af de største problemer i udviklingen af nye behandlingsstrategier, at de underliggende mekanismer bag muskuloskeletal smerte ikke er helt forstået. Ny smerteforskning har fremhævet centralnervesystemet og dets rolle i opretholdelsen af kronisk smerte grundet maladaptiv neuroplasticitet. Det er dog stadig uvist, hvordan neuroplasticitet ændres under udviklingen fra akut til kronisk smerte og på hvilke tidspunkter disse ændring finder sted. Derfor var det første mål for dette ph.d.-projekt at undersøge karakteristika samt tidsforløbet af kortikale neuroplasticitetsændringer i forbindelse med længerevarende muskelsmerter. Derudover er nye interventioner, der kan ændre smerteneuroplasticitet, for nyligt blevet anbefalet til behandling af muskuloskeletale smerter. Derfor var det andet formål med dette projekt at modulere de kortikale ændringer som langvarig muskelsmerte fremkalder, ved flere sessioner af repetitiv transkraniel magnetisk stimulation (rTMS) på venstre dorsolaterale præfrontale kortex (DLPFC).

For at provokere langvarig muskelsmerte blev tre smertemodeller anvendt i raske forsøgspersoner: 1) Excentrisk træning blev brugt til udviklingen af forsinket muskelømhed (delayed-onset muscle soreness, DOMS) (Studie I), 2) muskelsmerter induceret ved gentagende intramuskulære injektioner af nerve growth factor (NGF) (Studie II), og 3) muskelsmerter induceret via en kombination af gentagende intramuskulære injektioner af NGF og excentrisk træning (DOMS) (Studie III).

For at undersøge karakteristika og tidsforløbet af de kortikale excitabilitetsændringer blev motor-evokerede potentialer (MEPer), induceret af transkraniel magnetisk stimuletion (TMS), og somatosensorisk evokerede potentialer (SEPer), fremkaldt af elektrisk nervestimulering, indsamlet før og efter anvendelse af de tre smertemodeller (Studie I, II og III). Disse to neurofysiologiske målinger blev valgt da de genereres i de sensomotoriske kortikale regioner, og deres ændringer er tidligere blevet fortolket som tegn på neuroplasticitet.

Til modulering af smerteneuroplasticitet, blev flere sessioner af rTMS af det venstre DLPFC anvendt under langvarig muskelsmerte induceret af intramuskulære injektioner af NGF (Studie III). Det venstre DLPFC blev valgt, da det har vist sig at spille en vigtig rolle i smerteopfattelse samt smertereduktion.

Resultaterne fra det første og andet studie viste, at muskelsmerter induceret af DOMS, intramuskulære injektioner af NGF, og en kombination af de to, er i stand til at fremkalde langvarig muskelsmerte i op til 20 dage, muskelhyperalgesi og funktionsnedsættelse. Endvidere blev de kortikale excitabilitetsændringer undersøgt: Mens DOMS hæmmede den kortikale motoriske excitabilitet, blev den øget af

intramuskulære injektioner af NGF. Derudover hæmmede intramuskulære injektioner af NGF både den frontale og centro-parietale kortikale sensoriske excitabilitet, mens DOMS kun hæmmede den centro-parietale kortikale sensoriske excitabilitet. Sammenfattende tyder disse resultater på, at DOMS og muskelsmerter, induceret af NGF, provokerede forskellige kortikale sensomotoriske ændringer.

Resultaterne fra det tredje studie viste, at flere sessioner af rTMS af det venstre DLPFC var i stand til at modulere kortikale motoriske og sensoriske ændringer under muskelsmerte, induceret af intramuskulær injektioner af NGF, såvel som reduceret hyperalgesi, smerteintensitet og graden af funktionsnedsættelse.

Afslutningsvis viste resultaterne af dette ph.d.-projekt for første gang lovende resultater vedrørende muligheden for at provokere og modulere smerteinduceret kortikal neuroplasticitet over flere dage sammen med en smertelindrende effekt af rTMS stimulering af det venstre DLPFC.

ACKNOWLEDGEMENTS

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I would like to thank the co-authors of this project: Dr. Siobhan Schabrun, Dr. David Seminowicz, Prof. Laura Petrini and Dr. Matteo Zandalasini, for their valuable contribution to develop the aims of this project and to improve the quality of my papers.

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I want to acknowledge all of my colleagues in CNAP, SMI and the staff of Aalborg University for their contribution. My special appreciation goes to Dennis Boye Larsen, Silvia Lo Vecchio and Ning Qu. Dennis has been a great help on developing the experimental setup and the analysis and, Silva and Ning have been great friends during these years in Aalborg.

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Finally, I am deeply thankful for my girlfriend, Yin Ka Lam, my friends and my family. During these years, they encouraged all my decisions and they were always cheering me up and stood by me despite the long distances.

PREFACE

This PhD thesis summarizes the research work realized from November 2015 to September 2018 at the Center of Neuroplasticity and Pain (CNAP), Aalborg University (Denmark). A stay abroad was carried out at the Pain Center from the Hospital das Clínicas, University of São Paulo (Brazil), as part of external collaboration with CNAP.

This project was fully funded by the Danish National Research Foundation.

The present research project aims at reversing pain neuroplasticity induced by muscle pain developing over days (long-lasting muscle pain model) in heathy subjects. In order to obtain this goal, three steps were necessary: i) to provoke long-lasting muscle pain; ii) to probe changes in corticomotor excitability and sensory cortical excitability induced by long-lasting muscle pain; iii) to test the modulatory effects of consecutive daily sessions of repetitive transcranial magnetic stimulation (rTMS) to left dorsolateral prefrontal cortex (DLPFC) during long-lasting muscle pain.

This thesis is divided in 5 chapters. The first chapter presents a brief introduction on provoking, probing, and modulating pain neuroplasticity. The second chapter defines the experimental pain models used to provoke long-lasting muscle pain and the time-course of pain manifestations. The third chapter illustrates the neurophysiological tools used to probe cortical neuroplasticity during long-lasting muscle pain. The fourth chapter describes the analgesic and neuromodulatory effect of high frequency rTMS to DLPFC during long-lasting muscle pain. Finally, the thesis is completed in the fifth chapter with a brief conclusion and future perspectives. Suggestions of different methods to provoke, probe and modulate pain neuroplasticity are proposed and, the translation of these experimental findings to chronic musculoskeletal pain is highlighted.

The primary content of this thesis is based on 3 original papers, which have been published in international peer-reviewed journals.

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CHAPTER 1. INTRODUCTION

Musculoskeletal pain poses one of the major health-related burdens on human population and it is the main cause of disability worldwide[1]. Despite several years of pain research, long-term management of MSK pain still remains inefficient[1].

One of the main problems in planning and developing long-term therapeutic strategies is that the pathophysiological mechanisms causing pain are not completely understood[2]. Although the recent development of imaging techniques, the association between pain and tissue abnormalities remains poor[3–9], indicating that the pathoanatomical origin may not be sufficient to explain pain chronicity. Recently, a stronger relationship has been described between pain intensity and pain duration, and central sensitization[4,10], impaired motor control[11,12] and psychosocial factors[13–16]. Consequently, the role of the nervous system in chronic musculoskeletal pain have been highlighted[3,4,17], leading to the introduction of the so-called "maladaptive pain neuroplasticity". This pathophysiological mechanism is derived from the hypothesis that intense and prolonged nociceptive inputs provoke dysfunctional plastic changes of the nervous system [2,17,18].

1.1. WHAT IS NEUROPLASTICITY?

Neuroplasticity is the capacity of neurons to change in function, form and number [19,20]. Neuroplasticity is the consequence of i) events in the external environment able to activate receptors; ii) the activities of neurons that are spontaneously active; and iii) factors and substances in the local environment able to modulate the neural activity [19]. In physiological conditions, adaptive neuroplasticity results in changes in the synaptic connection strength between neurons, and it is a fundamental mechanism for improving brain functioning. For instance, it represents a critical neural substrate for learning and memory [19–21].

1.2. WHAT IS MALADAPTIVE PAIN NEUROPLASTICITY?

Maladaptive neuroplasticity is the pathological side of neuroplasticity, and it is based on an imbalance synaptic function of the nervous system[17]. The results of maladaptive neuroplasticity is a loss of coordination and function of the nervous system, causing disability and reduction of quality of life[17]. In the recent years, maladaptive neuroplastic changes during the process of pain chronification have been described from the peripheral to the cortical levels (structural and functional changes)[17,22]. Therefore, it has been suggested that intense and prolonged nociceptive inputs from a pathological tissue may lead to dysfunctional neuroplastic changes[17,22]. Indeed, based on several neurophysiological and neuroimaging measurements, the dysfunctional activity of the nervous system, accompanied by structural remodeling, have been observed in patients affected by chronic musculoskeletal pain[17,22].

However, the causality and the time-course between pain and maladaptive neuroplasticity is still unknown since no longitudinal studies, assessing the neural function before the pain becomes chronic and in different stages of the disease (i.e., <6 weeks and >3 months), exist. Therefore, it is still not known *how* neuroplasticity is impaired during the transition from acute to persistent pain and *when* these neuroplastic changes appear.

A simplistic approach, to reduce complexity between pain and neuroplasticity, is to apply experimental persistent pain models in healthy subjects. The main scientific advantage of using these models is to create causality and to provide information about the temporal profile of neuroplasticity during the transition from acute to persistent pain. Besides, since the researcher strictly controls the stimuli provoking pain, this approach offers the opportunity to experimentally investigate pain neuroplasticity, avoiding other confounding factors and co-morbidities connected to clinical pain conditions.

1.3. HOW TO PROVOKE PAIN NEUROPLASTICITY?

Temporary and reversible neuroplastic adaptations have been experimentally described in response to several different external stimuli, such as anesthetic blocks[23], electrical stimulation[24], immobilization[25,26], repetitive transcranial magnetic stimulation[27] and motor training[28–30]. Similarly to them, short-lasting painful stimulation induced experimentally in healthy subjects results in changes in neural excitability, due probably too extensive nociceptive inputs entering the nervous system[31,32].

Different methods can provoke experimental muscle pain[2,33]. Based on the time profile, the pain models can be divided based on short-lasting (few minutes) or long-lasting (few days) pain models. In this project, repeated injections of intramuscular injection of nerve growth factor (NGF), a neurotrophic protein released physiologically during an inflammatory process[34], and eccentric exercise-induced delayed onset muscle soreness (DOMS) have been used since both models can provoke prolonged muscle pain over several days. Indeed, a previous study has shown that multiple intramuscular injections of NGF are capable of inducing progressive muscle pain up to 21 days[35]. Importantly, NGF-induced muscle pain simulates the time-course (slow development of muscle pain) and processes involved in the transition to persistent musculoskeletal pain, such as hypersensitivity to mechanical pain and temporal summation of pressure, and thus provides a realistic model for investigating long-lasting muscle pain [35–38].

In contrast, eccentric exercise-inducing DOMS provokes muscle pain up to 5-6 days[39,40], amd it can be applied only a single time because it produces training effects[41]. The mechanism underlying this kind of muscle pain is related to ultrastructural muscle damage caused by tissue overloading, and it results in the

release of several algesic substances, such as bradykinin, prostaglandins and NGF [42,43].

1.4. HOW TO PROBE PAIN NEUROPLASTICITY?

Several different neurophysiological and neuroimaging techniques have been used to probe pain neuroplasticity in healthy subjects such as functional magnetic resonance imaging (fMRI)[44], magneto- (MEG) and electro- (EEG) encephalography[45–47], and transcranial magnetic stimulation (TMS)[31,48]. Indeed, altered cortical excitability has been recorded not only during acute pain but also when pain vanished[49], indicating that nociceptive inputs induce temporary and reversible neuroplastic changes. More specifically, evoking motor evoked potentials (MEPs), as produces by TMS, Le Pera et al.[31] showed an inhibition of the primary motor cortex (M1) during 5-10 minutes of acute muscle pain and around 30 minutes after the pain disappeared[31,48]. Besides, Rossi et al.[45] showed inhibition of early sensory evoked potentials (SEPs) induced by low-threshold afferents from the ulnar nerve after injecting levo-ascorbic solution into the first dorsal interosseous muscle. The inhibition of early SEPs lasted around 30 minutes after the pain disappeared[48], confirmed similar temporary neuroplastic changes in the somatosensory cortical areas.

Recently, neuroplastic changes have also been described after applying repeated injections of NGF into the extensor carpi radialis brevis (ECRB) muscle in healthy subjects[38]. In contrast with acute pain induced by hypertonic saline injection, injections of NGF induced altered motor cortex organization and impaired function characterized by expansion of motor cortex excitability, that is present few days after developing muscle pain[38]. Consequently, this experimental pain model provided, for the first time, the opportunity to investigate the neuroplastic adaptations across several days.

1.5. HOW AND WHERE TO MODULATE PAIN NEUROPLASTICITY?

Different type of non-invasive cortical stimulations have been proposed to therapeutically induce cortical neuroplasticity in neurological and psychiatric disorders[50,51]. For instance, TMS consists of electromagnetic pulses inducing electrical currents in the cortex via a coil placed on the head[51,52]. The application of repeated electromagnetic stimuli to a single scalp position is called “repetitive transcranial magnetic stimulation” (rTMS)[28]. These stimuli lead to temporary cerebral modulation through the modification of cortical excitability[28], changes in blood flow to the stimulated area[53–56], and release of several neurotransmitters such as dopamine, serotonin, opioids, gamma-aminobutyric acid (GABA) and glutamate[57–60]. Additionally to the local action, TMS acts on distant structures via several brain connections[61–63]. For this reason, this technique has been proposed as a

possible intervention able to modulate maladaptive neuroplasticity in several neurological and psychiatric conditions[51].

In the context of experimental pain, several different areas of the brain have been shown to be active[44]. Pain-related brain activation has been shown in the primary sensory cortex, anterior cingulate cortex (ACC), insula, prefrontal, and motor regions[64]. In addition, the left dorsolateral prefrontal cortex (DLPFC) shows abnormal function in chronic pain populations[65,66], and it is frequently activated in experimental pain studies.

Based on evidence that left DLPFC morphology and function reflect chronic pain conditions, and it is linked to pain regulation, this cortical region has been suggested as a therapeutic target[67]. Indeed, several studies have also shown that 10 Hz rTMS to this area can temporary reduce acute or chronic pain[60,68–70].

1.6. AIMS AND GOALS OF THE PH.D PROJECT

The three goals of this work were 1) to probe *the clinical manifestations* of long-lasting muscle pain models; 2) to probe *the nature and the temporal profile* of cortical excitability adaptations in response to long-lasting muscle pain and 3) to investigate whether *5-daily sessions of rTMS* over the left DPFC modulate the clinical manifestations and the cortical excitability adaptations induced by long-lasting muscle pain.

Three steps were necessary to achieve these goals:

i) To provoke muscle pain applying three different long-lasting experimental pain models: 1) eccentric exercise-induced DOMS, 2) intramuscular injections of NGF-induced muscle pain and 3) a combination between NGF-induced muscle pain and eccentric exercise-induced DOMS (Chapter 2).

ii) To probe cortical excitability by motor evoked potential (corticomotor output) and sensory evoked potentials (sensory cortical integration of afferent inputs) during the three long-lasting muscle pain models (Chapter 3).

iii) To modulate cortical excitability changes induced by long-lasting muscle pain (intramuscular injections of NGF) applying consecutive 5-daily sessions of 10 Hz left DLPFC rTMS (Chapter 4).

Dissertation outline and Papers:

Fig 1 summarizes the research approach used to provoke, probe and modulate pain neuroplasticity and the connections between the studies.

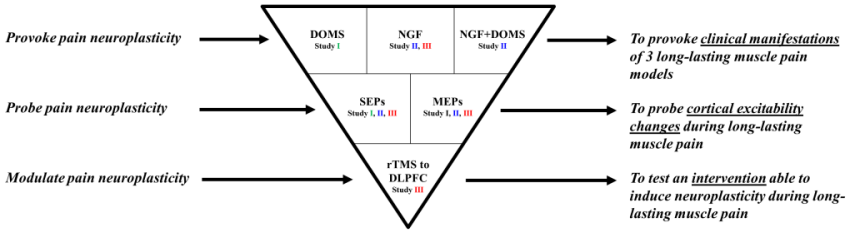


Fig. 1 Dissertation outline.

Primary papers:

Study I: Enrico De Martino, Laura Petrini, Siobhan Schabrun, Thomas Graven-Nielsen *Cortical somatosensory excitability is modulated in response to several days of muscle soreness.* Journal of Pain, 2018.

Study II: Enrico De Martino, Matteo Zandalasini, Siobhan Schabrun, Laura Petrini, Thomas Graven-Nielsen *Experimental muscle hyperalgesia modulates sensorimotor cortical excitability, which is partially altered by unaccustomed exercise.* PAIN, 2018.

Study III: Enrico De Martino, David Seminowicz, Siobhan Schabrun, Laura Petrini, Thomas Graven-Nielsen *Repetitive transcranial magnetic stimulation on left dorsolateral prefrontal cortex modulates the sensorimotor cortex function in the transition to sustained muscle pain*. NeuroImage, 2018

These papers will be referred to from hereon as named above (Study I, Study II and Study III).

Secondary paper:

Supplement Paper I: David Seminowicz, **Enrico De Martino**, Siobhan Schabrun, Thomas Graven-Nielsen. *Left dorsolateral prefrontal cortex repetitive transcranial magnetic stimulation reduces the development of long-term muscle pain.* PAIN, 2018.

This paper will be referred to from hereon as named above (Supplement Paper I). The effects of pain-induced neuroplasticity are addressed in the Study I and Study II (Chapter 2 and 3) while the modulatory effect of daily sessions of left DLPFC rTMS on the NGF pain model is addressed in the Study III and Supplement Paper I (Chapter 4).

CHAPTER 2. PROVOKING PAIN NEUROPLASTICITY

To probe the clinical manifestations of long-lasting muscle pain, three different models have been used in this project: 1) eccentric exercise-induced DOMS (Study I); 2) intramuscular injections of NGF (Study II and Study III); 3) Combined intramuscular injections of NGF and eccentric exercise induced-DOMS (Study II).

2.1. ECCENTRIC EXERCISE-INDUCED DOMS

Eccentric exercise-induced DOMS is recognized as an effective endogenous technique for inducing musculotendinous hyperalgesia[41,71,72] due to damage of the ultrastructural and cytoskeletal components of muscle fibers[40]. Muscle pain and hyperalgesia peak around 24–48 h after the exercise, followed by reduced range of movement and muscle strength in the affected muscle group[71]. However, when muscle pain is recovered, the second bout of exercise is not able to induce a similar muscle pain and muscle hyperalgesia, because of a training effect of the overload[41]. Importantly, resting pain is not a feature of this pain model, mimicking muscle hyperalgesia to mechanical pressure, muscle pain during contraction and stretching, attenuation of force parameters, and functional disability typical of musculoskeletal pain disorders.

To induce DOMS in this project, repetitive eccentric contractions were performed from maximal wrist extension to maximal wrist flexion. Briefly, one bout consisted of five repetitions separated by 1-min rest period. The bout began with a load of around 90% of the maximal voluntary contraction (MVC) and was repeated until the subject was not able to control the contraction. Then, the weight was reduced in steps of around 10% MVC until a load of around 50% MVC in the final bout (Study I and Study II).

To define the temporal profile of pain characteristics and cortical excitability adaptations in response to long-lasting muscle pain, the Study I comprised five identical long sessions on four different days (fig 2). In each session, data were collected in the following sequence: 1) Pain related questionnaires, 2) neurophysiological testing and 3) quantitative motor and sensory assessments. Additional information about the study design is reported in Study I.

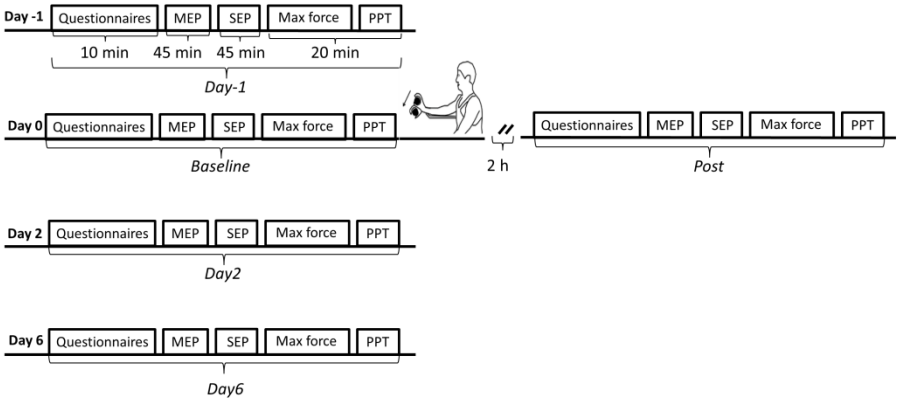


Fig 2: Clinical and neurophysiological outcome measures were collected on Day-1, Baseline, Post, Day 2 and Day 6 (Study I).

2.2. NERVE GROWTH FACTOR-INDUCED MUSCLE PAIN

Several studies have shown that inflammation can produce essential changes in the sensitivity of neurons from the nerve endings to the cortical neurons[17,73,74]. One of the main neurotrophic protein that is released during an inflammatory process and can influence neural function is NGF[34]. When NGF is experimentally injected into a muscle, increased sensitivity to mechanical pressure has been reported for several days[36,37]. Besides, multiple injections of NGF can induce muscle pain until 21 days[35], giving the opportunity to investigate the effect of muscle pain over 3 weeks. Finally, similar to exercise inducing DOMS, pain at rest is not a feature of this pain model, mimicking, therefore, the deep tissue hyperalgesia, functional disability and the pain location typical of mild/moderate lateral epicondylalgia (LE) until 21 days. In study II and III, 5µg/0.5 mL injections of NGF into the ECRB muscle were applied 2 or 3 times to provoke pain along the right forearm (ultrasound guided).

To probe the temporal profile of cortical excitability adaptations in response to progressively developing muscle pain, Study II comprised three identical long sessions and a short session on four different days (Fig 3). As in Study I, the long session consisted of 1) Pain related questionnaires, 2) neurophysiological testing and 3) quantitative motor and sensory assessments. The short session consisted on 1) pain related questionnaires, and 2) quantitative motor and sensory assessments. Besides, questionnaires were also sent by email on Day 6, 8, 10, 12, 14, 17 and 20. Additional information about the study design is reported in Study II.

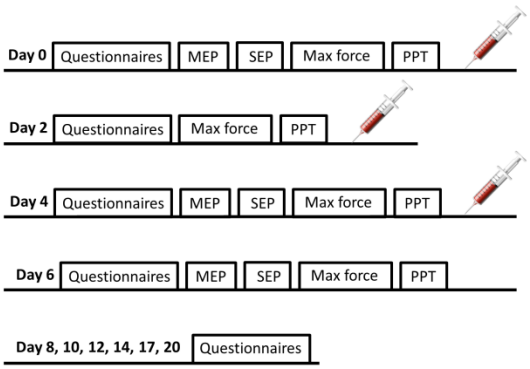


Fig 3: clinical and neurophysiological outcome measures were collected on Day 0, Day 2, Day 4 and Day 6 (study II).

2.3. COMBINED NGF AND DOMS MODELS

Repeated injections of NGF and eccentric exercise-induced DOMS were combined to achieve a long-lasting muscle pain (until 20 days), deep tissue hyperalgesia, attenuation of force parameters, functional disability and pain around the lateral epicondyle. Only a previous studies combined a single injection of NGF and DOMS to investigate the additive effects of these two pain models[75]. According to that study, the combination of the two models induced higher intensity of muscle pain and pain sensitivity to mechanical pressure compared with DOMS model[75].

To probe the temporal profile of cortical excitability adaptations in response to progressively developing muscle pain induced by NGF and eccentric exercise, a combined NGF and DOMS model was used using the same study design of Study II (Fig 4).

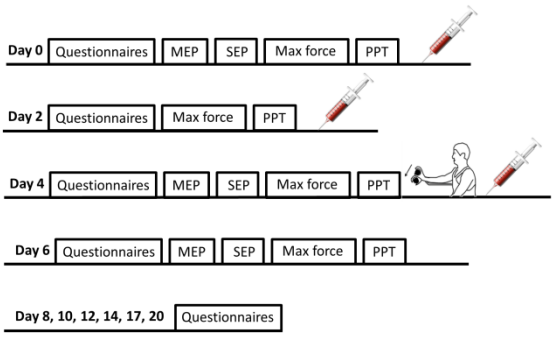


Fig 4: Clinical and neurophysiological outcome measures were collected on Day 0, Day 2, Day 4 and Day 6 (Study II). Note: at Day 4 eccentric exercise-induced DOMS was applied before the NGF injection.

2.4. QUATIFING INTENSITY, FUNCTIONAL LIMITATION AND LOCATION OF MUSCLE PAIN

Consistent with previous pain model studies (appendix B), different questionnaires were used to quantify the temporal profile of the clinical manifestations of long-lasting muscle pain in Study I, II, III and Supplement Paper I.

- A modified 7-point Likert scale was used to assess muscle pain intensity[37,71].
- Patient rated tennis elbow evaluation (PRTEE) was used to measure the functional disability[76].
- Body charts were used to quantify location and spatial distribution of perceived muscle pain[37,71].

The subjects were requested to complete the questionnaires at the beginning of each experimental session (Study I, II, III) or through email diaries (Study II, Supplement Paper I). Detailed information about the questionnaires is reported in Study I.

2.5. PAIN INTENSITY TO MECHANICAL PRESSURE

Mechanical pressure is one of the modalities used to assess pain sensitivity. Importantly, pressure pain threshold (PPT) has been extensively used to investigate pain sensitivity during DOMS and NGF pain models[37,38,41,71,72,77], and the present work showed the excellent reliability of these measures (ICC = 0.84) (Appendix A). In the current work (Study I, II and III), PPTs was slowly increased until the subject detected the first sensation of pain and then pressed a button. To quantify the local and widespread effect of muscle pain, PPTs were recorded bilaterally at the extensor carpi radialis (ECR) muscle and tibialis anterior (TA) muscle.

2.6. MAXIMAL WRIST EXTENSOR FORCE

To quantify the effect of DOMS and NGF on maximal voluntary contractions (MVC), wrist extension force was collected using a force sensor (Fig 5)[71,78]. The present work showed the excellent reliability of these measures (ICC = 0.88) (Appendix A). To date, previous studies have shown that DOMS reduced the wrist maximal force[71,72,77] while intramuscular injections of NGF have been reported inconsistent results[38,78].

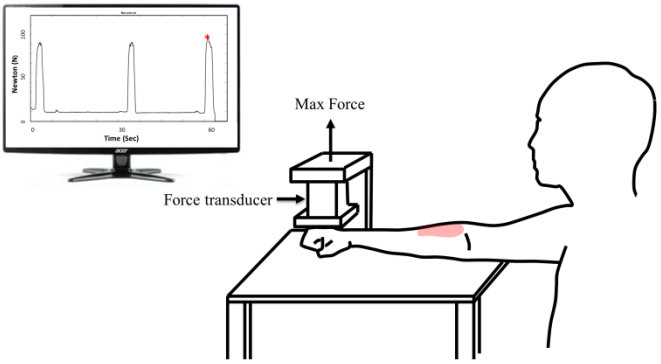


Fig 5: The subject performed three maximal contractions and the force transducer recorded the maximal wrist extension force (Study I, II and III).

2.7. COMPARISON BETWEEN THREE MUSCLE PAIN MODELS

Consistent with previous studies[39,41,71,72], eccentric exercise-induced DOMS provoked moderate muscle pain (muscle pain: ~4) (Fig 6), and mild functional disability (disability: ~25) at Day 2 (Fig 7). At Day 6, muscle pain and functional disability were almost completely recovered (Study I).

In a previous study[37], a single injection of NGF into ECRB muscle provoked mild muscle pain up to 1 week after the injection (muscle pain: ~3 and disability: ~20). When two injections (48 h interval within the injections) were applied into ECRB muscle[38], moderate muscle pain up to 2 weeks was described (muscle pain: ~4 and disability: ~25). Study II showed that the third injection of NGF into ECRB muscle was able to extend muscle pain until 3 weeks after the first injection (Fig 6). However, the intensity of muscle pain and the function disability were similar to 2 injections of NGF (muscle pain: ~4 and disability: ~25) (Fig 7), indicating that an additional injection extended the duration of muscle pain but not pain intensity. In contrast, when eccentric exercise was applied in a NGF pain model (NGF+DOMS group), the intensity of muscle pain (muscle pain: ~5) and the functional disability (disability: ~35) increased compared with the NGF only (Study II). However, the duration of muscle pain and functional disability were not affected by the combined model (Fig 6 and Fig 7).

The muscle pain area was localized along the radial site of the right elbow in all groups (Study I and Study II) (Fig 8). Combined NGF + DOMS models showed more extensive areas of muscle pain compared with the NGF group (Study II)

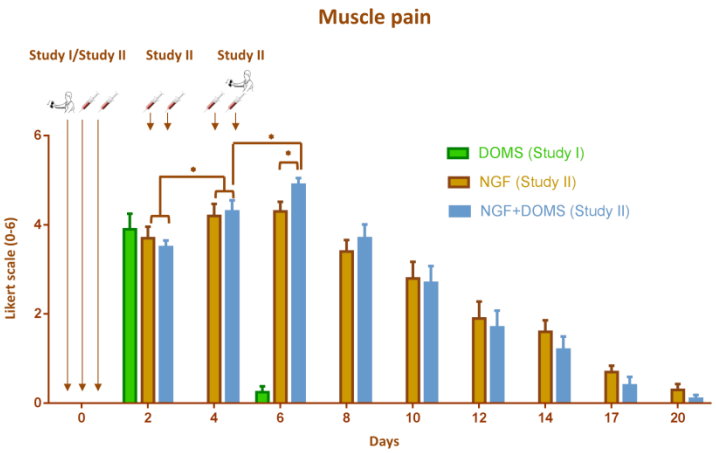


Fig. 6: Mean (± SEM, N = 12) Likert scores of muscle pain for DOMS, NGF and NGF+DOMS groups. Note: DOMS group performed eccentric exercise at Day 0. NGF group and NGF+DOMS group received 3 NGF injections on Day 0, Day 2 and Day 4. NGF+DOMS group performed eccentric exercise on Day 4. Significant differences in muscle pain between Groups and Days are illustrated by * ($P < 0.05$) (statistical analysis Study II).

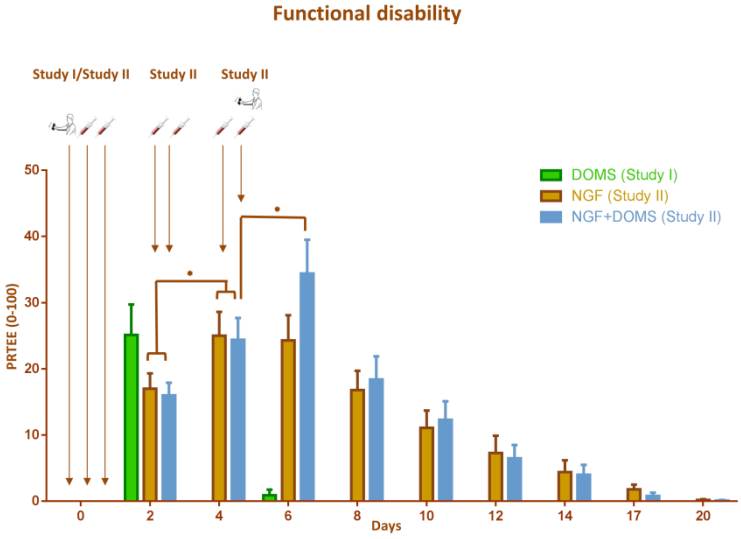


Fig. 7: Mean (± SEM, N = 12) patient-rated tennis elbow evaluation (PRTEE) for DOMS, NGF and NGF+DOMS groups. Significant differences in PRTEE questionnaire between Groups and Days are illustrated by * ($P < 0.05$) (statistical analysis Study II).

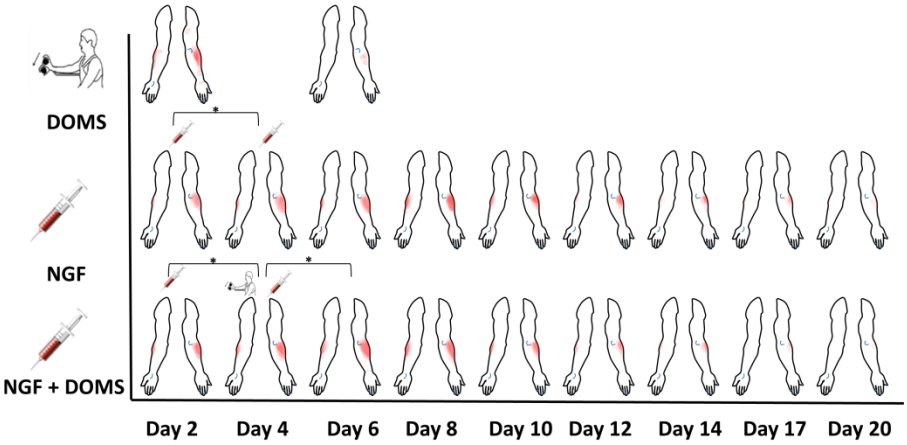


Fig. 8: Areas of muscle pain for DOMS, NGF and NGF+DOMS groups. Significant differences in body charts between Groups and Days are illustrated by * ($P < 0.05$) (statistical analysis Study II).

Similar to with previous studies decreased sensitivity to mechanical pressure is commonly reported in response to muscle pain induced by eccentric exercise[41,71,72,77] and intramuscular injections of NGF[37,38] (Fig 9). In the Study I, the peak of muscle hyperalgesia was two days post exercise, and it was completely recovered six days after the exercise. Repeated injections of NGF were able to maintain similar levels of muscle hyperalgesia in Day 2, Day 4 and Day 6. Interestingly, the combination of intramuscular injections of NGF and DOMS was not able to additionally increase muscle hyperalgesia, likely because of NGF-receptors saturation in the forearm muscle (study II).

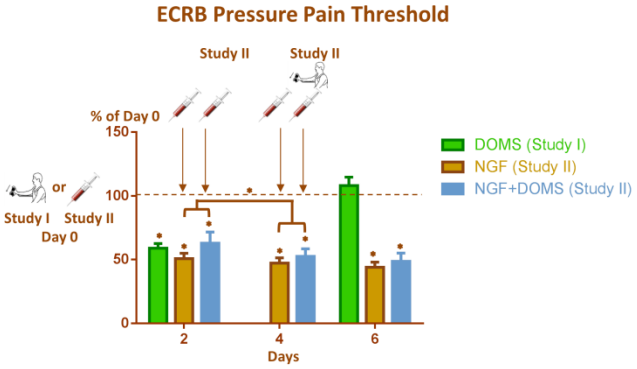


Fig. 9: Mean (± SEM, N = 12) normalized pressure pain threshold (% of Day 0) for DOMS, NGF and NGF+DOMS groups. A significant difference in pressure pain threshold compared with Day 0 and between Days is illustrated by * ($P < 0.05$) (Statistical analysis Study I and Study II).

The decrease of maximal force is commonly reported during DOMS, but controversial findings have been reported after injections of NGF. The Study I and the Study II (NGF + DOMS) confirmed that DOMS reduced the maximal force (~20% reduction compared with Day 0) while NGF induced a minimal reduction of maximal force (less than 5% of reduction compared with Day 0) (Fig 10). Considering that muscle pain and the area of pain are very similar between the two models (Likert scale: ~4), damage of muscle fibers may explain the difference between the two models.

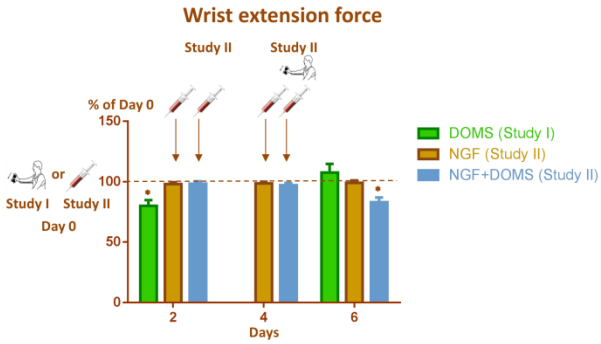


Fig. 10. Mean (± SEM, N = 12) normalized wrist extension maximal force (% of Day 0) for DOMS, NGF, and NGF+DOMS groups. A significant difference in maximal wrist extension force compared with Day 0 is illustrated by * ($P < 0.05$) (Statistical analysis Study I and Study II).

In summary, the DOMS model and NGF model induced similar intensity of muscle pain, functional disability and muscle hyperalgesia, however, the reduction of maximal force is only evident in the DOMS model. Repeated injections of NGF can extend the duration of muscle pain and functional disability up to 20 days while DOMS induced muscle pain and functional disability until 6 days.

The combination of 3 NGF intramuscular injections and eccentric exercise-induced DOMS allows provoking more intense muscle pain, larger muscle pain areas, and functional disability at day 6 as well as reduction of maximal force. However, the duration of muscle pain and muscle hyperalgesia were not affected by the combined model.

2.8. MAIN FINDINGS ADDING TO THE CURRENT KNOWLEDGE

- 3 injections of NGF induced muscle pain until 20 days.
- 3 injections of NGF did not increase the peak intensity of muscle pain, area of muscle pain and muscle hyperalgesia compared with 2 NGF injections.

- 3 injections of NGF combined with DOMS induced an increase of pain intensity, functional disability and area of muscle pain compared with only 3 injections of NGF, but did not extend the pain duration and muscle hyperalgesia.
- 1, 2, or 3 injections on NGF did not reduce the maximal force.
- Reduction of maximal force induced by DOMS on a pre-sensitized muscle was the same reduction as DOMS without pre-sensitization.

CHAPTER 3. PROBING CORTICAL PAIN NEUROPLASTICITY

Following a transient stimulus, such as electric, visual, auditory or tactile, the nervous system generates a series of electrical potentials with latencies ranging from few milliseconds to hundreds of millisecond according to the type of nervous fibers. By placing recording electrodes over specific anatomical locations, these electrical potentials can be collected and processed[79].

In the current work, SEPs, provoked by electrical stimulation of the radial nerve, and MEPs, evoked by TMS to the ECRB, have been collected and analyzed to probe neuroplastic changes induced by muscle pain across several days. Classically, cortical neuroplasticity has been demonstrated in the somatosensory and motor cortical areas after a motor learning task[21,29,80,81]. For instance, applying TMS, Pascual-Leone et al. demostrated that the cortical motor map of the muscles involved in a motor task became progressively larger until explicit knowledge was learnt, illustrating a rapid functional plasticity of motor cortical areas[21]. Similarly, several authors have described that the centro-parietal SEPs decreased and frontal SEPs increased following 20 minutes of repetitive typing[80,82,83], indicating rapid functional plastic changes in the cortical areas related to sensorimotor integration of afferent inputs.

In pain research, evoking MEPs, Le Pera et al.[31] showed an inhibition of the M1 during 5-10 minutes of acute muscle pain and around 30 minutes after the pain disappeared[31,48]. Similarly, Rossi et al.[32] showed an inhibition of early SEPs after muscle pain into upper and lower limbs[32,45]. In addition, Schabrun et al., demonstrated that the inhibitory effect lasted for several minutes after the pain vanished[48]. In recent years several other authors showed neuroplastic effects induced by acute muscle pain in the corticomotor output and sensory cortical excitability (Appendix C shows a list of papers using MEPs and SEPs to probe cortical excitability changes induced by experimental pain; systematic review[49]).

3.1. MOTOR EVOKED POTENTIALS

To probe corticomotor output changes induced by long-lasting muscle pain, MEPs evoked by TMS to ECRB have been used (Study I, Study II and Study III). TMS generates a current in the cerebral cortex able to stimulate the axons of the neurons in M1[50] (Fig 11). To record the motor response, surface recording electrodes were located in a bipolar configuration along the muscle fibres of the ECRB muscle with the reference placed on the olecranon[38].

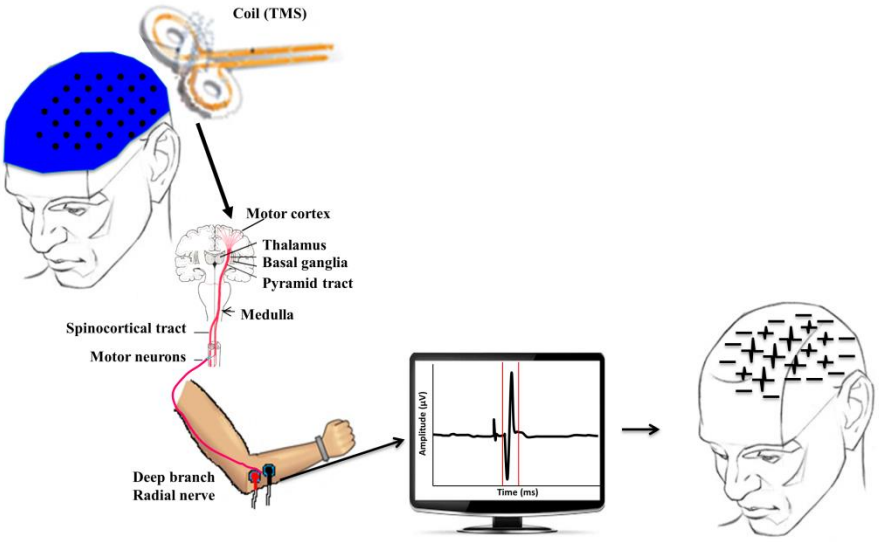


Fig. 11: The participants were seated with a swimming cap marked with a 1 × 1 cm grid. Recording electrodes were placed along the ECRB muscle and referred to the olecranon (not displayed in the image).

Three neurophysiological measures have been collected in this progect: Rest Motor Threshold (rMT), MEPs and Motor Maps:

1) rMT was the lowest intensity of the stimulator at which 5 out of 10 stimuli applied at the hot spot of the muscle at rest evoked a response with a peak-to peak amplitude higher than 50 μV[50].

2) MEPs were collected at 120% of rMT over the hot spot of ECRB muscle at rest to evaluate corticomotor excitability[50].

3) A motor map is defined as the territory where MEPs can be induced using a fixed stimulation intensity. In this project the TMS intensity was 120% of the individual's rMT and 5 stimuli at each site of the grid were delivered in a pseudo-randomly order[38,84,85] (Fig 12 and 13).

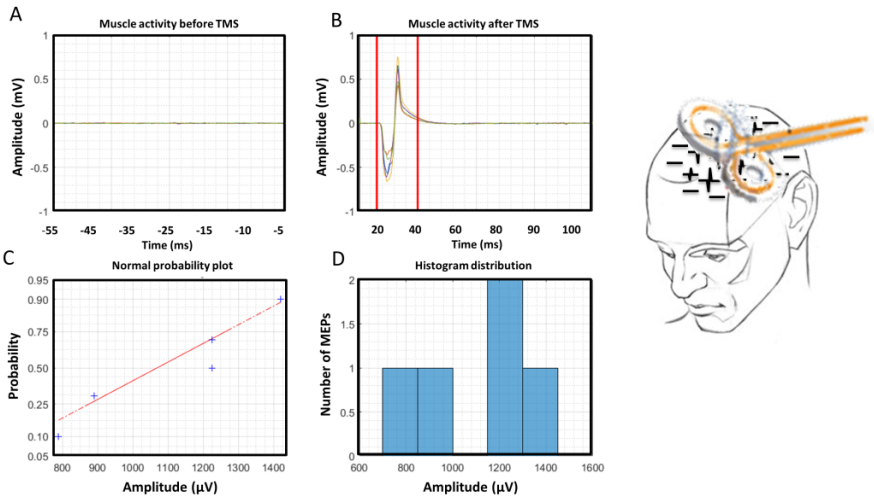


Fig 12: Illustrative example of 5 pulses delivered in the center of a motor map. 5 peak-to-peak MEPs were combined and displayed to check the absence of muscle activity before the TMS pulse (A), the MEP in the time window between 20 and 40 ms after the stimulation (red lines) (B). Trial-to-trial variability in peak-to-peak MEP amplitude was checked by probability plot (C) and histogram (D).

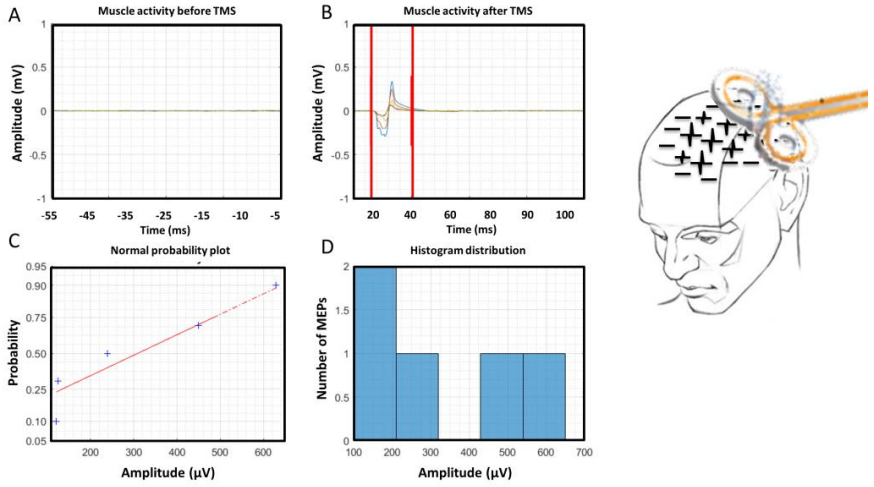


Fig 13: Illustrative example of 5 pulses delivered in the border of a motor map. The peak-to-peak MEPs were combined and displayed to check the absence of muscle activity before the TMS pulse (A), the MEP in the time window between 20 and 40 ms after the stimulation (red lines) (B). Trial-to-trial variability in peak-to-peak MEP amplitude was checked by probability plot (C) and histogram (D). **Note:** the lower peak-to-peak amplitude, the longer latency and the high variability of the response on the border of the map compared to the center of a map (fig 12). Wasserman et al., [86] suggested that the periphery of the muscle representation, with its lower density of corticospinal neurons, may generate fewer descending impulses in response to a standardized stimulation and require a longer time to achieve the temporal summation necessary for activation of spinal motoneurons[87].

The border of the motor map was considered when no MEPs were evoked in the grid site. The number of active map sites (*map area*) and map volume were calculated off-line by an in-house matlab code. Briefly, if the average peak-to-peak amplitude of the MEPs evoked at that site was higher than 50 μV, the site was considered "active" (Study I, II and III). The *map volume* was the mean of all active sites (Study I, II and III). The centre of gravity (*CoG*) was defined as the amplitude-weighted centre of the map and was calculated by $\frac{\sum Vi \cdot Xi}{\sum Vi}$; $\frac{\sum Vi \cdot Yi}{\sum Vi}$; where Vi represents mean MEP amplitude at each site with the coordinates Xi (latitude of CoG), Yi (longitude of CoG) [86].

Therefore, each motor map produced four outcomes: a map volume (sum of MEPs), a map area (number of active sites), longitude and latitude of centre of gravity referred to 0,0 (vertex) (Fig 14). More details about the methodology are reported in Study I.

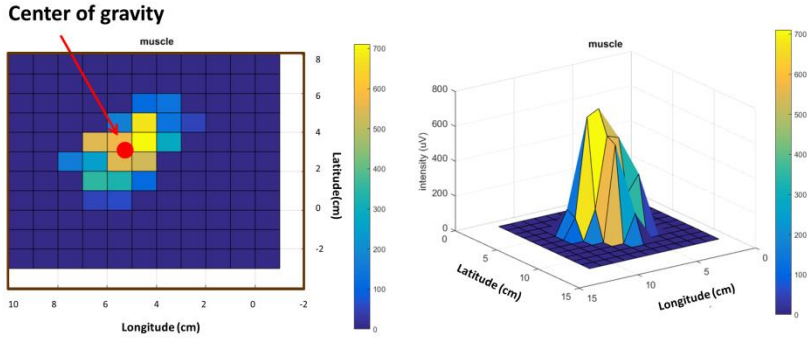


Fig 14: illustrative example of superior and lateral view of a 3D motor map of a subject. Note: 0,0 is referred to the vertex of the head (Cz). A motor map generally shows discrete amplitude peaks, or "hot spots", closely spaced (yellow and orange squares). These points represent low threshold areas where corticospinal neurons projecting to the particular muscle are most concentrated [86].

It is important to highlight that the methodology selected for this project makes impossible to determine the exact level of the excitability changes along the motor pathway. In fact, the amplitude of the MEP reflects the motor cortex and spinal motoneuron excitability. Therefore, the interpretation of the changes described in Study I, II and III were limited by the unspecificity of the outcome.

The present work confirmed the excellent reliability of rMT (ICC=0.94) and CoG latitude (ICC=0.86), the fair to good reliability of MEP in the hot spot (ICC=0.65), motor cortical volume (ICC=0.67), motor cortical area (ICC=0.71) and CoG longitude (ICC=0.44) (Appendix A).

3.2. CORTICOMOTOR NEUROPLASTICITY

The two TMS components affected by long-lasting muscle pain were the map volume (sum of all MEPs amplitude) and the map area (number of active sites) (Study I and Study II, Fig 15).

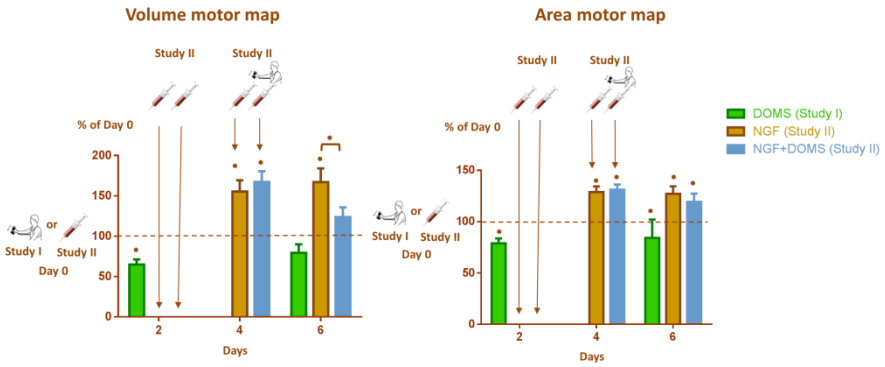


Fig 15. Mean (± SEM, N = 12) normalized volume motor map an area motor map (% of Day 0) for DOMS, NGF and NGF+DOMS groups. A significant difference in motor map volume and motor map area compared with Day 0 and Groups illustrated by * ($P < 0.05$) (Statistical analysis Study I and Study II). NOTE: Study I no recordings at Day 4.

Study I showed that muscle pain induced by eccentric exercise provoked a reduction of both motor map volume and area. Based on previous studies showing changes at spinal and peripheral level[88] but not at cortical level[89], the attenuation of the motor map excitability has been interpreted as a peripheral and/or spinal inhibitory effect provoked by muscle damage induced by eccentric exercise (Study I). In contrast, two injections of NGF facilitated the motor map excitability (Day 4), as previously reported[38]. In addition, Study II showed that a third injection of NGF maintained the facilitation of motor map excitability at Day 6 (NGF group). However, when eccentric exercise was applied, inhibitory effect of the motor map excitability was detected (NGF+DOMS group).

As explained by Schabrun et al.[38], the increase of motor map excitability during muscle pain induced by NGF may be a sign of neuroplastic changes underpinning the search for a new movement strategy. Indeed, an increase of motor map excitability has been shown during motor learning, and when a new motor strategy was acquired, the motor map excitability reduced[21]. A similar pattern has been described in the first phases of prolonged muscle pain as a new motor strategy is sought[38]. In fact, several studies have demonstrated an increased movement variability and muscles activity of the low back during experimental muscle pain[12,38,90] but decreased variability in patients with chronic low back pain[12,38,91]. Interestingly, the increase of motor map between Day 4 and Day 6 was very similar, suggesting that additional time may be needed before the motor map reduces.

In contrast, a reduction of the motor map excitability was found when DOMS was provoked in a pre-sensitized muscle, suggesting that the inhibitory spinal and/or peripheral effects of DOMS interfered with cortical facilitation induced by NGF.

The results of Study I and Study II suggested that muscle pain induced by NGF and eccentric exercise-induced DOMS provoked different adaptations of the motor map excitability, probably driven by different cortical and spinal mechanisms. While DOMS induced a depression of the motor map excitability, NGF-induced muscle soreness induced an increase of the motor map excitability.

3.3. SENSORY EVOKED POTENTIALS

SEPs are the neural responses to sensory stimuli recorded using electroencephalography (EEG)[92]. A stimulator was used to deliver 2 blocks of 500 electrical stimuli of 1 ms duration at a rate of 2 Hz. Stimulus intensity was set at 3 times the perceptual threshold detected in each session. To specifically activate the superficial branch of the radial nerve[93], the cathode was located on the right radial styloid process while the anode was placed two cm proximal. To check the correct location of the electrodes, participants were asked to indicate on their hand the area of the electrical sensation induced by the stimulation. If the participants did not point to the first and second finger, the anode electrode was relocated medially or laterally. This branch of the radial nerve has been selected in this project because the radial nerve innervates all wrist and fingers extensor muscles (structures targeted by the pain models).

To probe the neuroplastic changes induced by muscle pain in the frontal and parietal sensory cortical areas, SEPs have been recorded using an EEG cap including 64 recording electrodes (Study I, II and III). The recording electrodes active during the electrical stimulation were the F3, F1, Fc3, Fc1, C3, C1, Cp3, Cp1, P3 and P1 scalp sites and the electrical signals were referred to the electrical signal recorded on contralateral earlobe. This configuration was selected to optimize the resolution of the frontal and centro-parietal evoked potentials[94]. To minimize the displacement of the recording electrodes over different sessions, the EEG cap was mounted according to 10-5 system with Cz orientated to the vertex of the head[95]. The vertex of the head was defined as the interception between nasion-inion and the inter-aural lines.

The electrical signal was sampled at 2400 Hz, amplified (50000x), band-pass filtered off-line at 5-500Hz, divided in epochs of 400 ms (time windows -100 ms before the electrical stimulation to 300 ms after) and all traces were visually inspected for artefacts. Any contaminated epochs were manually rejected while the artefact-free epochs were averaged (Fig 16) (EEGlab).

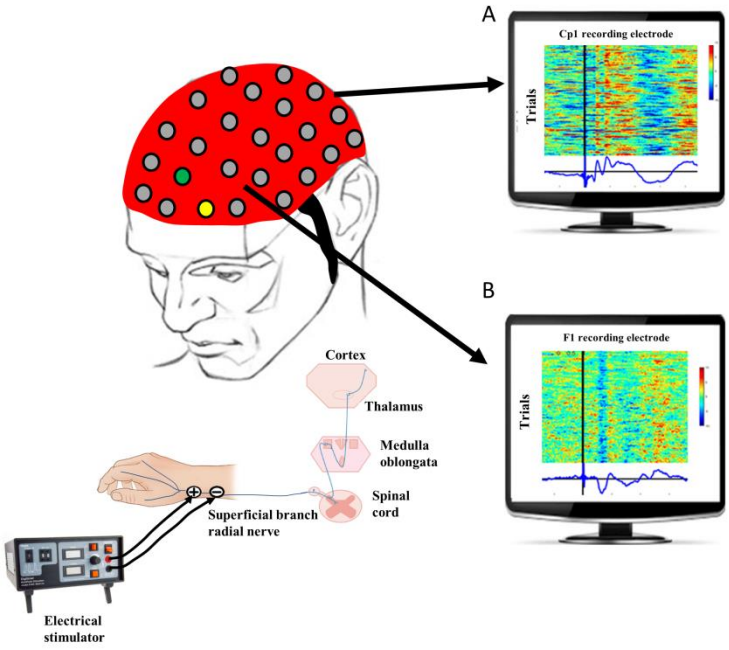


Fig 16: The electrical signal recorded, filtered off-line at 5-500Hz, divided in epochs based on the electrical stimulation and cleaned from artefacts (EEGlab). A and B show centro-parietal (Cp1) and frontal recording electrodes (F1) of a subject. All trials (epochs) are plotted in the time-domain (amplitude ±10μV) and averaged.

The peaks P14, N18, P22, N30, P45 and N60 in the frontal leads and P14, N20, P25, N33, P45 and N60 in the parietal traces[96] were automatically identified by an home-made program running on MatLab (Fig. 17).Visual check confirmed the correct peaks and, finally, the pre-stimulation interval was used to normalise the peak amplitude.

The amplitudes and latencies of each peak were imported in statistical software for the statistical comparisons.

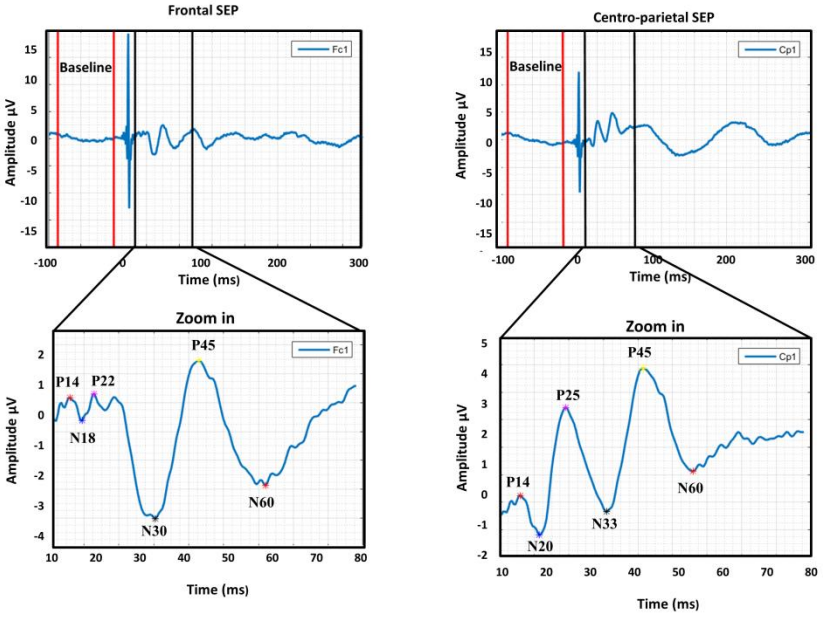


Fig. 17: The traces were separately plotted and local peaks of each recording electrodes in the data vector were automatically found in specific time windows. Visual inspection confirmed the correct identification of the peaks. The pre-stimulation interval (between the red lines) was used to normalise the peak amplitude (subtracting the mean amplitude in the interval from -100 ms to -20 ms before the electrical stimulation)

The present work confirmed the excellent reliability of P25 (ICC=0.84), P45 (ICC=0.95) and N60 (ICC=0.77), and fair to good reliability of N20 (ICC=0.58) and N30 (ICC=0.63) (Appendix A).

3.4. SOMATOSENSORY CORTICAL NEUROPLASTICITY

Only the early SEPs (between 10-80 ms) collected over the contralateral centro-parietal and frontal cortices have been extracted from the electrical signal. Previous studies have shown that these SEPs represent the earliest afferent inputs in the primary sensory (S1)[97], supplementary motor area (SMA) and premotor cortex (PMC)[97–100]. In addition, these neural components have been shown affected by functional neuroplastic changes induced by motor learning[80,82,83], immobilization[101], deafferention[23,102], pharmacological manipulations[103], repetitive transcranial magnetic stimulation[104,105] and acute muscle pain[45,48,49], making it reasonable to hypothesize that these neurophysiological measurements should also be affected by long-lasting muscle pain.

The long-lasting muscle pain models used in this project are characterized by the absence of pain at rest, while injections of algesic substances, such as hypertonic saline, used in previous studies provoke acute muscle pain[45,48]. Because acute muscle pain is accompanied by a loss of position sense and reduction of stimulus

perception, the depression of centro-parietal SEPs has been previously discussed as an effect of cortical gating of afferent inputs caused by acute pain[32,45]. In contrast, the absence of pain at rest during the electrical stimulation in the present project could not produce any cortical gating, but the cortical excitability changes have been interpreted as a sign of neuroplasticity of cortical processing of somatosensory afferents.

The two SEPs components affected in this project were: N30 and P45. Indeed, the combined results of Study I and II showed that muscle pain provoked by injections of NGF reduced the peak amplitude of N30 while DOMS did not show any N30 effect (Fig 18).

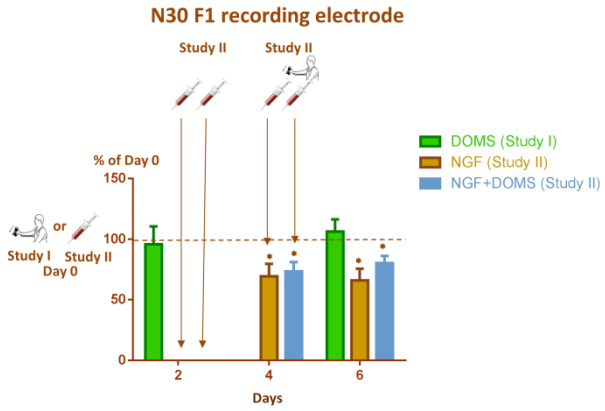


Fig 18. Mean (± SEM, N = 12) normalized N30 from F1 recording site (% of Day 0) for DOMS, NGF and NGF+DOMS groups. DOMS group performed eccentric exercise at Day 0. A significant difference in N30 peak amplitude in F1 recording site compared with Day 0 illustrated by * ($P < 0.05$) (Statistical analysis Study I and Study II).

Evidence from human studies have demonstrated that sensory inputs reach PMC and SMA either after synapsing in S1[106] or via parallel independent pathway from the thalamus[107,108]. It is well know that the N30 SEPs reduced during execution, observation and imagination of a movement ipsilateral to nerve stimulation[81,109–111]. In contrast, the N30 SEPs increased during execution of repetitive movements contralateral to nerve stimulation[80,82,83,105,112,113]. Importantly, using intra-cortical recording electrodes in epileptic patients, the PMC and the SMA have been shown to be the main generators of N30 SEPs[114]. Moreover, the depression of the N30 SEP component has been demonstrated in different neurological diseases, such as Parkinson disease[115]. This SEP component has also been linked to dopamine function since single doses of L-Dopa and apomorphine in Parkinson's patients normalized the N30 amplitude[116,117]. Finally, inhibitory or facilitatory rTMS paradigms delivered to PMC and SMA modify this frontal component[105]. Based on these evidences, it has been suggested that this cortical component represents the

functionality of a complex interhemispheric cortico/subcortical network linking basal ganglia, thalamus, supplementary and pre-motor cortices[105,118]. The results of Study II demonstrated that long-lasting muscle pain provoked by NGF was able to modify the N30 SEPs, probably interfering with some aspects of the motor planning or the motor execution.
However, at 30 ms of latency a second SEP generator from centro-parietal areas overlaps the frontal N30 SEPs. This second generator produces both the frontal N25 potential (not considered in this project) and the parietal P25 response[119]. Consequently, it is possible that in the NGF group the decrease of the frontal negative potential (probably N25) corresponds to the increase of the parietal P25 response, likely caused by a shift of the tangential source generating both responses (N25/P25) (Study III). Consequently, the observed N30 SEP modifications may also represent a parietal phenomenon.

The results of Study II suggest that excitability changes provoked by NGF-induced muscle pain were evidenced by the decrease amplitude of the N30 SEP. The decrease of this early-latency SEP component, in the absence of changes to other earlier and later components, indicates a likely frontal cortical site for pain plasticity, however a parietal phenomenon cannot be excluded.

The results of Study I and Study II showed that muscle pain provoked by both injections of NGF and eccentric exercise induced similar increase of peak amplitude of P45 (Fig 19).

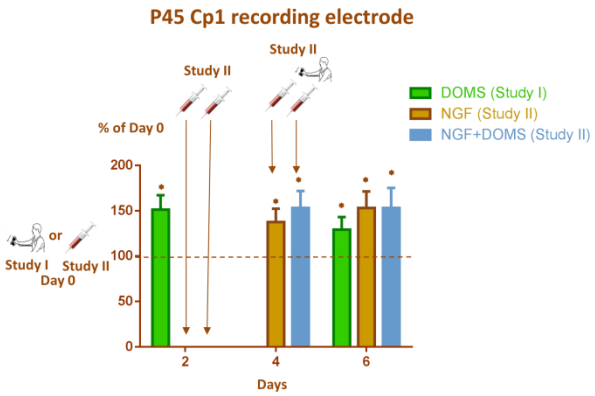


Fig 19. Mean (± SEM, N = 12) normalized P45 from Cp1 recording site (% of Day 0) for DOMS, NGF and NGF+DOMS groups. A significant difference in P45 peak amplitude in Cp3 recording site compared with Day 0 illustrated by * ($P < 0.05$) (Statistical analysis Study I and Study II).

Intracortical and scalp recording studies have demonstrated that the earliest evoked potentials after the electrical stimulation of a nerve have an S1 origin[97,120]. Although still debated, P45 recorded by scalp electrodes may reflect S1 activity[120]. Besides, S1 may be involved in the process of pain [121] and, particularly, in the sensory-

discriminative aspect of pain[120,122,123]. For instance, based intracortical recording studies, laser-evoked stimulations have shown to activate area 1 of S1[124,125]. Finally, inhibitory or facilitatory rTMS paradigms delivered to S1 modified the tactile stimuli and pain threshold, indicating that S1, in particular area 1, may play a role in some aspect of pain perception. However, P45 amplitude is also affected by attention[126,127], therefore it cannot be completely excluded that the P45 amplitude increase, described in Study I and Study II, can be explained by changes in the subject's attention to the affected territory.

Excitability changes provoked by NGF-induced muscle pain and DOMS were evidenced by the increase amplitude of the P45 SEP. The increase of this mid-latency SEP component, in the absence of changes to earlier components, suggests a centro-parietal cortical site for pain plasticity. However, changes in attention to the affected territory cannot be excluded.

3.5. MAIN FINDINGS ADDING TO THE CURRENT KNOWLEDGE

- DOMS is followed by corticomotor inhibition of the ECRB muscle.
- Muscle pain induced by 3 injections of NGF revealed similar increase of corticomotor excitability at Day 6 compared with 2 injections at Day 4.
- Application of DOMS on a pre-sensitised muscle injected by NGF depressed the corticomotor excitability.
- Muscle pain induced by eccentric exercise, two and three injections of NGF induced a similar increase of P45 SEPs.
- Muscle pain induced by two and three injections of NGF induced the same decrease of the peak amplitude of N30 SEPs.
- The application of DOMS on a pre-sensitized muscle did not alter the SEP.

CHAPTER 4. MODULATING PAIN NEUROPLASTICITY

rTMS is based on the application of repetitive trains of TMS to target specific cortical areas[128,129]. When a train of stimuli is delivered in specific time profile, changes in cortical excitability can be provoked and they have been interpreted as a sign of neuroplasticity[28]. However, the nature and the duration of the neuroplasticity induced by rTMS dependents on the interaction between the stimulation frequency, intensity, train duration and number of applications[129,130]. Classically, low frequency rTMS protocols (lower than 1Hz) inhibit cortical excitability while high frequency rTMS protocols (higher than 5 Hz) facilitate cortical excitability[129,131–134] (for detailed information on previous rTMS studies and cortical excitability based on MEPs and SEPs see Appendix D).

Briefly, to induce cortical excitability changes that last longer than the stimulation period (between 30 minutes and 1 hour)[129], high stimulus intensities are needed (around rMT), high numbers of stimuli (more than 500) and periods of several minutes (between 10 and 30 minutes) (Appendix D). One approach to extend the duration of cortical neuroplasticity is to apply multiple daily sessions of rTMS paradigm[135,136]. Based on animal models, multiple applications of rTMS enhance the lifetime of synaptic neuroplasticity[137]. Similar effect has been shown in healthy subjects, with daily rTMS sessions producing long-lasting neuroplastic changes, longer than the effects seen following a single application (around 1 hour)[135,138]. However, more importantly, clinical studies, investigating the therapeutic value of rTMS, use multiple stimulations over consecutive days in order to achieve long-lasting therapeutic effects (few days)[135].

4.1. THE ROLE OF THE LEFT DLPFC IN COGNITION AND PAIN

The dorsolateral prefrontal cortex (DLPFC) is a brain region implicated in emotion, cognition and behavior[67,139]. The left DLPFC is expanded in humans compared with other primates, indicating a role in complex cognitive processes[67,140]. Recently, the left DLPFC has been suggested to play an important role in pain suppression and detection (for a detailed review, see[67]). Based on the results of neuroimaging studies, nociceptive stimuli have shown a strong activity of the left DLPFC[141] in healthy subjects and chronic musculoskeletal pain conditions are commonly associated with decreased of left DLPFC gray matter and reduced function[65,142,143], reflecting probably a hypo-metabolic state. In addition, pain-relief interventions can reverse this structural and functional abnormality[66], confirming that pain interferes with this cortical function. Interestingly, 10 Hz left DLPFC rTMS has been applied as a therapeutic target in experimentally induced skin pain[60] and post-surgical pain[68,69], indicating that nociceptive and anti-nociceptive synaptic transmission can be modulated by 10 Hz rTMS stimulation to the left DLPFC. The mechanisms by

which 10 Hz left DLPFC rTMS can induce pain relief is not unclear[67]. A first possible mechanism may be the activation of the descending modulatory endogenous opioidergic system[144]. For instance, based on diffusion tensor imaging, a recent study showed the existence of an anatomical circuitry from the periaquaductal grey and the nucleus cuneiformis to the left DLPFC[145]. A second possible mechanism is that the analgesic effects derived from the left DLPFC rTMS occur through modulation of the cognitive function and mood state[67,146]. Indeed, 10Hz-rTMS over the left DLPFC provokes secondary changes in the left parahippocampal gyrus, the right insula, the right cingulate gyrus, the ipsilateral subgenual anterior cingulate cortex and medial orbitofrontal cortex[57,59].

In summary, the activity of left DLPFC may modulate pain perception and pain suppression, and two possible mechanisms have been proposed: 1) the activation of the descending modulatory endogenous opioidergic 2) the modulation of the cognitive function and mood state.

4.2. HOW TO TARGET THE LEFT DLPFC

A technical challenge to perform rTMS to the DLPFC is the appropriate location of the coil over the scalp[147,148]. While the location of the M1 is based on a measurable response of the MEPs, the stimulation of the DLPFC does not elicit any neurophysiological response. For this reason, alternative approaches based on scalp measurements have been developed for locating the DLPFC[148]. One of the earliest and most widely used approach is the "5 cm rule", where the motor hotspot for the first interosseous muscle is first identified applying TMS on the motor cortex, and then the coil is moved 5 cm forward to this site[149]. However, the "5 cm rule" fails to stimulate the DLPFC in 1/3 of the patients undergoing treatment[148,150]. Based on a MRI study[151], indicating an discrepancy of around 2 cm between the site identified with the 5 cm rule, a modified "7 cm rule" have been adopted, although these approach likewise appears not very reliable as well[148,152]. Therefore, an alternative approach, based on the 10–20 EEG electrode placement system, has been developed[148] to localize the left DLPFC.

The algorithm, named as "BeamF3", provides the localization of the left DLPFC based on 3 measurements: left tragus- right tragus distance, nasion-inion distance and head circumference [147,148]. The BeamF3 algorithm has been compared with the MRI-guided neuronavigation and provide a reasonable approximation for locating the left DLPFC in a majority of subjects[148] (used in Study III and Supplemetary paper I).

4.3. 10 HZ RTMS TO THE LEFT DLPFC

The rTMS protocol used in Study III and Supplementary paper I consisted of 1 session per day for 5 consecutive days (from Day 0 to Day 4) (Fig. 20). Each intervention consisted of 80 trains of 5 second pulses with a frequency of 10 Hz and an interval of 10 seconds between each train. The total amount of pulses was 4000

per session[60,68] and the total number of stimulations for the entire treatment was 20000. The stimulation intensity used was 110% of the rMT of the FDI muscle and the coil was located at the left DLPFC according to the BeamF3 algorithm[147,148].

The pain model selected to induce long-lasting muscle pain consisted of two injections of NGF at Day 0 and Day 2 since detailed clinical and neurophysiological manifestations of this model has been previously investigated[38].

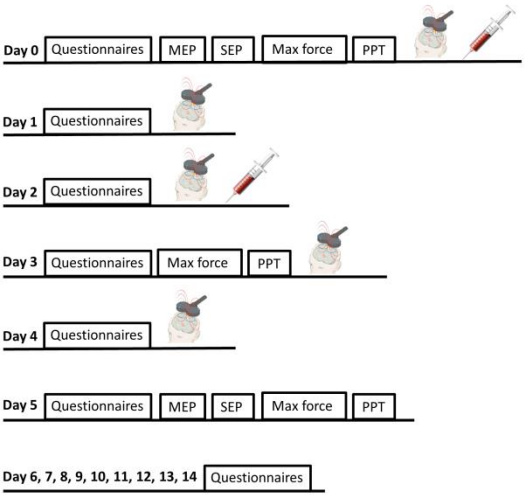
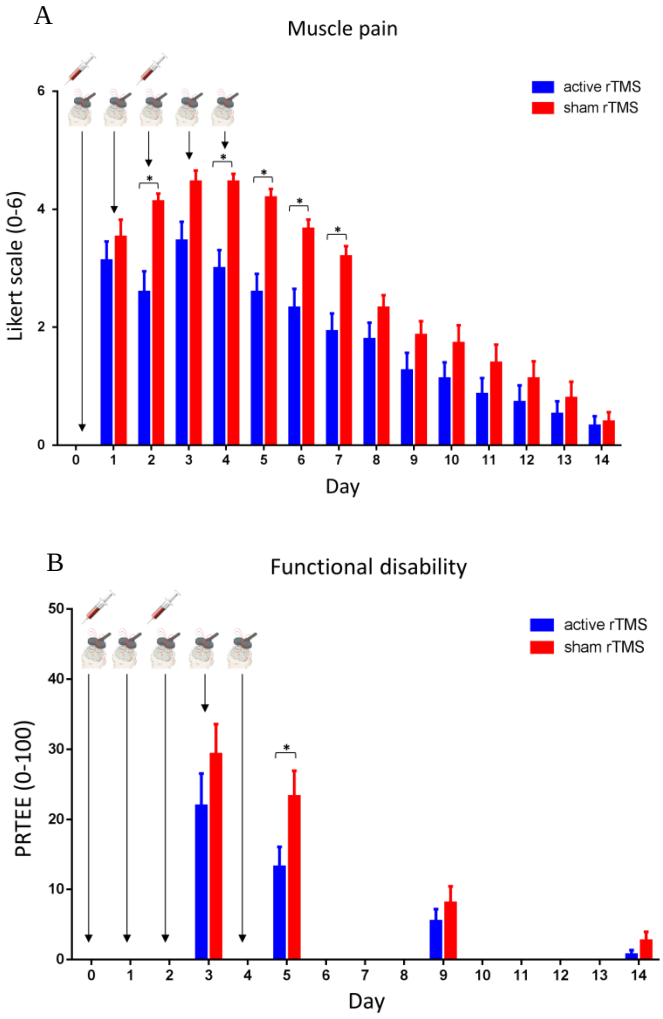


Fig 20: After assessments at Day 0, Day 1, Day 2, Day 3, and Day 4 participants received active or sham 10 Hz left DLPFC rTMS. Pain related questionnaire regarding muscle pain (Likert scale) was completed on Days 0 to 14, while functional disability (PRTEE) and pain distribution (body chart) were completed on Days 0, 3, 5, 9, and 14. At Day 0 and Day 5, neurophysiological testing (motor evoked potentials and sensory evoked potentials) and quantitative motor and sensory assessments (wrist extensor force and pressure pain thresholds) were assessed. At Day 3, quantitative motor and sensory assessments were also assessed.

4.4. EFFECTS OF 10 HZ LEFT DLPFC RTMS ON LONG-LASTING MUSCLE PAIN

Pain intensity is one of the strongest predictor of the transition from acute to chronic pain[153,154], therefore, interventions able to decrease pain intensity during the first stages of development may have the clinical application to prevent chronic pain after injury and tissue damage (Supplement Paper I). The results of Study III and Supplement Paper I showed that five consecutive days of left DLPFC rTMS reduced pain intensity (Fig. 21 A), functional disability (Fig. 21 B), spatial distribution (Fig. 21 C) and muscle hyperalgesia to mechanical pressure (Fig. 21 D) induced by two injections of NGF. Importantly, these changes outlasted to 3 days after the intervention period (Fig. 21 A) but the duration of muscle pain was not affected by

the treatment. Maximal wrist extension force was not affected by NGF and by the treatment (Fig. 21 E).



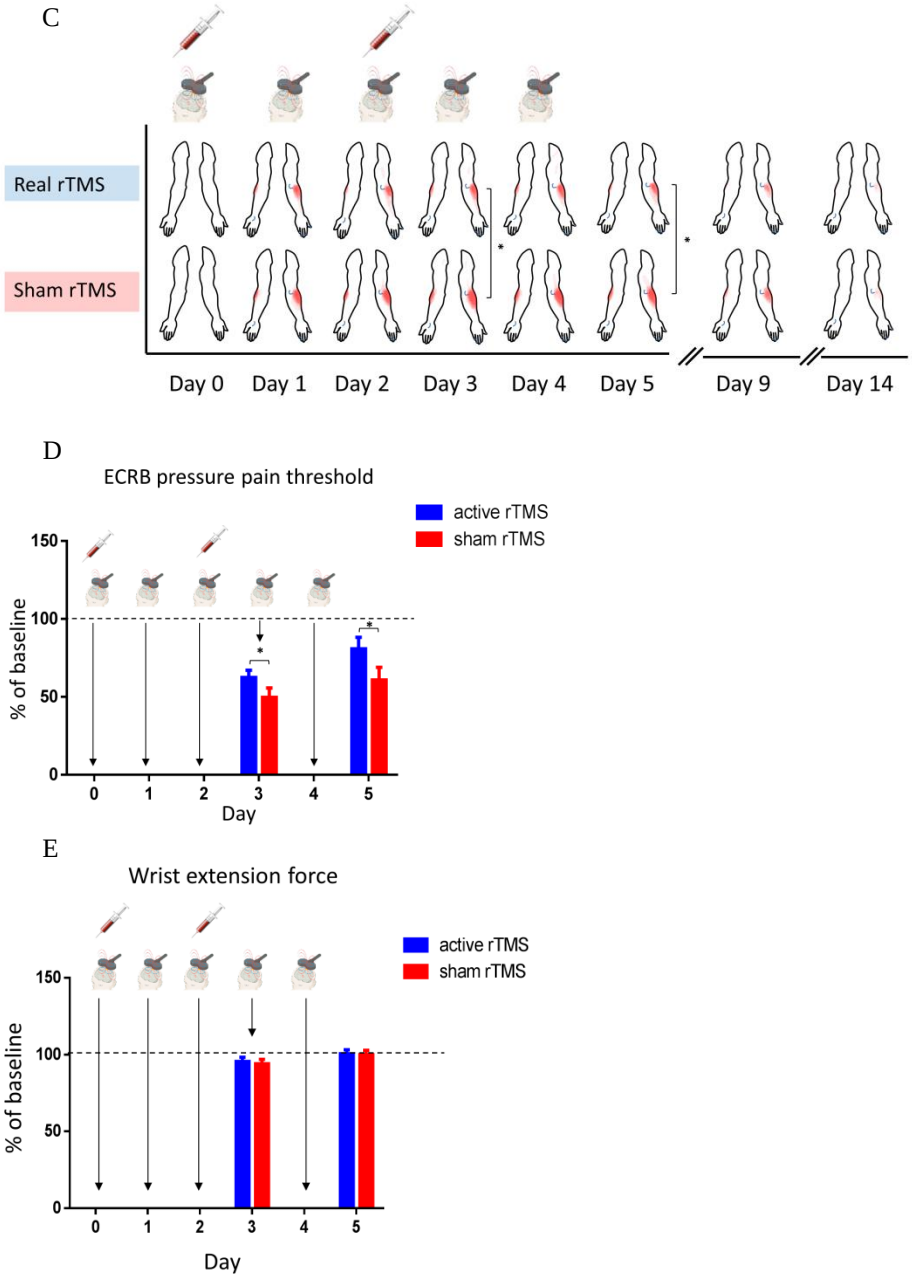


Fig 19. Mean (± SEM, N = 15) Likert scores of muscle pain (A), PRTEE scores (B), pain areas (C), muscle pain sensitivity to mechanical pressure (D) and maximal wrist extension force (E) following NGF injections on Day 0 and Day 2. Note: 10 Hz left DLPFC rTMS occurred on Days 0, 1, 2, 3, and 4.

4.5. EFFECTS OF 10 HZ LEFT DLPFC RTMS ON CORTICOMOTOR EXCITABILITY

A previous study has demonstrated that muscle pain provoked by two intramuscular injections of NGF induced an increase of motor cortical excitability at Day 4[38]. These results have been confirmed and expanded in Study II. In Study III, the sham group showed a facilitation of motor map excitability at Day 5. In opposite, when 10 Hz left DLPFC rTMS was applied, the motor map volume decreased (Fig 22) while the map area did not expand. A first explanation of this reduction of the motor map excitability reported in Study III was that the multiple stimulations of left DLPFC during muscle pain induced a pain relief and, consequently, a modulatory effect on the motor map excitability. A second explanation was that the reduced motor map excitability was caused by the multiple acute pain sensations that the participants experienced during active rTMS (Study III). Indeed, the MEPs depression has been observed during and after acute muscle pain[49]. In this study, the procedural score of pain from the 1st to the 5th session in the active rTMS group were 5.8 ± 0.8, 4.3 ± 0.7, 3.9 ± 0.6, 3.5 ± 0.6 and 2.9 ± 0.5, respectively (Supplement paper 1).

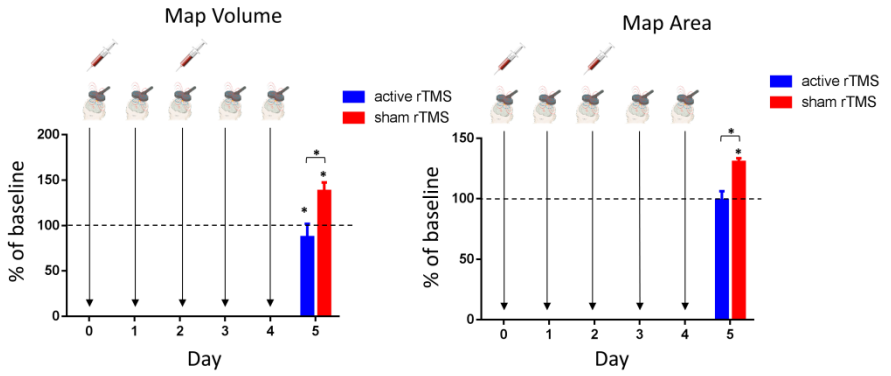


Fig 22. Mean (± SEM, N = 15) normalized map volume and map area (% of Day 0) in both groups. NGF injections were performed on Day 0 and Day 2 and 10 Hz left DLPFC rTMS occurred on Days 0, 1, 2, 3, and 4.

4.6. EFFECTS OF 10 HZ LEFT DLPFC RTMS ON SOMATOSENSORY CORTICAL EXCITABILITY

In Study III, the sham group showed a decrease of the N30 SEP similar to the N30 SEP changes described in Study II (Day 4 and Day 6) (Fig 23). In contrast to the sham group and the results of Study II, 10 Hz left DLPFC rTMS increased the N30 SEP (Study III).

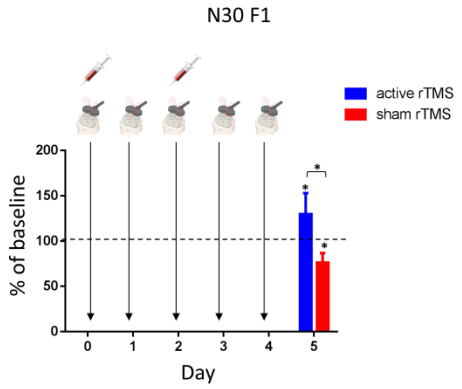


Fig 23. Mean (± SEM, N = 15) normalized N30 peak amplitude in F1 recording site (% of Day 0) in both groups. NGF injections were performed on Day 0 and Day 2 and 10 Hz left DLPFC rTMS occurred on Days 0, 1, 2, 3, and 4.

Opposite to the N30 SEP, an increase of the P45 SEP was found in both groups (Fig 24). When muscle pain was induced by NGF and eccentric exercise was used subsequently, increased P45 SEP was observed (Study I and Study II). Interestingly, left DLPFC rTMS did not modulate the P45 SEPs (Study III) although the DLPFC has modulatory reciprocal connections to the associative sensory cortex but not directly with S1[155].

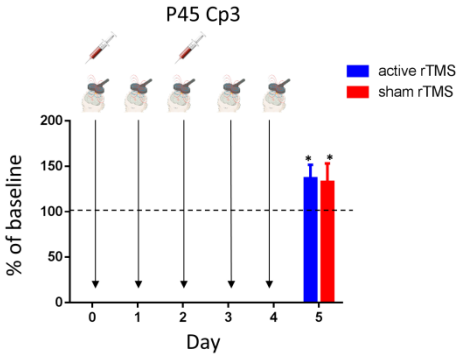


Fig 24. Mean (± SEM, N = 15) normalized P45 peak amplitude in Cp3 recording site (% of Day 0) in both groups. NGF injections were performed on Day 0 and Day 2 and 10 Hz left DLPFC rTMS occurred on Days 0, 1, 2, 3, and 4.

In summary, 5-daily sessions of 10 Hz left DLPFC rTMS reduced motor map excitability, normally increased by long-lasting muscle pain, and increased N30 SEPs. No changes were found on the P45 SEPs. These results suggested that daily sessions of 10 Hz left DLPFC rTMS modulate the cortical excitability induced by muscle pain across several days, likely by the frontal-basal ganglia network.

4.7. MAIN FINDINGS ADDING TO THE CURRENT KNOWLEDGE

- 10 Hz left DLPFC rTMS reduced the peak pain intensity on muscle pain provoked by NGF.
- The analgesic effect lasted at least three days after the end of the treatment.
- 10 Hz left DLPFC rTMS did not reduced the duration of muscle pain induced by NGF.
- 10 Hz left DLPFC rTMS modulated the effects provoked by NGF on the motor map excitability.
- 10 Hz left DLPFC rTMS modulated the effect induced by NGF on the frontal cortical excitability.
- 10 Hz left DLPFC rTMS did not modulate the effect provoked by NGF on the centro-parietal cortical excitability.

CHAPTER 5. CONCLUSION

The three goals of this PhD project were: 1) to characterize the nature and temporal manifestations of three long-lasting pain models (Study I and Study II), 2) to probe the nature and temporal cortical excitability changes during long-lasting muscle pain (Study I and Study II); 3) to modulate pain neuroplasticity using daily sessions of left DLPFC rTMS (Study III).

The results of this project showed that NGF and DOMS provoked similar pain intensity, moderate functional disability and reduction of pain sensitivity to mechanical pressure. The combination of NGF and DOMS are able to induce higher pain intensity but not to extend the duration of muscle pain compared with the only NGF or DOMS. Finally, only DOMS is able to reduced maximal force.

Both repeated injections of NGF and eccentric exercise-induced DOMS produced several neuroplastic effects in the corticomotor excitability: While NGF facilitated the motor map excitability DOMS depressed it. Moreover, only injections of NGF altered the sensorimotor integration of sensory afferents in the frontal cortex while both NGF and DOMS modified the sensory processing in the centro-parietal cortex.

Finally, daily sessions of left DLPFC rTMS induced analgesic effects and modulated pain neuroplasticity induced by NGF in motor and premotor cortices but not the centro-parietal cortex (Fig 25).

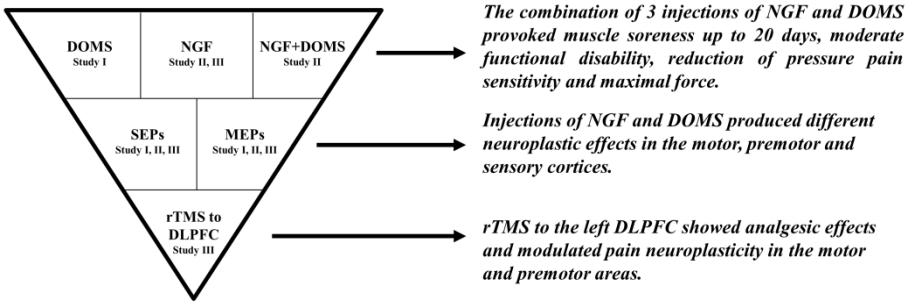


Fig 25. Dissertation outline with main findings.

5.1. FUTURE PERSPECTIVE

1) Improvement of pain models used to simulate the clinical sensorimotor features of musculoskeletal pain. For instance, combination of repeated intramuscular injections of NGF, eccentric exercise-induced DOMS and repeated intramuscular injections of hypertonic saline may be able to mimic several sensorimotor features of musculoskeletal pain, such as long-lasting muscle hyperalgesia (pain duration), force deficits and episodic acute exacerbation (high pain intensity) of muscle pain. However, experimental prolonged muscle pain models cannot replicate other

features as anxiety, negative mood, increased fatigue, fear of movement, and fear of re-injury typical of chronic pain[156]. It is important to note that all these missing features may play a crucial role in the development of maladaptive pain neuroplasticity. Consequently, the combination of these experimental pain models with "sleep deprivation" [157] and tasks provoking "stress-induced hyperalgesia" [158,159] may also mimic some different neuropsychological features of chronic musculoskeletal pain and may help to investigate maladaptive pain neuroplasticity.

2) Applying more specific neurophysiological techniques to evaluate cortical neuroplasticity may help to understand the mechanisms behind pain-induced neuroplasticity and, therefore, to tailor specific neuromodulatory interventions. For instance, intra-cortical inhibition and intra-cortical facilitation may help to investigate mechanisms such as $GABA_A$, $GABA_B$ and glutamate-NMDA receptors. Both receptors have been described affected in experimental muscle pain[160] and clinical musculoskeletal pain condition[161,162]. Moreover, oscillatory models in the frequency domain during muscle pain can suggest thalamo-cortical disinhibition based on alpha oscillations[163]. For instance, preliminary evidences have shown reduced alpha oscillations during experimental pain[163] and clinical pain[164–166], suggesting that altered thalamo-cortical activity may be associated with pathological pain.

3) Applying different rTMS paradigms at different cortical levels. For instance, theta burst stimulation (TBS) has gained much interest in the last 10 years because of its efficacy and its short stimulation period[135]. In fact, the main advantage of TBS is the shorter duration of the stimulation (less than 3 minutes) and fewer numbers of pulses (600 or 1200 pulses) compared with the 'classical' 10Hz-rTMS (from 1500 to 4000 pulses in 15-20min)[60,144,167,168]. Moreover, a stronger analgesic effect[169], and more reproducible neuroplastic effects[170,171] have been reported with TBS. Consequently, therapeutic potential of multiple daily sessions of TBS could become a valid therapeutic option to induce prolonged neuroplastic changes and analgesic effects.
Finally, different cortical areas can be targeted by the intervention, such as M1, S1, the insula or the anterior cingulate cortex. For instance, M1 stimulations have shown significant long-term analgesic effect with repeated high frequency rTMS sessions in neuropathic and non-neuropathic pain patients[51,168].

5.2. FOCUS ON TRANSLATION

In this project the main reason to induce long-lasting muscle pain in wrist extensors muscle was to mimic some clinical sensorimotor features seen in lateral epicondylalgia (LE). LE is a debilitating musculoskeletal condition characterized by pain in the area of the lateral humeral epicondyle. LE affects around 3% of the general population[172], with peaks of 15% in workers at-risk industries[173] or in athletes[174] using repeated upper limb and hands movements. Besides to the clinical pain and disability characteristics, bilateral sensorimotor impairments have been shown in patients with LE. Based on cross-sectional studies, altered joint position sense[156,175], reduced maximal grip and wrist extension force[162,175], slower reaction

time and speed of movement[176], local and widespread mechanical hyperalgesia[177,178], bilateral cold hyperalgesia[179,180] have been described in lateral epicondylalgia patients, suggesting that the nervous system may play a role in the chronicity of this disease.
Based on that, recent studies have also investigated and shown that the excitability and organization of the cortical and subcortical areas were altered in patients affected by lateral epicondylalgia. For instance, evidence of spinal cord hyperexcitability[181], motor cortex hyperexcitability[182], less intra-cortical inhibition[162], and less intra-cortical facilitation in the M1 contralateral to the affected ECRB muscle[162] have been shown in chronic lateral epicondylalgia patients compared with healthy controls.

Similar clinical and neurophysiological findings have been also described in other chronic musculoskeletal pain conditions such as back pain[161,183–186], knee pain[187–189], and shoulder pain[190] and they have been also interpreted as signs of maladaptive pain neuroplasticity.

Based on the interpretation of these results, interventions able to non-invasively modulate cortical neuroplasticity can be a reasonable tool for the future management of musculoskeletal pain conditions. More specifically, future clinical studies applying rTMS in the early stage of a musculoskeletal pain conditions are necessary to evaluate whether rTMS applied in the first stage of this disease may reduce pain chronicity.

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APPENDICES

Test-retest reliability of MEPs, SEPs, PPT over the right ECRB muscle and maximal wrist extensor force (unpublished data)

Test-retest reliability of MEPs, SEPs, PPT over the right ECRB muscle and max wrist extension force was performed in 22 healthy volunteers. 12 volunteers participated in Study 1 (Fig 2: data collected at Day -1 and Day 0) and 10 volunteers participated in Study II and Study III (Day 0).

Statistical analysis:

Absolute and relative reliability were calculated to assess test-retest reliability of the following measurements 1) motor evoked potentials (rMT, MEPs, map volume, map area, CoG latitude and CoG longitude), 2) sensory evoked potentials (N20, P25 and P45 on Cp3 EEG recording site and N30 and N60 on F1 EEG recording site), 3) right ECRB PPT and 4) right max wrist extension force.

The absolute reliability was calculated using 1) Inter-individual and intra-individual coefficient of variation (CV)[191] and 2) Bland Almond analysis[192].

1. Inter-individual and intra-individual CV was calculated with the following formula:
    - Coefficient of variation intra-individual (CV_{intra}= $SD_{intra\text{-}individual}$/$mean_{intra\text{-}individual}$*100). Then, the average of the CV for each subject was calculated.
    - Coefficient inter-individual between two baseline sessions ((CV_{inter} = $SD_{mean\ of\ difference}$/($mean_{baseline1}$+$mean_{baseline2}$)/2) *100).
2. Bland almond analysis was plotted to inspect the homoscedasticity and reported all raw data; bias was the difference between 2 baseline plots against individual mean of the two baselines. Upper and lower limits of agreement (LoA) were showed as ±1.96 standard deviation (SD) of difference between sessions; (Table 1 and Figure 1).

The relative reliability was assessed using intra-class correlation coefficient (ICC)[193]. Absolute agreement and 95% confidence interval (IC) were fixed. ICC was calculated using a two way random model; single measurement (3,1) and average measurement (3,k) were reported[191]. ICC values above 0.75 were considered

'excellent' reliability, in the interval between 0.40–0.75 fair to good reliability and less than 0.40 poor reliability[194].

Results:

The results for rMT, CoG latitude, PPT over the right ECRB muscle, max wrist extension force, N60 in F1 recording site, P45 and P25 in Cp3 recording site exhibited excellent reliability. MEP in the hot spot, motor cortical map, motor cortical area N30 in F1 recording site, N20 in Cp3 recording site and CoG longitude exhibited the fair to good reliability (Table 1).

Measure Reliability			**Absolute reliability**	**Relative reliability**	
	CV (%) intra	**CV (%) inter**	**Bland almond analysis**	**ICC (3, 1)**	**ICC (3, k)**
MEP			**Bias (lower LoA/ upper LoA)**	**(95% confidential interval)**	
rMT	2.9	6.1	0.73 (-3.6/5.0)	0.94 (0.86/0.97)	0.97 (0.92/0.99)
MEP	16.1	38.0	-42.03 (-496.6/412.5)	0.65 (0.33/0.84)	0.79 (0.5/0.913)
Map area	7.8	23.4	-0.12 (-7.8/7.5)	0.71 (0.41/0.87)	0.83 (0.58/0.93)
Map Volume	14.1	40.1	53.88 (-3855.8/3963.5)	0.67 (0.35/0.85)	0.80 (0.52/0.92)
Latitude CoG	2.5	6.1	0.01(-0.71/0.73)	0.86 (0.71/0.94)	0.93 (0.83/0.97)
Longitude CoG	16.2	40.3	0.12 (-1.2/0.92)	0.44 (0.05/0.72)	0.61 (0.1/0.84)
SEP					
N20 (Cp3)	20.7	40.8	0.2 (-0.83/1.23)	0.58 (0.23/0.8)	0.73 (0.37-0.89)
P25 (Cp3)	16.1	39.3	0.01 (-0.99/1)	0.84 (0.65/0.93)	0.91 (0.79/0.96)
N30 (F1)	15.0	38.8	0.11(-1.31/1.53)	0.63 (0.31/0.83)	0.77 (0.47/0.90)
P45 (Cp3)	12.5	21.6	0.04 (-0.9/0.82)	0.95 (0.89/0.98)	0.98 (0.94/0.99)
N60 (F1)	18.4	37.4	0.09 (-0.91/1.09)	0.77 (0.54/0.86)	0.85 (0.70/0.93)
Clinical					
Max wrist force	4.67	15.81	-1.2 (-44.25/41.86)	0.88 (0.73/0.95)	0.93 (0.84/0.97)
PPT Right	9.57	22.35	-0.09 (-96.76/96.59)	0.84 (0.65/0.93)	0.91 (0.79/0.96)

ECR

Table 1 reports absolute reliability (intra and inter-subject CV, Bland Almond analysis) and relative reliability measures (ICC).

Conclusion:

Compared to previous test-retest studies (intra-individual and inter-individual variability), similar results have been found in MEPs, motor cortical volume[195,196], frontal and centro-parietal SEPs[193,197] and PPT[198,199] evaluated in separate sessions. In addition to previous results, the present findings indicate that these neurophysiological and clinical outcomes are also reliable when combined.

Appendix A. Overview of studies investigating the effects of DOMS and NGF models on the elbow

A summary of studies provoking long-lasting muscle pain models in the wrist extensor muscles is showed in the table below. A range of terms (pain, musculoskeletal pain, persistent pain, delayed onset muscle soreness, nerve growth factor, experimental muscle pain, hyperalgesia, lateral epicondylalgia; tennis elbow, elbow pain), grouped by main search terms, were used in combinations to search the following databases: Pubmed, Scopus and Web of Science.

The table aims at highlighting the temporal profile of the measurements, the outcomes used to describe the pain model and the main findings.

Authors and Journal	**Paper Title**	**Pain model and Temporal Profile**	**Outcomes used to describe pain model**	**Main findings**
Leger et al., **Medicine & science in sports & exercise (2001)**	Muscle function at the wrist after eccentric exercise	DOMS model (eccentric exercise). 5 sessions: Day 0 (Pre), 24 h (Day 1), 48 h (Day 2), 96 h (Day 4), and 240 h (Day 10).	Muscle pain intensity (Likert scale), Pain area distribution (body chart), Maximal wrist extension force	Eccentric exercise by wrist extensors induced muscle pain around lateral epicondyle of the homerous and reduced maximal force of the wrist extensor muscles.
Slater et al., **Eur J Pain (2003)**	Experimental deep tissue pain in wrist extensors—a model of lateral epicondylalgia	DOMS model (eccentric exercise). 3 sessions: Day 0, Day 1 and Day 7	Muscle pain intensity (Likert scale), Pain area distribution (body chart), Pressure pain thresholds (pressure algometer), Maximal wrist extension force	Muscle pain, mechanical hyperalgesia pr pressure and force reduction support the use of DOMS as an experimental model simulating the clinical sensorimotor correlates of lateral epicondylalgia.
Slater et al., **Manual therapy (2006)**	Effects of a manual therapy technique in experimental lateral	DOMS model (eccentric exercise). Two groups: control vs	Muscle pain intensity (Likert scale), Pain area distribution (body chart), Pressure pain	No significant differences in pain intensity, pain distributions, mechanical hyperalgesia to

	epicondylalgia (DOMS model)	"mobilization-with-movement" intervention. 3 sessions: Day 0, Day 1 and Day 7	thresholds (pressure algometer), Maximal wrist extension force	pressure or force attenuation.
Fernández-Carnero et al., **Medicine & science in sports & exercise (2009)**	Pressure Pain Sensitivity Mapping in Experimentally Induced Lateral Epicondylalgia	DOMS group (eccentric exercise). 3 sessions: Day 0 (before and immediately after), and Day 1 (24 h after eccentric exercise)	Pressure pain thresholds (pressure algometer) were assessed over 12 points forming a 3 x 4 cm matrix	The most sensitive localizations for PPT assessment corresponded to the muscle belly of the ECRB.
Delfa de la Morena et al., **Journal of Strength and Conditioning Research (2013)**	Pressure pain mapping of the wrist extensors after repeated eccentric exercise at high intensity.	DOMS group (eccentric exercise). **First test round:** 3 sessions: Day 0 (before and immediately after), and Day 1 (24 h after eccentric exercise) **Second test round performed 7 days later:** 3 sessions: Day 0 (before and immediately after), and Day 1 (24 h after eccentric exercise)	Pressure pain thresholds (pressure algometer) were assessed over 12 points forming a 3 x 4 cm matrix	A lack of hyperalgesia underlined adaptation after the second test round of eccentric exercise performed 7 days after the initial test round.
Bergin et ., **Pain Medicine (2015)**	Movement Evoked Pain and Mechanical Hyperalgesia after Intramuscular Injection of Nerve Growth Factor: A Model of Sustained Elbow Pain	NGF model (1 injection): Four experimental sessions: Days 0, 2, 4, and 10, and completed a daily diary of their elbow pain from Day 0 to Day 10	Muscle pain intensity (Likert scale), Pain area distribution (body chart), Pressure pain thresholds (pressure algometer),	A single intramuscular injection of NGF induces sustained elbow pain that is lasting for up to one week.

Schabrun et al., **Cerebral cortex (2016)**	Motor Cortex Reorganization and Impaired Function in the Transition to Sustained Muscle Pain	NGF model (2 injections): Four experimental sessions: Days 0, 2, 4, and 14.	Muscle pain intensity (Likert scale), Pain area distribution (body chart), Pressure pain thresholds (pressure algometer), Grip force	Two NGF injections resulted in a progressive increase in pain and disability up to 2 weeks.
Mista et a., **J Pain (2016)**	Effects of Prolonged and Acute Muscle Pain on the Force Control Strategy During Isometric Contractions	NGF model (1 injection): Three experimental sessions: Days 0, 2, 4	Maximal wrist extension force	No impairement of the maximal force after injection of NGF.

Appendix B. Overview of studies probing experimental pain on the upper limb using MEPs and SEPs

A summary of studies examining the cortical excitability in response to experimental muscle pain models is showed in the table below. A range of terms (pain; musculoskeletal pain, persistent pain, experimental pain, motor cortex; motor evoked potential, transcranial magnetic stimulation, evoked potential, sensory evoked potentials), grouped by main search terms, were used in combinations to search the following databases: Pubmed, Scopus and Web of Science.

The table aims at highlighting the muscle pain models, the outcomes used to describe the cortical excitability and the main findings.

Authors	Paper title	Muscle pain model	Cortical excitability	Main findings
Le Pera et al., **Clin Neurophysiology (2001)**	Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain	Injection of hypertonic saline into the right ADM (short-lasting pain model)	MEPs	Tonic muscle pain inhibited the corticomotor excitability.
Rossi et al., **Clinical Neurophysiology (2003)**	Early somatosensory processing during tonic muscle pain in humans: relation to loss of proprioception and motor 'defensive' strategies	Ascorbic acid injection in the right first dorsal interosseous muscle (short-lasting pain model)	SEPs.	Reduction of the post-central N20-P25-N33 complex.
Svensson et al., **European Journal of Pain (2003)**	Suppression of motor evoked potentials in a hand muscle following prolonged painful stimulation	Hypertonic saline in the FDI muscle (short-lasting pain model)	MEPs (stimulus response curves)	Muscle pain is followed by a depression of MEPs. These changes are at least in part due to a depression of the excitability of the motoneurones

				in the spinal cord.
Del Santo et al., **Brain Research (2007)**	Corticospinal drive during painful voluntary contractions at constant force output	Injection of ascorbic acid in the muscle belly of ADM and BIC (short-lasting pain model)	MEP	Acute pain during voluntary isometric contractions increased the MEP in both proximal and distal upper limb muscles (opposite effect to rest MEPs).
Schabrun et al., **J Pain (2012)**	Muscle Pain Differentially Modulates Short Interval Intracortical Inhibition and Intracortical Facilitation in Primary Motor Cortex	Hypertonic saline in the FDI muscle (short-lasting pain model)	Short interval intracortical inhibition (SICI) and intracortical facilitation (ICF)	SICI was increased following pain, but not during pain. ICF was decreased both during and after pain when compared with the pre-pain condition
Schabrun et al., **Neuroscience (2013)**	Temporal association between changes in primary sensory cortex and corticomotor output during muscle pain.	Hypertonic saline in the FDI muscle (short-lasting pain model)	SEPs and MEPs	Pain reduces sensory processing (SEPs) before motor output is altered (MEPs).
Schabrun et al., **Plos One (2015)**	New Insight into the Time-Course of Motor and Sensory System Changes in Pain	Hypertonic saline in the FDI muscle (short-lasting pain model)	SEPs and MEPs	S1 processing (SEPs) and corticomotor output (MEPs) are co-modulated in association with muscle pain.

Schabrun et al., **Cerebral cortex (2016)**	Motor Cortex Reorganization and Impaired Function in the Transition to Sustained Muscle Pain	NGF injection into ECRB muscles (long-lasting pain model)	MEPs, TMS mapping, SICI, ICF, interhemispheric inhibition	Reorganization of M1 characterized by: 1) increased map excitability, 2) reduced intracortical inhibition, 3) increased intracortical facilitation, 4) reduced interhemispheric inhibition from the "affected" to the "unaffected" hemisphere.

Appendix C. Overview of studies probing the neuroplastic effect of a single session rTMS using MEPs and SEPs

A summary of studies examining the cortical excitability (MEPs and SEPs) in healthy subjects applying different rTMS parameters is showed in the table below. A range of terms (Sensory cortex; Motor cortex; cortical excitability, motor evoked potential, transcranial magnetic stimulation, evoked potential, sensory evoked potentials, repetitive transcranial magnetic stimulation, rapid-rate transcranial magnetic stimulation), grouped by main search terms, were used in combinations to search the following databases: Pubmed, Scopus and Web of Science.

The table aims at highlighting the cortical area targeted by rTMS, frequency, intensity, number of pulses, duration of the intervention, cortical excitability after-stimulation and duration of cortical excitability after the stimulation.

Study	Cortical area targeted by rTMS	Frequency	rTMS intensity	No. of rTMS pulses	Duration of interventions	Cortical excitability after-stimulation	Duration of cortical excitability after-effects
Pascual-Leone et al., Brain (1994)	M1	1 Hz	100-220% RMT	20	20 sec	No MEP changes	Not evaluated
		5-10 Hz	100-220% RMT	20	2-4 sec	↑ MEP amplitude	~ 3 min
		20 Hz	100-220% RMT	20	1 sec	↑ MEP amplitude	~ 3 min
Chen et al., Neurology (1997)	M1	0.9 Hz	115% RMT	810	15 min	↓ MEP amplitude	~ 3 min
		0.1 Hz	115% RMT	360	1 h	No MEP changes	Not evaluated
Berardelli et al., Exp Brain Res (1998)	M1	5 Hz	120% RMT	100	5 min	↑ MEP amplitude	Not evaluated
Rollkin et al., Muscle Nerve (1999)	left DLPFC	5 Hz	90% RMT	60	12 sec	↓ MEP amplitude	~ 8 min
Siebner et al., Neurology (1999)	M1	1 Hz	90% RMT	1800	30 min	No changes on stimulus-response curve	Not evaluated

Wu et al., Neuroscience Letters (2000)	M1	5 Hz and 15 Hz	120% RMT	30	6 sec, 2 sec	↑ MEP amplitude, ↑ICF and ↓ ICI	~ 1.5 min
Muellbacher et al., Clin Neurophysiology (2000)	M1	1 Hz	115% RMT	900	15 min	↓ stimulus-response curve	Not evaluated
Maeda et al., Clin Neurophysiology (2000)	M1	1 Hz	90% RMT	240	4 min	↓ MEP amplitude	24 hours
		10 Hz	90% RMT	240 (3 trains intreval 72s)	4 min	No MEP changes	24 hours
		20 Hz	90% RMT	240 (6 trains interval 38s)	4 min	↑ MEP amplitude	24 hours
Stefan et al. Brain (2000)	M1	PAS 25	150% RMT	90 pairs (ISI = 25ms)	30 min	↑ MEP amplitude	~ 30 min
Touge et al. Clin Neurophysiology (2001)	M1	1 Hz	95% RMT	1500	25 min	↓ MEP amplitude	~ 30 min
Gerschlager et al., Neurology (2001)	PMC	1 Hz	90% AMT	1500	15 min	↓ MEP amplitude	~ 30 min
	left DLPFC	1 Hz	90% AMT	1500	15 min	No MEP changes	~ 60 min
Enamoto et al., Clin Neurophysiology (2001)	M1	1 Hz	110% AMT	200	200 s	↓ SEP amplitude (N20/P25 and P25/N33)	~ 60 min
	S1	1 Hz	110% AMT	200	200 s	↑ SEP amplitude (N20/P25 and P25/N33)	~15 min
Romero et al., Clin Neurophysiology (2002)	M1	1 Hz	90% RMT	600	10 min	↓ MEP amplitude	~ 10 min
Di Lazzaro et al., Exp Brain Res (2002)	M1	5 Hz	120% RMT	20	4 s	↑ MEP amplitude	~ 2 min
Fitzgerald et al., Clin neuroph (2002)	M1	1 Hz	85 and 115% RMT	900	15 min	both ↓ MEP amplitude	Not evaluated
Tsuji and Rothwell, Journal of	M1	PAS 20	105% RMT	180 pairs (ISI = N20)	30 min	↑ SEP amplitude (N20/P25,	~15 min

Physiology (2002)						P25/N33)	
Satow et al., neurology (2003)	M1	0.9 Hz	90% RMT	900	15 min	No changes SEP	Not evaluated
Grunhaus et al., International Journal of Neuropsychopharmacology (2003)	left DLPFC	10 Hz	90% RMT	1200	12 min	↑ MEP amplitude	~30 min
Gilio et al. (2003) J Physiol	M1	1 Hz	117% RMT	900	15 min	↑ MEP amplitude in **contralateral** M1	~ 20 min
Wolters et al. (2003) J Physiol	M1	PAS 25	130% RMT	90 pairs (ISI = 25 ms)	30 min	↑ MEP amplitude	Not evaluated
	M1	PAS 10	130% RMT	90 pairs (ISI = 10 ms)	30 min	↓ MEP amplitude	~ 75 min
Lyer et al., The Journal of Neuroscience (2003)	M1	6 Hz-primed 1 Hz rTMS	90% RMT and 115% RMT	No reported + 600	10 + 10 min	↓ MEP amplitude	~ 60 min
Chouinard et al., J. Neurophysiol (2003)	PMC	1 Hz	90% rMT	900	15 min	↓ MEP amplitude	Not evaluated
Wolters et al. J Physiology (2005)	S1	PAS N20	150% RMT	180 pairs (ISI = N20)	30 min	↑ SEP amplitude (N20/P25, P25/N33)	~ 30 min
	S1	PAS N20-20ms	150% RMT	180 pairs (ISI = N20-20 ms)	30 min	↓ SEP amplitude (N20/P25, P25/N33)	~ 30 min
Huang et al. Neuron (2005)	M1	cTBS	80% AMT	300	20 s	↓ MEP amplitude	~ 20 min
	M1	cTBS	80% AMT	600	40 s	↓ MEP amplitude	~ 60 min
	M1	iTBS	80% AMT	600	190s	↑ MEP amplitude	~ 20 min
Olivieri et al., Neuroscience Letters (2005)	Cereb.	1 Hz	90% RMT	600	10 min	↑ MEP amplitude	~ 30 min
Lang et al. (2006) Neurology	M1	1 Hz	115% RMT	900	15 min	↓ MEP amplitude	~ 20 min
	M1	1 Hz	90% RMT	900	15 min	↓ MEP amplitude	~ 20 min

Urushihara et al., NeuroImage (2006)	PMC	1Hz	85% RMT	250	250s	↑ frontal N30 SEP	Not evaluated
	PMC	0.2 Hz	85% RMT	250	250s	No SEP changes	Not evaluated
Ishikawa et al., Clin Neurophys (2007)	M1	cTBS	80% AMT	600	40 s	↑ MEP amplitude and ↓ SEP amplitude (P25/N33, N33/P40)	~ 40 min
	S1	cTBS	80% AMT	600	40 s	no changes MEP amplitude and ↓ SEP (P25/N33)	~ 15 min
Katayama et al., Clin Neurophys (2007)	M1	iTBS	80% AMT	600	190s	No SEP changes	~ 30 min
	S1	iTBS	80% AMT	600	190s	↑ SEP amplitude (N20/P25)	~ 30 min
Fierro et al., Exp Brain Reseach (2007)	Cereb ellum	1 Hz	90% RMT	900	15 min	↑ MEP amplitude	~ 20 min
Hosono et al., Clin Neurophys (2008)	PMC	1 Hz	85% RMT	375	5 min	↑ frontal N30 SEP	~ 10 min
Gentner et al., Cerebral Cortex (2008)	M1	cTBS	70% RMT	600	40 s	↓ MEP amplitude	~ 30 min
Stefan et al., NeuroImage (2008)	M1	cTBS	80% AMT	600	40 s	↓ MEP amplitude and ↑ contralateral MEP	~ 5 min
Koch et al., Clin Neurophys (2008)	Lat. cereb	cTBS	80% AMT	600	40 s	↑ MEP amplitude	~ 15 min
	Lat. cereb	iTBS	80% AMT	600	190s	↓ MEP amplitude	~ 15 min
Suppa et al., J Physiol (2008)	M1	cTBS	80% AMT	600	40 s	↓ MEP amplitude and ↑ contralateral MEP	~ 30 min
Todd et al., Exp Brain Res (2009)	M1	10 min 2/6Hz + cTBS	90% RMT and 70% AMT	600	40 s (2 min interval)	No MEP changes	~ 30 min

	M1	cTBS + iTBS	70% RMT + 70% RMT	1200	5 min (2 min interval)	↓ MEP amplitude	~ 30 min
Huang et al., Clin Neurophysiol (2009)	PMC	cTBS	80% AMT	600	40 s	↓ MEP amplitude	~ 60 min
Premji et al., BMC Neurosci (2010)	S1	iTBS	80% AMT	600	190s	↑ bilateral N20/P25 SEP	~ 15 min
Rothkegel et al., Clinical Neurophysiology (2010)	M1	5 Hz (six blocks of 200 pulses each with an intertrain interval of 60 s)	90% AMT	1200	8 min	↓ MEP amplitude	~ 15 min
Fierro et al., Exp Brain Reseach (2010)	left DLPFC	5 Hz	90% RMT	1800	8 min	No MEP changes	~ 30 min
Gamboa et al., Exp Brain Res (2010)	M1	Prolonged ITBS (PiTBS)	80% AMT	1200	390s	↓ MEP amplitude	~ 60 min
	M1	Prolonged cTBS (PcTBS)	80% AMT	1200	80s	↑ MEP amplitude	~ 60 min
Katayama et al., Clin Neurophys (2010)	S1	cTBS	80% AMT	600	40 s	No SEP changes	~ 30 min
	S1	iTBS	80% AMT	600	190s	↑ SEP amplitude (N20/P25)	~ 30 min
Doeltgen et al., Clin Neurophysiology (2011)	M1	cTBS	65% and 70% RMT	300	20s	↑ MEP amplitude (70% RMT); ↓ MEP amplitude (65% RMT)	~ 30 min
Doeltgen et al., Exp Brain Res (2011)	M1	iTBS-primed cTBS	80% AMT	600	190 s + 40 s (2 min interval)	↓ MEP amplitude	~ 30 min
Premji et al., PlosOne (2011)	S2	cTBS	80% AMT	600	40 s	↑ bilaterally MEP amplitude	~ 60 min
	S2	iTBS	80% AMT	600	190s	↑ controlateral MEP amplitude	~ 60 min

Di Lazzaro et al., J Neurophysiol (2011)	M1	PAS 25	150% RMT	90	30 min	↑ MEP amplitude	~ 30 min
	M1	PAS 10	130% RMT	90	30 min	↓ MEP amplitude	~ 30 min
	M1	1 Hz	110% RMT	900	30 min	↓ MEP amplitude	~ 30 min
	M1	5 Hz	90% RMT	900	15 min	No MEP changes	~ 30 min
	M1	cTBS	80% AMT	600	40 sec	↓ MEP amplitude	~ 30 min
	M1	iTBS	80% AMT	600	190 sec	↑ MEP amplitude	~ 30 min
Doeltgen et al., European Journal of Neuroscience (2012)	M1	tDCS-primed cTBS	80% AMT	600	40 sec	↓ MEP amplitude	~ 60 min
Torta et al., PlosOne (2013)	M1	cTBS	80% RMT	600	40 sec	No SEP changes	Not evaluated
	S1	cTBS	80% RMT	600	40 sec	No SEP changes	Not evaluated
Legon et al., Brain stimulation (2013)	SMA	cTBS	80% AMT	600	40 sec	↓ frontal N30 SEP, no changes MEP amplitude	~ 30 min
Jacobs et al., Brain stimulation (2014)	S1	cTBS (30Hz)	55% RMT	600	40 sec	↑ MEP amplitude	~ 45 min
	M1	cTBS (30Hz)	55% RMT	600	40 sec	↓ MEP amplitude	~ 30 min
Goldsworthy et al., Brain Stimulation (2014)	M1	cTBS	70% RMT and 80% AMT	600	40 sec	↓ MEP amplitude	~ 30 min
Goldsworthy et al., Cerebral cortex (2014)	M1	spaced cTBS	70% RMT	1200	2*40 sec interval 10 min	↓ MEP amplitude	~ 120 min
Neva et al., Behavioural Brain Research (2014)	PMC	iTBS	80% AMT	600	190 sec	↑ MEP amplitude	Not evaluated
Brown et al., Behavioural Brain	PMC	cTBS	80% AMT	600	40 sec	↓ frontal N30 SEP and N60 SEP	~ 30 min

Research (2015)	Right DLPFC	cTBS	80% AMT	600	40 sec	↑ frontal N30 SEP and ↓ frontal N60, ↑parietal P25 and P40	~ 30 min
Opitz et al., Frontiers in Human Neuroscience (2015)	left DLPFC	cTBS	80% AMT	600	40 sec	↓ frontal N30 SEP and ↓P40	~ 25 min
Goldsworthy et al., Clin Neurophisiol (2016)	M1	cTBS	70% RMT	600	40 sec	I/O curves (peak ↓ 150%)	~ 30 min
	M1	iTBS	70% RMT	600	190sec	I/O curves (peak ↑110%%)	~ 30 min
Tse et al., scientific reports (2018)	M1	spaced iTBS	80% AMT	600	190 sec	↑ MEP amplitude	Not evaluated

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