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Larsen, Dennis Boye

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BY
DENNIS BOYE LARSEN

DISSERTATION SUBMITTED 2019

AALBORG UNIVERSITY
DENMARK

PROBING AND MODULATING PAIN-INDUCED CORTICOMOTOR EXCITABILITY REDUCTION BY ENGAGING PREMOTOR CORTEX ACTIVITY IN HUMANS

PHD THESIS

by

Dennis Boye Larsen

AALBORG UNIVERSITY
DENMARK

Dissertation submitted

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PhD supervisor: Associate Prof. Shellie A. Boudreau, Ph.D.,
Aalborg University

PhD co-supervisor: Prof. Thomas Graven-Nielsen, Ph.D., dr.med.,
Aalborg University

PhD committee: Associate Professor Andrew J. T. Stevenson (chairman)
Aalborg University
Scientific Director of the Center for Interdisciplinary
Research in Rehabilitation and Social Intergration
(CIRRIS) Catherine Mercier
University Laval
Dr., PhD, Reader in Rehabilitation Neuroscience
Michael J. Grey
University of East Anglia

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CV

Dennis received a B.Sc. degree in medicine, and a M.Sc. degree in translational medicine from Aalborg University. Subsequently, he went into Ph.D. training under the supervision of Associate Prof. Shellie A. Boudreau, at Center for Neuroplasticity and Pain, Aalborg University.

His focus has been on probing and modulating corticospinal excitability, as measured by non-invasive brain stimulation. In this respect, the focus has been to understand the plasticity of corticospinal excitability reduction in response to acute experimental muscle pain. The main methods used to probe the corticospinal system have been transcranial magnetic stimulation, electromyography, and acute experimental muscle pain as induced by hypertonic saline injections. Additionally, to modulate corticospinal excitability response to acute experimental muscle pain, external models known to modulate corticospinal excitability were applied, more specifically, a working memory two-back task and action observation combined with motor imagery. He has been involved in supervising and assessing 3rd semester medicine students in student projects as well as running neurophysiology workshops for the 1st semester Pain Master's. Furthermore, he has been involved in several dissemination activities through congress activity, abstract and poster submission for international congresses, publications in international peer-reviewed journals outside the PhD topic, and acted as reviewer for a peer-reviewed journal.

ENGLISH SUMMARY

Chronic musculoskeletal pain is a major societal problem due to the impact on quality of life and the large financial burden. Arguably, a main reason why chronic musculoskeletal pain management is still suboptimal is that the underlying mechanisms remain undecided.

Over the last three decades our understanding of the influence of sensorimotor changes in response to acute and chronic muscle pain has improved. Nonetheless, technological limitations, controversial findings, and knowledge gaps contribute to no overwhelmingly successful rehabilitation regimes for individuals living with chronic musculoskeletal pain.

In this respect, the aim of this PhD project was to apply and test novel approaches for modulating the well-known phenomenon of a reduced motor cortical response following a painful episode. This PhD project utilized a well-established pain model for inducing localized transient pain and aimed to modulate the ensuing reduced motor cortical response by engaging the prefrontal and premotor areas of the brain. Premotor cortex activation has been shown able to facilitate primary motor cortex (M1) excitability. Therefore, the objectives of the PhD project were to (1) establish a robust model for inducing a reduction in corticomotor excitability and (2) modulate pain-induced reduction in corticomotor excitability by engaging premotor cortex activity.

The first study demonstrated and characterized a robust hypertonic saline pain-induced reduction in corticomotor excitability in the small hand, but not forearm musculature, indicating that despite shared corticomotor representation, differential responses can be elicited. The second study showed that performance of a two-back task was ineffective, possibly due to influences related to prefrontal, subcortical, and/or intracortical mechanisms, in modulating the pain-induced reduction in corticomotor excitability, but enhanced pain perception. Finally, the third study provided the first evidence that action observation combined with motor imagery successfully modulated pain-induced reduction in corticomotor excitability, possibly through premotor cortex activation facilitating M1 excitability.

In conclusion, the current PhD thesis provides novel evidence on how to modulate pain-induced reduction in corticomotor excitability in the acute phase of muscle pain by action observation and motor imagery. This contributes to our understanding of the malleability of the motor system, and that an easily delivered task such as action observation combined with motor imagery is warranted in future research in managing musculoskeletal pain.

DANSK RESUME

Kroniske muskuloskeletale smerter er et stort samfundsmæssigt problem grundet indflydelsen på livskvalitet og den store økonomiske byrde. Der kan argumenteres for at hovedårsagen til at behandlingen af kroniske muskuloskeletale smerter stadig er suboptimal, er fordi de underlæggende mekanismer stadig er uafklarede.

Over de sidste tre årtier har vi fået øget forståelse for indflydelsen af sensorimotoriske ændringer ved akutte eller kroniske muskelsmerter. Ikke desto mindre, bidrager teknologiske begrænsninger, kontroversielle fund og mangel på viden til at der ikke findes overvældende succesfulde rehabiliteringsordninger for individer der lever med kroniske muskuloskeletale smerter.

Derfor var målet med dette PhD projekt at anvende og teste nye tilgange til at modulere det velkendte fænomen hvor the kortikale motoriske respons reduceres efter en smertefuld episode. Dette PhD projekt anvendte en veletableret smerte model der inducerer lokaliseret forbigående smerte og forsøgte at modulere den efterfølgende reduktion i kortikalt motor respons ved at aktivere de præfrontale og præmotoriske områder af hjernen. Kortikal præmotorisk aktivering kan facilitere den kortikale primær motoriske (M1) excitabilitet. Derfor var målsætningerne for PhD projektet at (1) etablere en robust model for smerte-induceret reduktion af kortikomotorisk excitabilitet og (2) modulere smerte-induceret reduktion af kortikomotorisk excitabilitet ved at aktivere kortikal præmotorisk aktivitet.

Det første studie demonstrerede samt karakteriserede en robust saltvandssmerte-induceret reduktion i kortikomotorisk excitabilitet i småhånds- men ikke underarmsmuskulatur, der indikerede at selvom de to muskulaturer deler kortikomotorisk repræsentation, kan forskellige ændringer fremprovokeres. Det andet studie viste at en two-back opgave, muligvis på grund af påvirkning af præfrontale, subkortikale, og/eller intrakortikale mekanismer, ikke kunne modulere smerte-induceret reduktion i kortikomotorisk excitabilitet, men i stedet forøgede smerteføelelsen. Det tredje studie viste, for første gang, at action observation kombineret med motor imagery kunne modulere den smerte-inducerede reduktion i kortikomotorisk excitabilitet, muligvis gennem kortikal præmotorisk aktivering der faciliterede M1 excitabiliteten.

Som konklusion tilføjer denne PhD afhandling ny evidens på hvordan man kan modulere smerte-induceret reduktion i kortikomotorisk excitabilitet i den akutte fase af muskel smerte, ved action observation og motor imagery. Dette bidrager til vores forståelse af hvordan motor systemet kan formes, og en opgave som action observation kombineret med motor imagery, der let kan leveres til forsøgspersoner, er berettiget yderligere forskning i hvordan man kan behandle muskuloskeletale smerter.

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My deepest gratitude goes to Center director of CNAP, Prof. Thomas Graven-Nielsen, for allowing me to explore my curiosity in pain neuroplasticity research. Prof. Thomas Graven-Nielsen has been paramount to the studies presented in the present PhD thesis, but also teaching me valuable insights into academia through his own extensive experience.

I met Associate Prof. Shellie A. Boudreau during my Master's education where we did our first work together, and thanks to her, I decided to apply for a PhD scholarship at CNAP. Shellie has been my greatest inspiration and an amazing mentor throughout my PhD, and I feel lucky to call her my colleague and friend. Words cannot express the gratitude and admiration I hold for her, and how her support and continuous effort to push my limits have made me a better researcher and person.

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The acknowledgments section is not extensive enough for me to be able to thank all the wonderful people at CNAP and SMI individually, but my sincerest gratitude goes to all the people I have met over the years. Thank you to the CNAP and SMI secretariat and administration, for making everyone's lives easier every single day of the year! You guys are doing amazing work for all of us and are highly appreciated.

Special thanks go to Enrico De Martino with whom I shared many good times both during lab training and in personal conversations at and outside of work. Thank you for being a good friend, you will go far in anything you set your mind to. Megan McPhee and Line Bay Sørensen also deserve a wholeheartedly felt thank you for all the good times we have spent together over the years. Both of you are brilliant and will go far in anything you wish, and you have made the time as a PhD much more enjoyable! A special thank you also goes to current and former office-mates and friends, Rósa Hugosdóttir, Steffan Wittrup Christensen, and Ning Qu who have been great company and have always been up for cake and banter. Fellow PhDs Alessandro Ranieri, Rocco Giordano, Daniele Riccio, and Post-doc Silvia Lo Vecchio have all been a big part of my last three years, and I thank you for being amazing individuals. Finally, a special thanks to Associate Prof. Kristian Kjær Petersen for believing in me and supporting me into my next step of my career. I look forward to work with you!

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PREFACE

The work for this PhD thesis was performed between August 2015 and December 2018 at Center for Neuroplasticity and Pain (CNAP), SMI®, Department of Health Science and Technology, Aalborg University, Aalborg, Denmark. The Danish National Research Foundation (DNRF121) is gratefully acknowledged for stipend and project support.

The PhD thesis contributes to our understanding by filling knowledge gaps of the effects of pain on the corticospinal system and explores novel methods to modulate pain-induced corticospinal excitability reduction.

The first chapter of the thesis briefly introduces the overarching issue associated with managing musculoskeletal pain, and the general concept of neuroplasticity of the motor system. This is further substantiated by exploring the concepts, and their interaction, of adaptive and maladaptive pain neuroplasticity of the motor system. The second chapter covers established experimental protocols that allow for probing and provoking the motor system. These experimental protocols include e.g. tasks known to engage premotor cortex activity such as action observation and motor imagery, and acute and chronic pain models. The third chapter discusses the impact of muscle on pain-induced reduction in corticomotor excitability and different possibilities for inducing and modulating neuroplasticity of the motor system. The current thesis findings are considered with respect to the large body of literature specifically investigating methods for restoring motor function through exercise, non-invasive brain stimulation, and specific for the thesis, modulation through non-primary motor areas. The fourth chapter delves into the main results and findings of the current PhD thesis, and put them into perspective for future studies. These perspectives are discussed in relation to basic and clinical research and sum up how the current thesis adds to our current knowledge on pain-induced neuroplasticity of the motor system.

The PhD thesis is based on three original papers, one of which has been published and the remaining two currently under review in international peer-reviewed journals.

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CHAPTER 1. INTRODUCTION

1.1. MUSCULOSKELETAL PAIN AND THE CENTRAL NERVOUS SYSTEM

We have all experienced soreness or pain in our muscles at one point or another due to exercise or injury. Fortunately, most of us return to a pain free state and normal function after recovery. However, for some, muscle pain or musculoskeletal pain persists even after the original injury or trauma have dissipated or been corrected. In fact, musculoskeletal pain conditions such as neck and back pain, accounted for approximately 18.5% of years lived with disability (YLD) in 2015 [1]. These numbers remained unchanged in 2017, where musculoskeletal pain was still the main contributor to YLD, especially in working-age males and females (20-54 years) [2].

A major factor to the large number of individuals living with chronic musculoskeletal pain is that the mechanisms underlying the acute-to-chronic pain transition are elusive [3,4]. For instance, the degree of tissue damage is not the main driver of pain intensity perception [5,6]. Instead, the duration (from hours to months) that the nervous system has been exposed to constant barrages of nociceptive input, may partly explain the development of musculoskeletal pain chronicity [7]. Indeed, pain duration has been shown to predict for example phantom limb and residual pain development after amputation, [8], post-surgery pain [9], and acute-, subacute- and chronic low-back pain [10]. If the duration of nociceptive input to the central nervous system is a factor in later pain chronification, gradual changes along the neuraxis may subsequently predispose individuals to develop chronic pain. The current and prevailing motor adaptation to pain theory accounts for this notion, describing a shift in biomechanical load onto associated and unassociated structures during painful movement [11]. This, in turn, provides the patho-anatomical basis for a persistent nociceptive drive to the central nervous system that may underlie the transition from acute-to-chronic pain [7,11]. Indeed, a recent opinion paper on musculoskeletal pain treatment, implied that nociceptive-driven changes in sensorimotor cortices, may underlie the chronification after the initial peripheral insult [3]. This notion is well supported and there is ample evidence that motor [12–20] and sensory [21–24] cortices are involved in experimental musculoskeletal pain [25].

It is therefore unequivocally clear that the sensorimotor areas of the human brain are involved in both acute and chronic musculoskeletal pain conditions. One missing part to the puzzle is to understand how to translate the fundamental knowledge of these sensorimotor changes into improved musculoskeletal pain management. Independent if the transition from acute-to-chronic pain is peripherally or centrally driven, the changes occurring in response to pain are unified under the term

neuroplasticity. The concepts of adaptive and maladaptive neuroplasticity, as it occurs in the motor pathways, will be explored in more detail in the next Sections.

1.2. ADAPTIVE NEUROPLASTICITY

The terms adaptive and maladaptive neuroplasticity of the motor system, describe how the nervous system structurally and functionally adapts to aid healing after injury [3] or possibly promote the development of chronic pain [11], respectively. Adaptive neuroplasticity of the motor system is often associated with advantageous (i.e. advantageous adaptive neuroplasticity) changes in e.g. motor performance or return towards normal function after musculoskeletal pain [26]. Animal studies have provided neurophysiological evidence on short and long-term anatomical and functional changes occurring when animals are exposed to e.g. motor skill learning [27,28]. In humans, motor practice has been shown to induce a facilitation in corticospinal excitability [29–31], expansion of trained muscle representation at the cortical level [32–34], and improved motor performance [13,30,31,35–38]. At present, these behavioral and neurophysiological manifestations of motor practice are believed to be of an advantageous character since they reflect improved motor performance, possibly related to the facilitation in corticospinal excitability and increase of muscle representation.

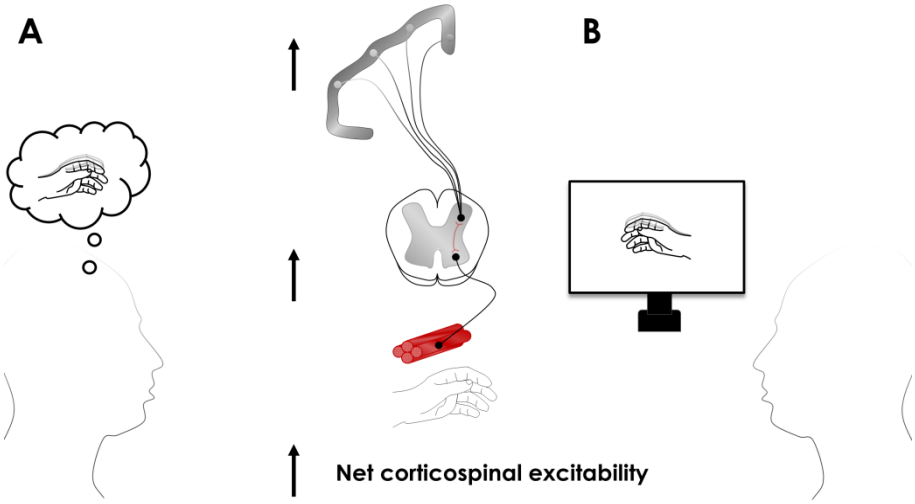

Figure 1. Concepts of AO and MI. *During MI (A), subjects are instructed to imagine performing a movement, e.g. with the index finger. During AO (B), subjects actively observe the movement on a screen Both techniques are performed without any overt volitional movement and facilitate corticospinal excitability.*

Another well-established technique for inducing facilitation in corticospinal excitability and muscle representations is that of motor preparation. A large and growing body of evidence suggests that motor imagery (MI), i.e. imagining a movement without any overt volitional exertion, can induce significant increases in neurophysiological measures such as MEPs (see e.g. [39–42]) and expansion of muscle representation [34] (Fig. 1, A). A similar method is action observation (AO), where observing movements yields a temporary increase in corticospinal excitability (see e.g. [43–45]) (Fig. 1, B). An influential review by Vogt et al. [46] sparked interest in combining AO and MI (AOMI), given the overlapping neuroanatomical structures during performance [47], and that one technique does not exclude the other [48]. The AOMI combination has since been shown to enhance corticospinal excitability facilitation, when compared to AO and MI separately [49–51]. The neuroanatomical structures involved were elucidated by functional magnetic resonance imaging (fMRI) studies, where consistent activation of e.g. the premotor cortex (PMC) has been reported [47]. It is therefore highly plausible that the effects of AOMI on M1 excitability are, at least partly, mediated through cortico-cortical connections between the PMC and M1. Indeed, earlier evidence in macaque monkeys have yielded the neuroanatomical link between the PMC and M1, since strong reciprocal connections from the dorsal and ventral PMC project to the proximal and distal upper limb muscle representations in M1 [52,53]. In humans, one of the first studies to explore such connection by TMS was Civardi and colleagues [54], who demonstrated that a low-intensity conditioning TMS pulse, 4-8 cm anterior to M1, could inhibit FDI excitability. Further corroborating evidence showed that repetitive TMS (rTMS) to the PMC can induce facilitatory [55] or inhibitory [56,57] effects on M1 excitability. These findings suggest that PMC can modulate M1 excitability. To date, the potential for harnessing the neuroplastic potential of one area of the brain, to affect another, remains unexplored in relation to PMC-to-M1 effects on pain-induced reduction in corticospinal excitability.

Our current knowledge on adaptive (and perhaps advantageous) neuroplasticity of the motor system includes motor practice and motor priming through imagery and action observation. As such, there is precedence for the use of non-rTMS paradigms to influence M1 excitability indirectly through engagement of e.g. the PMC but has not been investigated in relation to pain-induced reduction in corticospinal excitability. The use of AO and MI (or the combination of both; AOMI) has been shown effective in stroke patient rehabilitation, where the application of especially AO, yielded long-term improvements in motor function of the affected limb (for review on current state-of-the-art, see [48,58]). In musculoskeletal pain conditions, MI may become impaired [59] and may limit the applicability of AO in musculoskeletal

pain. Nonetheless, it currently remains unknown to which degree the temporary facilitation in excitability through AOMI performance affects musculoskeletal pain.

1.3. MALADAPTIVE NEUROPLASTICITY

Maladaptive neuroplasticity of the motor system, denotes the changes that occur during e.g. pain, and is characterized by changes in structural load on tissues that may be harmful in the long-term [11]. After amputation, extensive reorganization of the muscle representation is known to occur at the sensorimotor cortical level [60]. For example, amputation of the forearm and hand results in the corresponding muscle representations to be invaded by intact and adjacent M1 muscle representations [61]. When suffering from chronic low-back pain, muscle representations of the painful muscles become less distinct and overlap, resulting in a 'smudge' [15,16,62]. This 'smudging' effect has been associated with pain severity [17], however the mechanism is less clear. Emerging evidence on other chronic pain conditions such as patellofemoral pain has reported similar findings [63]. Furthermore, corticospinal excitability is reduced in patients with chronic migraine when assessed after performing a simple ballistic movement task [64]. Altogether, these results imply that muscle representations as well as the overall motor output are altered in chronic pain conditions.

In an experimental setting, we can provoke pain neuroplasticity through several different pain models. For instance, injection of the neurotrophic factor nerve-growth factor (NGF) is used to mimic and recreate movement-induced soreness and pain as seen during delayed-onset muscle soreness (DOMS) [65]. Traditional means to evoke DOMS using eccentric exercise also alters cortical motor- and sensory excitability for several days [23]. An important point must be made on the idea of advantageous adaptive neuroplasticity since it is based on context. The DOMS and NGF models are perfect examples of this. The DOMS model produces the 'classical' reduction in corticomotor excitability and shrinking of cortical motor representation of the sore muscle [23]. Conversely, NGF produces a large expansion of the muscle representation and facilitates M1 excitability when an acute pain exacerbation is evoked by hypertonic saline injection [66]. Therefore, despite having similar perception profiles (i.e. soreness/pain during movement but not at rest), the corticomotor response is opposite. In relation to DOMS, the reduction in corticomotor excitability and cortical motor representation shrinkage is believed to be a protective mechanism, to avoid further injury [11,67]. Contrarily, the increase in muscle representation and facilitation in corticomotor excitability induced by NGF injection, is ascribed as an adaptive mechanism, where the M1 is provoked to search

for a novel motor strategy [66,67]. Furthermore, limb immobilization has been shown to facilitate corticospinal excitability and increase cortical representations in both animals [68] and humans [69] (albeit the literature is rather contradictory, see e.g. [70–72]). In stroke patients, constraint-induced movement therapy has been used for rehabilitative purposes (restriction of volitional movement of the non-affected limb), and has been demonstrated to increase cortical muscle representation and facilitate corticospinal excitability of the affected abductor pollicis brevis muscle [73]. Even if these changes are associated with pathology (and it follows that they are likely maladaptive), the same processes that we acknowledge as being advantageous, may underlie the development of "maladaptive" movement behavior [11]. Therefore, it is important to recognize that different connotations are associated with the facilitation in corticomotor excitability, dependent on the setting in which it is used. The current thesis will mainly consider adaptive neuroplasticity as a way of counterbalancing pain-induced reduction in corticospinal excitability, and as such a potential advantageous type of neuroplasticity, but acknowledges that the term is based mainly on the context.

Whereas the NGF and DOMS experimental pain models produce longer-lasting soreness of the muscle, acute pain models result in short-lasting pain. For instance, the hypertonic saline model, when injected into the muscle, evokes localized pain and referred pain patterns [74]. Therefore, short-term pain-induced neuroplasticity of the corticomotor system can be probed to understand the characteristics of the acute phase of pain. The current thesis employed the hypertonic saline model to investigate acute effects of musculoskeletal pain, and if modulation of these effects is feasible.

1.4. THE INTERACTION BETWEEN ADAPTIVE AND MALADAPTIVE NEUROPLASTICITY

It is paramount to understand the interaction between, what is considered, adaptive neuroplasticity and maladaptive pain neuroplasticity of the motor system, to expand our knowledge on how to effectively treat musculoskeletal pain [3].

A vast body of work has demonstrated the interaction between motor control and the presence of experimental pain. For instance, in rats, carrageenan, which induces transient local inflammation, was shown to interfere with an instrumental spinal task (leg flex to avoid noxious stimulation of the tail), that outlasted the nociceptive input for up to 48 hrs [75]. In humans, it was shown that topical pain induced by capsaicin cream, did not interfere with motor skill acquisition of a locomotor perturbation

task, but affected the retention of the task 24 hrs later [38]. This is further supported by a similar study where the perturbation task was performed using the upper limb, and the application of capsaicin cream yielded a compensatory overshooting (to targets) that remained 24 hrs after motor skill acquisition [37]. A later study corroborated that local and remote heat pain applied during motor acquisition, had no impact on a finger-tapping task performance [76]. These lines of evidence suggest that motor performance is unaffected even when pain is applied during the motor learning acquisition phase, but rather, the acquired movement patterns may differ. Other studies suggest that the perception of pain may enhance motor skill learning. For instance, Dancey et al. [77] demonstrated that capsaicin-induced pain enhanced sensory processing and accuracy on a repetitive typing task as compared to a no pain group. Later, the same group provided more evidence on the enhancing effect of acute experimental pain on motor skill learning, in that the pain group consistently outperformed the no pain group [78,79]. At odds with these findings, an early study suggested that performing a tongue-protrusion task during pain interfered with task performance and the M1 excitability gains that would occur in the no-pain group [13]. In support, migraine-sufferers had lower gains in performance of a ballistic thumb task compared to healthy controls [64]. As such, the literature supporting the interaction between adaptive motor neuroplasticity and maladaptive pain neuroplasticity remains controversial, and may be ascribed to different methodologies, differences in pain location, and outcome measures.

An important study is that of Mavromatis et al. [80], who showed that capsaicin cream applied to the dorsum of the hand, had no effect on corticospinal excitability of the flexor carpi radialis and flexor digitorum superficialis muscles. However, when applied together with an acute experimental deafferentation protocol (cuff inflation) an enhanced corticospinal excitability facilitation was found [80]. This finding is important for two reasons. First, the study showed proximal upper limb muscles are unaffected by distally applied experimental pain, which is consistent with findings of the current PhD thesis [14] (see Section 3.1., Study I). Secondly, it is feasible to modulate MEPs and sensory-evoked potentials (SEPs) [22] by applying a competing neuroplasticity-inducing paradigm [30]. Therefore, the results show that in addition to M1-governed volitional movement, afferent feedback [30], attentional and cognitive influences [81,82], and activity in non-primary motor areas [54,55,57] can modulate corticospinal excitability.

1.5. AIMS AND OBJECTIVES OF THE PHD PROJECT

This chapter has introduced pertinent literature on the interaction between adaptive and maladaptive neuroplasticity of the motor system during pain. It is evident that pain influences the corticomotor response, whether that being measured through corticomotor excitability or motor behavior. However, the existing evidence also raises a fundamental question if interfering with the interaction between adaptive and maladaptive neuroplasticity is, not only feasible, but also advantageous. Very little is known on the possible advantageous effects of modulating pain-induced reduction in corticospinal excitability, and if attenuating this reduction improves e.g. motor skill acquisition when exerted during muscle pain. The only study that has specifically explored the possibility to affect MEP reduction in response to muscle pain, is that of Schabrun and colleagues [83]. They demonstrated that hypertonic saline-induced reduction in corticomotor excitability of the extensor carpi radialis brevis muscle was unaffected by performing a finger-tapping task immediately after pain-resolve. Pain-induced reduction in corticospinal excitability can be induced by a variety of pain inducing agents and/or methods and thus is considered a robust phenomenon. The idea of countering maladaptive neuroplasticity by using paradigms known within adaptive neuroplasticity remains largely unexplored. This is surprising given the possible clinical implications adaptive motor neuroplasticity may have on individuals suffering from chronic musculoskeletal pain [3,26]. The lack of evidence on the feasibility and possible advantage(s) of reversing or attenuating pain-induced reduction in corticospinal excitability outside of non-invasive brain stimulation paradigms forms the basis of the current PhD thesis.

The overall aim of the PhD project was to explore pain-induced reduction in corticospinal excitability in relation to acute experimental pain to elucidate approaches to counterbalance the reduction (Fig. 2).

As such, the project had two specific objectives.

(1) Probe pain-induced neuroplasticity of the motor system using a well-established acute experimental muscle pain model in two different upper limb muscles. Study I on Fig. 2 explored the effect of pain on primary motor cortex excitability in a forearm and hand muscles. The findings were then applied to Study II and III.
(2) Modulate corticomotor excitability through indirect influence of the PMC through a working memory task or action observation combined with motor imagery. Study II and III on Fig. 2 explored the impact of a two-back task and action observation combined with motor imagery on the perception of pain, and the pain-induced reduction in corticomotor excitability.

1.5.1. PAPERS ASSOCIATED WITH THE DISSERTATION

The current PhD thesis includes one internationally peer-reviewed paper, and two manuscripts accepted/in press and under revisions after peer-review. The first paper addresses the first objective whereas the second and third paper were written and designed to answer the second objective. Experimental designs for each study are shown in Fig. 3.

Study I: Larsen D.B., Graven-Nielsen T., Hirata R.P., Boudreau S.A. (2018) Differential corticomotor excitability responses to hypertonic saline-induced muscle pain in forearm and hand muscles. Neural Plasticity, Volume 2018, Article ID 7589601 (doi: 10.1155/2018/7589601)

Study II: Larsen D.B., Graven-Nielsen T., Hirata R.P., Seminowicz D., Schabrun S., Boudreau S.A. (2019) Corticomotor excitability is reduced by experimental muscle pain and remains unaffected by performing a working memory task. Under review/Revisions: Experimental Brain Research.

Study III: Larsen D.B., Graven-Nielsen T., Boudreau S.A. (2019) Pain-induced reduction in corticomotor excitability is counteracted by combined action-observation and motor imagery. Accepted/In Press: The Journal of Pain.

Fig. 2 conceptualizes the three studies and their relation to pain-induced reduction in corticomotor excitability.

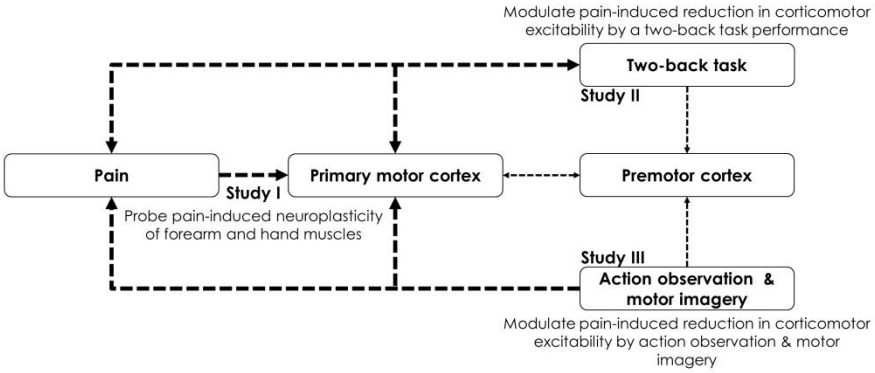

Figure 2. Conceptual overview of the dissertation studies. *Findings from Study I with regards to muscle choice were applied in Study II and Study III. Study II and III utilized tasks known to engage the premotor cortex (PMC), to modulate pain-induced reduction in corticomotor excitability.*

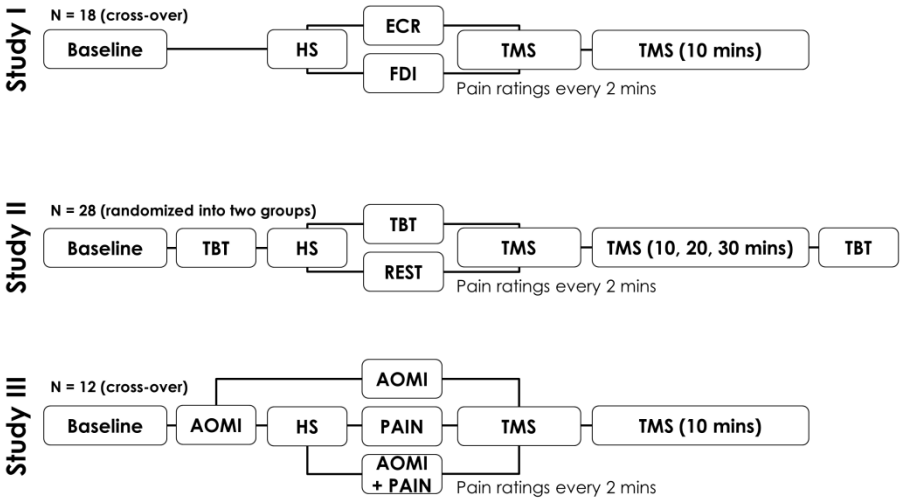

Figure 3. Experimental setups for Study I-III. *Study I and Study III followed the same principal design with 100 TMS stimulations throughout pain (Study I: ECR or FDI muscle, Study III: FDI muscle), AOMI (Study III), or AOMI+PAIN (Study III) and follow-up measures 10 mins after pain resolve. Study II included two groups with repeated measures, and TMS stimulations at pain-resolve, 10 mins, 20 mins, and 30 mins post-pain resolve.*

CHAPTER 2. PROBING AND PROVOKING PAIN NEUROPLASTICITY OF THE MOTOR SYSTEM

2.1. MOTOR-EVOKED POTENTIALS AS A MEASURE OF CORTICOSPINAL EXCITABILITY

One of the major advances in our understanding of motor neuroplasticity was the introduction of TMS as a research method [84,85]. A major advantage of TMS is that the minor sensations evoked during stimulation are generally well tolerated. TMS allows for rapid assessment of corticospinal excitability, which includes corticomotor-neuronal, spinal α-motoneuronal, and peripheral muscle fiber excitability [86,87]. The output is straight-forward since MEPs can readily be recorded from target muscles by measuring EMG activity [88], and several methods to extract information from these measures on corticospinal excitability is currently available [88,89]. In humans, TMS has revealed two important phenomena known as functional reorganization and corticospinal excitability changes. These two important phenomena have been demonstrated in relation to motor practice (see e.g.[31,90]) and motor skill learning (see e.g. [33,91]). For instance, Gallasch et al. [29] demonstrated that target training (goal-directed motor task) yields a concurrent facilitation in corticospinal excitability. This facilitation in corticospinal excitability may be mediated by a reduction in intracortical inhibition [29]. Furthermore, the cortical representation of an exercised muscle increases with training (see e.g. [33,92]). As such, the primary motor cortex (M1) is highly influenced by exteroceptive stimuli. The studies performed in the current thesis used MEPs recorded by placing surface EMG electrodes on the target muscles, conforming to the SENIAM recommendations for FDI and ECR muscle recordings (interelectrode distance of 20 mm; Study I-III).

2.2. MOTOR-EVOKED POTENTIALS IN STUDY I-III

Software for measuring MEP amplitudes is readily available, and measures both 100 ms before the magnetic pulse is induced, and 300 ms after the stimulation (Mr. Kick III, Aalborg University; Study I-III, Fig. 4). This allows the disqualification of MEPs based on pre-contraction of the target muscle, since pre-activation of the muscle before stimulation aggregates the magnitude of the MEP [93]. The current PhD thesis will mainly focus on peak-to-peak amplitude, which is well-described in terms of characteristics in healthy and pathological conditions [86,88]. Often, the resting motor threshold (RMT) is assessed according to standardized guidelines, and is

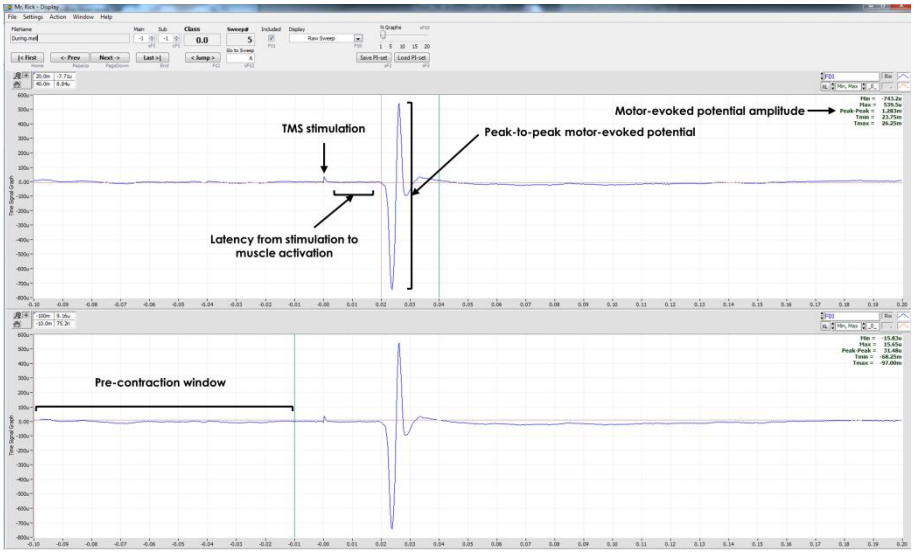

Figure 4. Graphical interface of Mr. Kick III (IP, Aalborg University). *Example of on-line feedback on latency to the motor-evoked potential (MEP) from stimulation time, peak-to-peak amplitude of the MEP, and pre-contraction are presented on-line, to ensure stable recording of corticospinal excitability of the target muscle. Reprinted with permission from Aalborg University*

defined as recorded peak-to-peak MEP amplitudes reading ≥ 50 μV in 50% of the stimulations (10-20) [94,95]. This is an important measure given the relative contribution to the compound MEP signal by direct (D-wave) or indirect (I-waves; I_1-I_3) corticospinal volleys [89]. For instance, utilizing 120% × RMT is known to mainly provoke indirect waves, and as such, providing an output related to transneuronal activation of the corticospinal neurons [89] (Study I and III). Another approach is to set a pre-determined peak-to-peak threshold, e.g. amplitude of ~1 mV amplitude MEPs in three consecutive trials (Study II) which yields a consistent baseline across the included sample, making it easier to tease out effects of any intervention applied in the study. In general, MEPs of the FDI muscle are known to be consistent within-subjects across weeks [96], attesting to the reproducibility value of MEPs as a measure of corticospinal excitability. On a between-subject basis, it is well-known that MEPs are highly variable [97]. This is countered by mainly evaluating changes of a given intervention, on a group basis (Study I-III).

2.3. EVIDENCE FOR THE PRIMARY ROLE OF M1 IN MODULATION OF CORTICOSPINAL EXCITABILITY

Since MEP amplitudes reflect the entire corticospinal tract and peripheral muscle fiber excitability, it is pertinent to discuss literature that has investigated the relative

contribution from cortical, spinal, and muscle structures to the overall excitability during pain. An exhaustive discussion on this topic is beyond the scope of the current thesis, but an introduction to the current knowledge on spinal and peripheral excitability changes in relation to pain is still warranted.

At the peripheral level, and in relation to MEPs, the muscle compound action potential (M-wave) describes motor axonal excitability. M-waves are evoked by delivering electrical pulses to a mixed nerve, and recording EMG activity at the muscle of interest [98]. At the segmental level, two main estimates are used to infer on the contribution of spinal α-motoneurons to corticospinal excitability, namely the Hoffmann reflex (H-reflex) and F-waves. The H-reflex reflects Ia afferent potentials, and is considered monosynaptic in nature, whereas the F-wave describes the antidromic activation of spinal motoneurons, and reflects pre-synaptic inhibition [99]. The H/M ratio signifies the proportion of activation the spinal α-motoneuron pool is capable of, and has been shown to be highly reliable intersession [100].

The three mentioned techniques have been employed to infer on the relative impact of spinal and peripheral contributions to corticospinal excitability during pain, and if the changes were occurring at the cortical level (from hereon, corticomotor excitability). For instance, when FDI pain is induced by hypertonic saline, the H-reflex is unaffected in the peak-pain period, whereas it reduces immediately post-peak pain [12]. Farina and colleagues [101] showed that topical capsaicin cream to the ECR muscle, did not affect the H/M ratio of the flexor carpi radialis during the painful period, and after application of capsaicin to the FDI, F- and M-waves of the muscle remained unaffected. These findings indicate that during noxious stimuli, the immediate response by the corticospinal system occurs at a cortical level, whereas spinal and/or peripheral changes may occur after the peak-pain phase. Similarly, no changes in M-wave excitability was noted in a later study using painful injection of hypertonic saline into the FDI muscle [102]. Non-painful transcutaneous electrical nerve stimulation (TENS) was shown to be mainly mediated by cortical motoneurons, since the H/M ratio did not change in response to TENS, while MEPs were strongly affected [103]. These studies all point towards a cortical origin of the reduction of MEPs, at least around peak-pain. An important disclaimer is that while most studies looking at pain effects on spinal motoneuronal pool excitability show little-to-no-change, these measures are not straightforward. For instance, F-waves are derived from only ~1% of the total α-motoneuron pool, and as such, gives an incomplete measure of spinal excitability [104]. The H-reflex only reflects a partial change in excitability of the entire spinal motoneuronal pool, and therefore attains similar limitation as the F-waves, albeit a higher percentage of α-motoneurons usually mediate the H-reflex response [99].

These inherent limitations to the H-reflex and F-wave measures must be taken into consideration when appraising the contribution of spinal effects on pain-induced corticospinal excitability reduction. Nonetheless, given the possible cortical origin

of pain-induced changes to the MEPs, from hereon, corticospinal excitability will be used when addressing the full corticospinal pathway, whereas corticomotor excitability will be used when selectively focusing on the motor cortical contribution during pain.

2.4. EXPERIMENTAL SHORT- AND LONG-TERM MUSCLE PAIN MODELS

To understand the immediate effects of pain on corticomotor excitability and its wider perspective in relation to clinical pain, experimental pain models mimicking, at least partly, the clinical manifestations are needed. The current thesis focuses on hypertonic saline and its use in experimental pain research as a short-duration (mins) muscle pain model but briefly discusses long-duration muscle pain models (hours to weeks).

A well-established acute muscle pain model is that of intramuscular injections of hypertonic saline [105]. An important feature of this pain model is, that the injection causes short-lasting and local muscle pain and can present with referred pain patterns as seen in clinical conditions [106–108]. In addition, delivery and location of the pain can be standardized across different protocols [106], underscoring its value in musculoskeletal pain research. The reported pain intensity of 5-6 on a numerical rating scale (NRS) is highly consistent across studies [12,14,74,83,102] (see also Study II-III). Hypertonic saline injections therefore yield similar experiences of pain intensity, independent of muscle choice, and perhaps even volume, since data from Study I suggested that 0.2 ml bolus (FDI) compared to 0.5 ml bolus (ECR), yielded similar pain intensity ratings.

Intramuscular injection of hypertonic saline has been extensively studied in relation to changes in corticomotor excitability. For instance, Le Pera and colleagues [12] demonstrated that infusion of hypertonic saline into the FDI muscle caused reductions in corticomotor excitability of both the FDI and the abductor digiti minimi (ADM) muscles. This suggests that hypertonic saline-induced pain affects homotopic muscles equally. Later, Schabrun et al. [21] indicated that sensory-evoked potentials were altered during and after pain, whereas MEPs only reduced after hypertonic saline-induced pain had resolved. These changes are likely mediated by intracortical mechanisms involving an increase in SICI and decrease in ICF, suggesting an enhanced inhibitory influence on corticomotor excitability [109]. Other models similar in duration, such as injection of ascorbic acid have been used and shown similar findings (see also Appendix A for an overview of the effect of acute experimental pain on corticomotor excitability). As such, short-duration muscle pain models allow testing the immediate response of the corticospinal pathway to intramuscular pain.

Research often distinguishes between endogenous and exogenous techniques to induce pain [107]. An excellent example of this division is that of the DOMS and the NGF model. When endogenously provoking DOMS through eccentric exercise, peak muscle soreness is usually reported 24-48 hours after the exercise [110]. During this period, corticomotor excitability reduces and the representation of the painful muscle shrinks [23]. In contrast, exogenous NGF injection model induces similar pain intensity ratings [65], but increases muscle representation size [24] and facilitates corticomotor excitability when further provoked by a hypertonic saline injection [79]. These two models exemplifies the adaptive properties of the corticomotor system in that when soreness/pain resolve after ~6-7 days, corticomotor excitability and muscle representation size returns to normal [23]. An increase in corticomotor excitability and muscle representation is often associated with motor training as earlier discussed (Section 1.3.). It has therefore been hypothesized that the NGF-induced increase in corticomotor excitability and muscle representation, may reflect a search for novel motor strategies, due to the sustained pain [66]. The mechanisms behind this facilitation in corticomotor excitability and increase of muscle representation have been proposed to be of cortical origin, more specifically mediated by ICI and ICF [66].

2.5. PAIN INTENSITY RATINGS & PAIN QUALITY

In the current PhD thesis, secondary outcomes included the assessment of the relationships between e.g. NRS ratings and MEP amplitudes (Study I-III). Each of the assessments are described and presented. Pain intensity was measured in Study I-III, whereas questionnaires including McGill's Pain Questionnaire (MPQ), Pain Catastrophizing Scale (PCS), mind-wandering scale (MWS), and the State-Trait Anxiety Inventory (STAI) were employed in Study II only. The two-back task (TBT) was used in Study II as an attempt to modulate the reduction in corticomotor excitability of the FDI muscle and will be discussed in relation to reaction time and accuracy (see Section 2.7).

Pain intensity ratings are traditionally measured using a visual analogue scale (VAS), where the level of pain is recorded on a 10 cm line. The line is anchored with '0' representing no pain and '10' representing worst pain imaginable. The numerical rating scale (NRS) is often employed when providing verbal ratings during e.g. acute experimental pain, which contains the same anchors as the VAS [111]. Given that participants verbally rated their pain in in Study I-III, the current thesis provides an overview of earlier findings of the NRS in relation to experimental pain.

The NRS is a well-established, validated, and unidimensional scale used to obtain the perception of pain intensity in experimental and clinical settings (for excellent

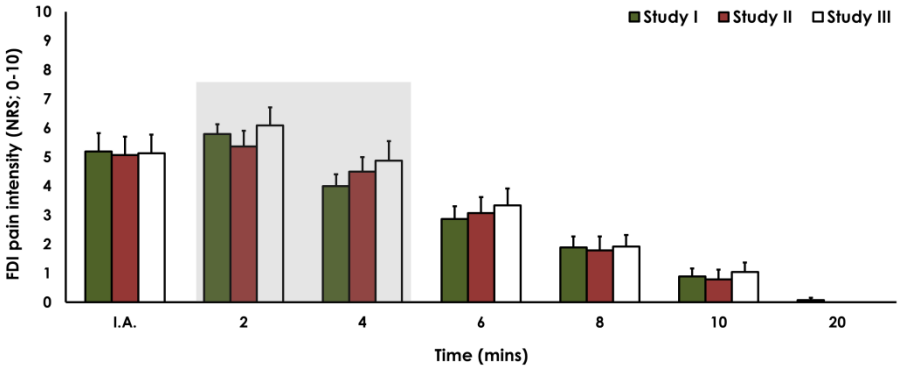

Figure 5. FDI NRS ratings across the three studies (Mean ± SEM). *The NRS ratings were highly consistent across the three studies in the present PhD thesis. The grey highlighted boxes indicate the NRS levels at peak-pain, which occurred 2-4 mins post-injection. All pain intensity ratings in the graph were obtained during rest and hypertonic saline-induced FDI pain.*

review, see [111]). Generally, the VAS and NRS are well-correlated [112], and perform equally in the acute clinical pain setting [113].

Additionally, the NRS has shown its efficacy in determining the minimal clinically important difference in recovery from musculoskeletal pain [114]. Based on these considerations, the NRS was chosen to be verbally recorded in all the current PhD studies. Earlier reports have recorded pain intensity ratings of ~5-6 in response to hypertonic saline injections (independent if bolus or infusion was used) [12,106,108,109]. The pain intensity across Study I-III, showed a highly consistent pattern of rated pain intensity ~5-6 at peak-pain, which steadily decreased over the next 15-20 mins (Study I-III; Fig. 5). As such, the hypertonic saline model is consistent in the perceived perception of pain across different studies.

Study I and Study III repeatedly showed that the pain intensity at peak-pain is not correlated with the magnitude of reduction in corticomotor excitability (Fig. 6-7), and is consistent with earlier studies [83,115]. This suggests that the nociceptive input drives the reduction in corticomotor excitability, but pain intensity is not likely to affect the extent of the reduction.

Study II surprisingly demonstrated that the TBT and the REST group differed in pain intensity ratings. This finding is at odds with earlier evidence demonstrating that performing e.g. a Stroop task [116,117], a three-back task [118] or an attention task [119,120] yields lower pain intensity ratings. Pain-related brain regions such as the S1, S2, and the posterior and anterior insula are linked to the attenuation of pain intensity when performing e.g. the Stroop or the three-back task [116,121–124]. These earlier studies mostly employed phasic heat pain models [116,118,119] or electrical stimulation [117], but given the moderate pain intensities reported, is unlikely to be the

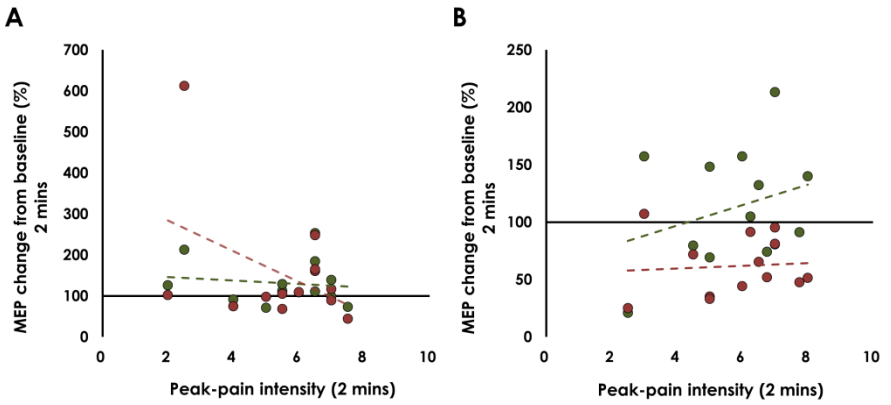

Figure 6. Study I correlations at 2 mins, between the percentage change in MEP magnitude from baseline and pain intensity. *No significant associations were found in Study I for the change in MEPs for either the ECR (green dots) or FDI (red dots), independent if the hypertonic saline was injected in the ECR (A) or the FDI (B) muscle.*

main explanation to the discrepant findings from Study II. Instead, the nature of the pain (deep muscle versus skin pain) may impact the perception of pain when performing the TBT, since an earlier study demonstrated that by sensitizing the skin with capsaicin cream prior to heat application (heat allodynia), an enhanced brain response related to areas such as the DLPFC could be seen when compared to similar intensity heat stimulation alone [125]. It is well-known that large interindividual differences in pain sensitivity exist [126]. However, the difference in pain perception cannot be attributed to pain catastrophizing, state or trait anxiety at baseline, or mindwandering during the task performance, as discussed further in Section 2.6. Furthermore, the two groups were age and gender-matched and can therefore not explain the difference in pain intensity ratings.

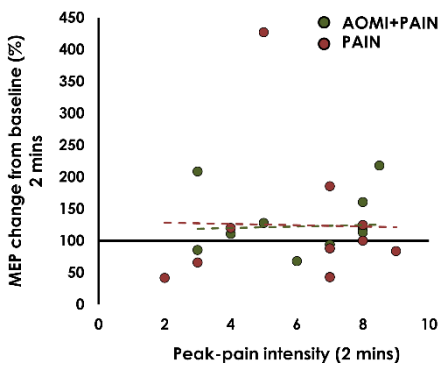

Figure 7. Study III correlation at 2 mins, between the percentage change in FDI MEP magnitude from baseline and pain intensity. *No significant associations were found in Study III for the change in MEPs for either the AOMI+PAIN session or the PAIN only session.*

To assess the quality of the pain, the McGill's Pain Questionnaire-short form 2 (MPQ-SF2) [127] was employed in Study II. The top five words in Study II were 'Sharp', 'Cramping', 'Aching', 'Heavy', and 'Numbness'. This is in line with earlier studies exploring hypertonic saline injection into the FDI [21,115,128], the ADM [109], and the ECRB [83] muscle. These findings further attest to the reproducible quality and intensity the hypertonic saline pain model induces in

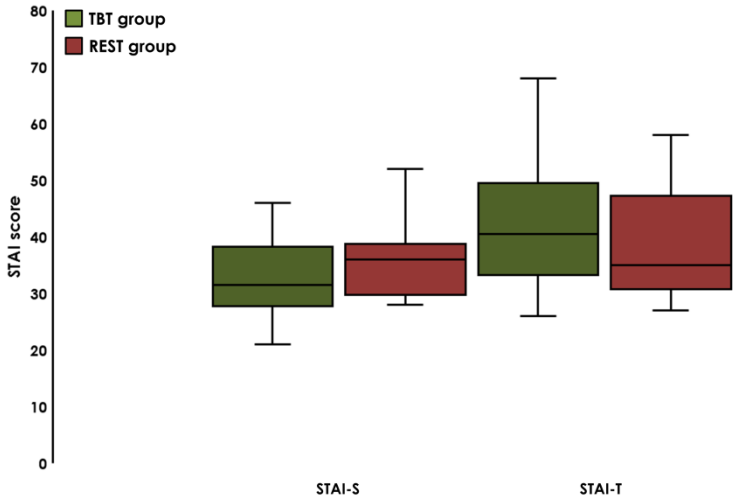

Figure 8. State and trait anxiety scores for the TBT (green) and the REST (red) group (median, 25th and 75th percentiles are presented as well as min/max values). *The two groups were similar in terms of state anxiety (STAI-S) and trait anxiety (STAI-T) at baseline, and are therefore unlikely to have had an impact on pain intensity ratings in Study II.*

healthy participants.

2.6. SUBJECT CHARACTERISTICS ASSESSMENTS

Three main questionnaires were employed in Study II to assess the level of pain catastrophizing in relation to a painful event (Pain catastrophizing scale: PCS) [129], state-trait anxiety inventory (STAI) [130] which assesses the degree of state (20 questions) or trait anxiety (20 questions), and the mind-wandering scale [131]. Discussing the wider applicability of the three questionnaires is beyond the scope of the current thesis. In brief, the PCS has been shown to be predictive of developing chronic musculoskeletal pain conditions such as chronic low-back pain [132]. It has also been shown to be consistently rated among subpopulations consisting of pain-free participants, chronic low-back pain patients, and fibromyalgia patients [133]. Furthermore, higher PCS scores suggest an enhanced pain perception to experimental pain [129] mediated by brain regions associated with attention, affective, and motor aspects of pain, such as the dorsolateral prefrontal cortex (DLPFC) [134]. The PCS is therefore an important instrument in assessing the cognitive influence on pain to either predict chronic pain development [132] or to control for a native catastrophizing response to e.g. hypertonic saline injection (Study II). The STAI has been employed in e.g. rheumatology [135] and to control for state or trait anxiety in clinical and healthy populations [136]. In clinical populations, state and trait anxiety have been shown to be elevated as compared to healthy controls in tension-type

headache [137] and experimental orofacial pain [138]. This is well-supported in that both trait and state anxiety have been associated with an increase in pain to e.g. noxious electrical stimulation [130,139]. Study II therefore included the STAI to ensure that the two randomized groups did not exhibit differences in state (at baseline) or trait anxiety. The TBT and REST groups rated their state and trait anxiety similar (Fig. 8). These findings suggest that state and trait anxiety had little impact on the results of Study III. Furthermore, no differences were found in the PCS score between the two groups in Study II (Fig. 9). As such, it is unlikely that pain catastrophizing played a key role in Study II.

In the presence of a noxious stimulus, our attention dynamically fluctuates towards the pain and away from it. This may partly explain interindividual differences in pain perception, given the intimate link between attention towards pain and activation of brain regions associated with the perception of pain (for review, see [131]). Since mindwandering may interfere with the attention towards the TBT [140], we sought to ensure that mindwandering would not impact the overall outcome on corticomotor excitability. We found that while the group that performed the TBT during pain had to put in more effort to still maintain task performance, there were no differences in the amount of mindwandering exerted. Moreover, most of the participants (independent if performing the TBT during pain or not) rated *2.1. external/sensory distractions* as the main reason for diverting their attention (Fig. 10). While *2.3. Mindwandering* was reported by the participants in both groups, there was no difference in the ratings. It is therefore unlikely to have had an impact on the difference in pain ratings. It is important to highlight that the scale is not

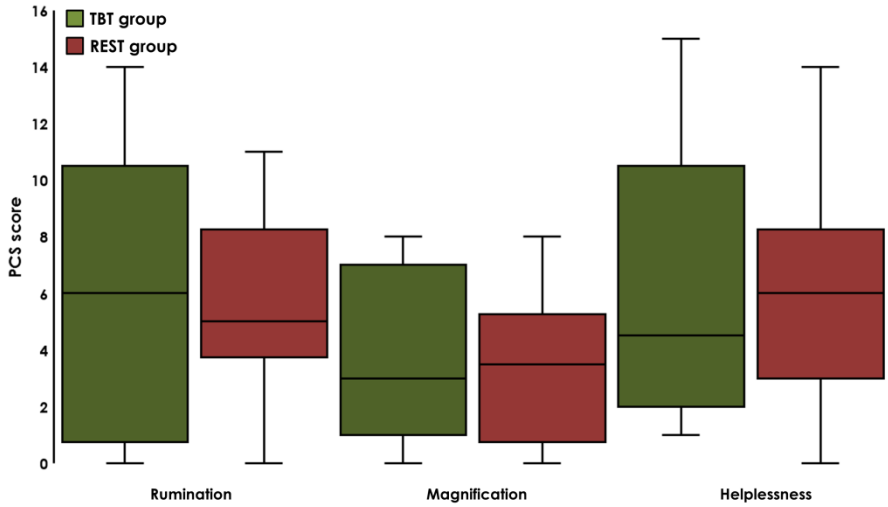

Figure 9. Pain catastrophizing scores within each category and both groups (median, 25th and 75th percentiles are presented as well as min/max values). *The two groups were similar in the three subcategories (Rumination, Magnification, and Helplessness) of the PCS.*

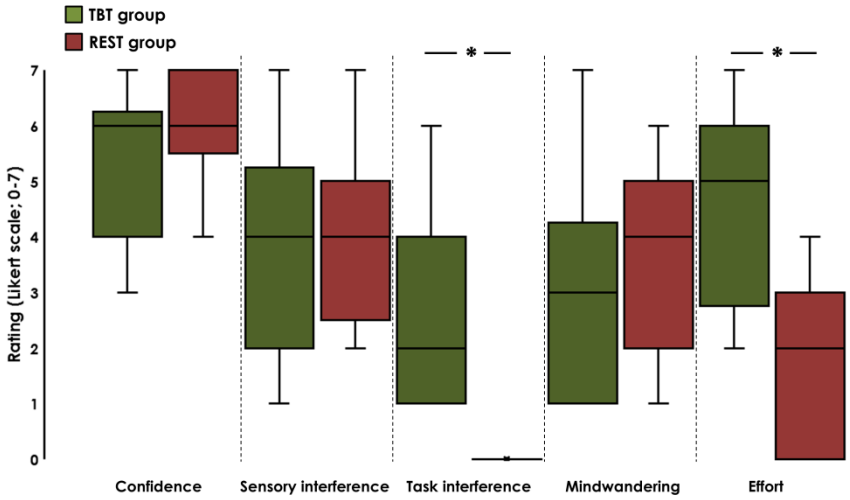

Figure 10. Likert scale data from the Mind-wandering scale (median, 25th and 75th percentiles are presented as well as min/max values). *The TBT and REST groups differed in effort needed to either (1) perform the TBT while in pain or (2) staying at rest. Furthermore, only the TBT group rated any task-related interference with their attention towards the task. *, $p < 0.05$*

validated and is not necessarily capturing the full aspect of mindwandering. Since the impact of mindwandering on pain perception is a relatively new area of research it would be premature to draw definite conclusions based on Study II. However, it is becoming evident that numerous brain regions associated with pain processing are engaged during mindwandering [141].

2.7. PAIN EFFECTS ON REACTION TIME AND ACCURACY IN A TWO-BACK TASK

Two commonly used behavioral outcomes for assessing learning in relation to motor preparation are reaction time (RT; for excellent systematic review on reaction time and motor preparatory processes, see [142]) and accuracy (ACC; [143]). In Study II the TBT demands the press of either a target key or a non-target key (response), depending on a presented letter, and assesses RT and ACC of the response. The parameters used for the TBT paradigm were adopted from Vermeij et al. [144]. Briefly, the participants were seated 90 cm (nasion to middle of the screen) away from a 17" monitor. A keyboard was used as the response box, and participants were instructed to press either numeric keypad '1' for targets or '2' for non-targets following every letter presentation. Non-targets reflect letters that were *not* shown two times back, whereas targets describe letters that *were* shown on the screen two times back. One

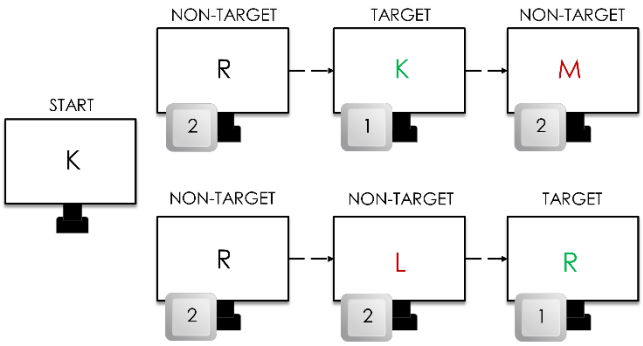

Figure 11. Two-back task performance in Study II. *Two examples of the presented letters and associated button presses. Each letter was shown for 3 s and separated by 500 ms of blank screen.*

round of the TBT consisted of 30 presented English letters including all consonants. Letters were presented for 3 s with interstimulus interval of 500 ms (Fig. 11). The RT time was measured as the time from letter presentation to keypad press, whereas ACC was measured as either correct or incorrect (target or non-target). To avoid ipsilateral motor activity of the painful side, the participants performed the TBT with their left non-dominant hand. RT and ACC were used as secondary outcomes in Study II to test for any learning effects and determine the influence of pain on TBT performance.

The RT and ACC were similar for targets and non-targets for the group performing the TBT while being in pain, and the group that remained at rest during pain (Study II; Fig. 12). A main effect of target ACC was found and thus the TBT and the REST groups improved equally (Fig. 12, Target ACC). The TBT engages working memory [145], the prefrontal areas, and specifically the DLPFC, and are believed to play a significant role in improving ACC and RT [146]. A recent meta-analysis on the role of DLPFC in improving ACC and RT concluded that neuromodulation through rTMS and tDCS exerts positive improvements on working memory in healthy and clinical populations (for review, see [143]). The DLPFC is connected with the anterior cingulate cortex [147], anterior insula [148], and the basal ganglia [149] and is thus positioned to have an integral role in improving ACC and RT. Since ACC improved when performing the TBT in Study II, this improvement may be driven by activation of prefrontal areas such as the DLPFC, and Study II adds that this is true even if pain is present. Various pain and motor learning studies support the idea that being in pain does not necessarily reflect a decrease in learning [13,37,150,151]. It is therefore unsurprising that the TBT and REST groups both improved, even if the TBT group had an additional 10 mins of performing the TBT.

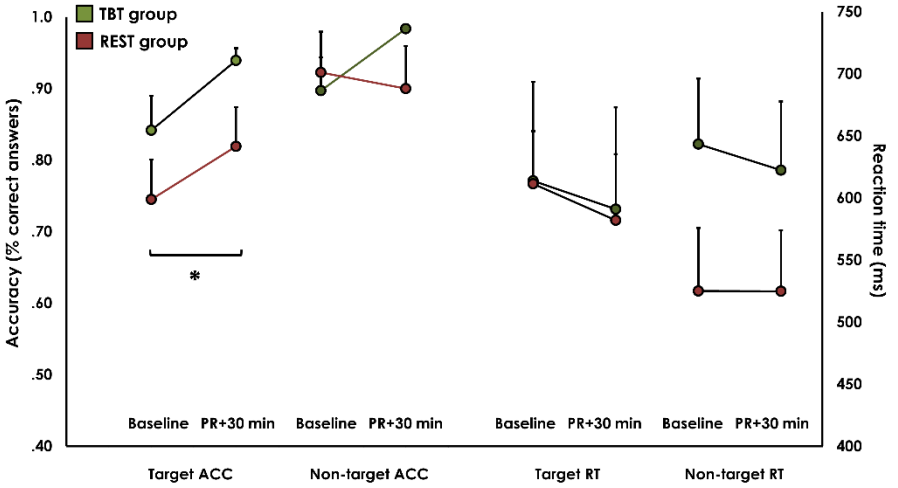

Figure 12. Study II: Accuracy and reaction time from the TBT (mean + SEM). *No differences in reaction time or accuracy was found between the two groups in ACC and RT for targets and non-targets. A main effect was detected for target ACC, indicating that both groups improved equally. *, $p < 0.05$*

The influence on corticomotor excitability are discussed in more detail in Chapter 3, and the rationale for employing the TBT to modulate corticomotor excitability during pain will be further expanded.

The presented results suggest that across the three studies, pain intensity was reproducible in intensity, and quality associated with the pain is in line with earlier reports. The degree of reduction in corticomotor excitability is rarely associated with the magnitude of pain intensity rating. This was further confirmed by Study I and III, where there were no associations between the two variables. In other words, even if the reduction in corticomotor excitability occur concomitantly to the pain induction, it is unlikely to drive M1 excitability reduction.

The psychophysical assessments on PCS and state and trait anxiety were similar in Study II. Pain was shown to exert a dominant effect on the effort needed to perform the TBT and was mostly related to external/sensory distraction due to the pain.

2.8. MAIN FINDINGS FROM STUDY I-III (PSYCHOPHYSICS AND TWO-BACK TASK PERFORMANCE)

- The hypertonic saline model is highly consistent in the induced pain quality and pain intensity across different studies

- The degree of reduction in corticomotor excitability of the ECR and FDI muscle is not associated with the pain intensity at peak-pain
- Pain catastrophizing, state and trait anxiety levels, and mindwandering were similar between the two groups in Study II, and are unlikely to have affected the pain perception difference found between the two groups
- Accuracy on the TBT improves even when pain is present, which was true for both groups in Study II

CHAPTER 3. PROBING AND MODULATING PAIN-INDUCED REDUCTION IN CORTICOMOTOR EXCITABILITY

This chapter will address the findings on the differences in pain-induced reduction in corticomotor excitability depending on muscle choice, and different methodologies to modulate pain perception by conventional non-invasive brain stimulation paradigms, and pain neuroplasticity of the motor system.

3.1. CONSIDERATIONS REGARDING TOPOGRAPHY OF SMALL HAND AND FOREARM MUSCULATURE – SUMMARY OF STUDY I

As discussed in Section 2.4., pain exerts equal reductions in corticomotor excitability of homotopic muscles such as the FDI and the ADM. Nonetheless, the current thesis presents the first findings on the relative impact of well-known body division topography [85]. Study I yielded results showing that the ECR muscle remained unaffected in response to both FDI and ECR muscle injections of hypertonic saline (Fig. 14, A), despite sharing a largely overlapping hotspot [152] (Fig. 13) and cortical motor representation [153]. This is in line with a recent paper highlighting the lack of effect of capsaicin cream applied to the dorsum of the hand on FCR and flexor digitorum superficialis corticomotor excitability [80]. Injection of hypertonic saline or application of capsaicin cream to the cheek was unable to affect MEPs of the masseter muscle, although the demand for pre-contraction of the jaw musculature may explain this discrepant finding [154]. Conversely, hypertonic saline injection into the flexor carpi radialis (FCR) muscle, has been shown to elicit reduction effects on FCR corticomotor excitability, in the same magnitude as seen for the FDI [12]. Furthermore, another recent paper suggested that MEPs were reduced by injection of hypertonic saline injection into the ECRB muscle [83]. These lines of evidence show that the effect of muscle pain on corticomotor excitability is not uniform, and may

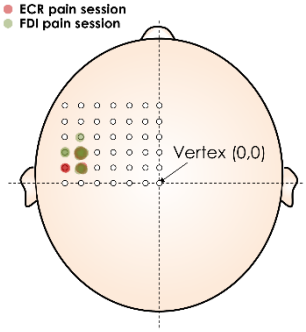

Figure 13. Visual representation of the hotspots used in Study I. *Hotspots were centered around X,Y coordinates 2,5 and 1,5 in both sessions.*

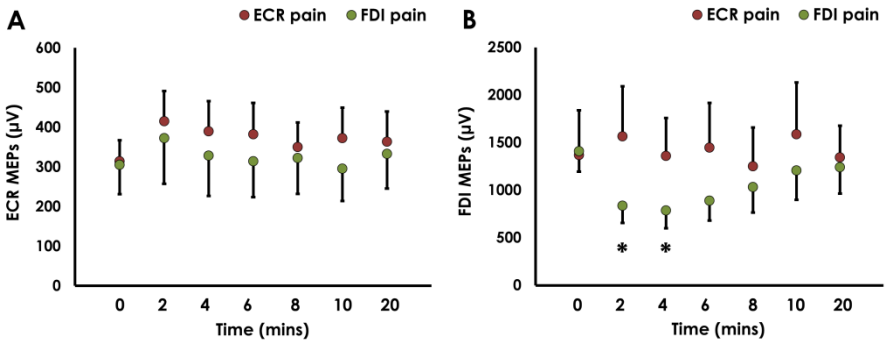

Figure 14. Motor-evoked potentials of the FDI and ECR muscles following injection of hypertonic saline injection (Mean ± SEM). *Injection of hypertonic saline into the ECR muscle did not yield any reduction in corticomotor excitability of the ECR or the FDI muscle (A, B; green and red dots). Contrarily, a significant drop in corticomotor excitability was found for the FDI muscle, when the injection was given in the FDI muscle (B; green dots)*

reflect differential corticomotoneuronal connections to the muscles, as noted by an earlier studies looking at excitation and inhibition of small hand and forearm musculature [93]. Such differential response to the same painful stimulus has not yet been systematically investigated but warrants further consideration in future studies.

In this respect, the current thesis adds to this knowledge by showing that the extensor carpi radialis (ECR) muscle is largely unaffected by hypertonic saline pain in the FDI muscle, and even the ECR muscle itself (Fig. 14, A; red dots). Conversely, injection of hypertonic saline into the FDI muscle produces a robust reduction in corticomotor excitability (Fig. 14, B; green dots). A defining property and important characteristic for this PhD thesis of the hypertonic saline injection model is that corticomotor excitability remains reduced even after pain has resolved [12,83,115] (see also Study II). The temporal profiling of pain-induced corticomotor excitability reduction, as clarified in study I (Fig. 14) and replicated in study III (Fig. 16), may have interfered with this lasting reduction in corticomotor excitability. This leaves open an important question if the impact of pain on corticomotor excitability can be modulated by continuous single pulse TMS, and more importantly, if the time at rest while experiencing pain plays a role on the magnitude of reduction in corticomotor excitability. In support of the pain-induced reduction in corticomotor excitability, Le Pera et al. [12] reported that at peak-pain (2 mins after hypertonic saline injection), FDI MEPs were strongly reduced and to the same magnitude as that of Study I and III (See also Appendix A for more studies investigating the effect of acute pain on corticomotor excitability). As such, it remains controversial at which time-point MEPs recover, if the sensory system impacts the relative timing of corticomotor excitability (as shown by Schabrun and colleagues [21]), and if continuous stimulation with single pulse TMS facilitates corticomotor excitability. Another explanation could be that while corticomotor excitability returned to baseline throughout the temporal profiling (Fig. 14, B), the continuous reporting of

the pain intensity may have influenced corticomotor excitability through attention or speech [81,82,155].

This subsection has highlighted important differences between the current thesis findings and earlier studies and warrants further investigations to clarify the effects of constant TMS stimulation throughout the duration of pain, on corticomotor excitability.

3.1.1. SAMPLE SIZE CONSIDERATIONS – STUDY I

One recurrent limitation in most studies investigating pain-induced reduction in corticomotor excitability is the sample size. A perfect example of the concern, and righteously so, is that of a comment received during preparation of Study I manuscript: *".. theories about transition from acute to chronic pain are based on very small sample sizes. To understand whether these (edit: effects of pain on corticomotor excitability) are just Type I errors (as there are some studies that show no effect)."* – Reviewer comment on Study I. The sample sizes are worthwhile reflecting upon, since the presented Studies I-III and earlier research [12,21,115,156], are indeed based on small samples (n = 10-20). Whereas most studies have demonstrated robust pain-induced reductions in corticomotor excitability, independent of pain modality [12,21,102], other evidence demonstrated that MEPs remained unaffected in response to e.g. orofacial pain [154] or forearm muscle pain (Study I). This may be due to methodological considerations such as the need for pre-contraction of jaw musculature to elicit MEPs during TMS. Such pre-contraction may mask the reduction that would otherwise occur in a resting muscle. However, the most recent meta-analysis and systematic review on pain-induced reduction in corticomotor excitability highlighted a moderate effect of pain on corticomotor excitability (Standardized Mean Difference; SMD = 0.52 [-0.01, 1.06]% at rest during pain) [67]. For Study I, the power calculation was based on the SMD of 0.52. With correlation among repeated measures of 0.8 (high interreliability of MEPs within each subject [96]), power pre-determined to 80% to show a difference for a within-group F-test (repeated measures analysis of variance) at $\alpha = 0.05$, 10 participants were needed (+2 for 20% dropout rate). Since we included 18 participants (where four subjects were lost to follow-up session), Study I was therefore well-powered to detect the differential response in muscle excitability to hypertonic saline-induced pain.

In summary, Study I showed that:

- A robust reduction in corticomotor excitability occurred due to a hypertonic saline injection and the FDI muscle was more susceptible to this reduction than the ECR muscle

- The ECR and the FDI corticomotor excitability differentially responded to a hypertonic saline injection.

3.2. MODULATING CORTICAL EXCITABILITY AND PAIN PERCEPTION BY NON-INVASIVE BRAIN STIMULATION

Two prominent methods to modulate cortical excitability are those of tDCS and rTMS [157]. Different configurations of tDCS exists, and it is generally accepted that anodal tDCS causes a facilitation in cortical excitability whereas cathodal tDCS yields a decrease in overall excitability [158]. This technique has been used to manage chronic pain in e.g. fibromyalgia [159–162], temporomandibular pain [163,164], and chronic non-specific low-back pain [165,166]. However, a recent Cochrane review [157] point towards the risk of bias through blinding that exists in the literature and questions the effect of tDCS for chronic pain management [167]. Therefore, tDCS will not be discussed in detail, but will be related to relevant discussions in Chapter 4.

The most well-established technique to drive neuroplasticity is rTMS. By applying trains of magnetic pulses at varying frequencies to specific areas of the cortex, cortical excitability can be readily modulated [168]. By keeping the frequency at 1 Hz or lower (low-frequency rTMS), it is possible to induce a reduction in cortical excitability, whereas employing 5 Hz or above (high-frequency rTMS), yields a facilitation in cortical excitability [169–171]. These properties of rTMS allow for testing intra- and interhemispheric connectivity between e.g. motor centers [55–57] or pain processing [172]. Additionally, rTMS has been shown effective in reducing pain perception in chronic pain populations such as neuropathic pain patients [173–176] and fibromyalgia [177,178]. Therefore, its clinical utility has been emphasized [179], albeit, as with tDCS, it seems that the issue on blinding (and therefore risk of bias) must be taken into consideration when appraising the effect of rTMS on alleviating chronic pain [157].

Several lines of evidence therefore support the use of non-invasive repetitive brain stimulation paradigms in alleviating pain, specifically in chronic pain populations. However, little is known on the aftereffects on corticomotor excitability after pain relief has been achieved, despite earlier research have linked musculoskeletal pain with sensory- and motor changes [15,16,20,23,24,180] which may be associated with pain severity [17]. The next subsection will delineate some of the key techniques outside of repetitive non-invasive brain stimulation available to modulate corticomotor excitability.

3.3. FACILITATING CORTICOMOTOR EXCITABILITY BY PARADIGMS OUTSIDE OF NON-INVASIVE BRAIN STIMULATION

Several paradigms are known to facilitate corticomotor excitability. These paradigms include motor practice [29,31,90], motor skill learning [13,181–183], motor imagery [42,43,184,185], and action observation [43–45]. Study III employed action observation (AO) combined with motor imagery (MI). This subchapter will focus on the facilitatory effects of AOMI [48] (See also Appendix B for selected pertinent literature).

During AO, participants observe a correctly executed movement whereas performing MI relies on the ability of the participant to correctly recruit the motor representations associated with the imagined movement. AO and MI independently engage and activate the cortical motor system, without yielding any overt movement, and can as such be considered motor simulations [186]. These motor simulations yield a facilitation of sensorimotor potentials [187] and corticomotor excitability of the involved muscles (for comprehensive review of AO, see [188] and for MI, see [189]) but not necessarily map representation [190]. Unsurprisingly, when performing AO and MI, the involved cortical neural structures and their activation largely overlap with each other but also with those of motor execution [47], and is true for both lower- [191] and upper limbs [192]. In macaque monkeys, mirror neurons of the PMC discharge during observation of movements by others [193,194], and may influence M1 excitability through strong reciprocal connections between the PMC and M1 [52,53]. In humans, inhibitory [56,57] or facilitatory [55] rTMS to the PMC reduces or facilitates M1 excitability, respectively. These findings suggest that M1 excitability may be modulated by directly activating the PMC. Performing AO and action imitation [47] recruits a vast network including e.g. frontal areas (BA 44 and 45), primary somatosensory cortex, and, important for Study III, the lateral dorsal PMC. Performing AO and MI can improve balance training [195], complex motor learning tasks [196], and even chronic pain, as shown for stroke patients [58]. Taube and colleagues [195] demonstrated that on a perturbed balance task, those that had performed MI or AOMI of postural exercises exhibited less postural sway than a non-MI/AOMI group. They also clarified that the improvements were attributed a supraspinal rather than a spinal excitability change. Moreover, observation of a simple repetitive thumb movement away from neutral position improves acceleration [197]. This is supported by a later study showing that improvements in error time, range of motion, and frequency when learning a novel complex motor skill task were more pronounced through AO than MI [196].

One consideration with regards to AO and MI is that they are unlikely to be performed independent of each other [46,48]. Several studies have therefore combined the two, and shown that AOMI exceeds the facilitatory effect on corticomotor excitability compared to AO and MI separately (see for example [49,51,198]). While

most research support the use of AO, MI, or combined AOMI in clinical conditions, given the possible benefits during e.g. motor rehabilitation [48,199] (see also Section 1.3.), little research on the effects of AOMI on pain-induced reduction in corticomotor excitability exists. This is surprising given the facilitating properties of AOMI on corticomotor excitability during performance, the easy delivery, and that participants in general respond well to the execution of AOMI. Despite lacking evidence on for reversing muscle pain-induced changes in corticomotor excitability, it is still being used as a physical therapy intervention [196]. However, at present, there is no supporting evidence that increasing corticomotor excitability also improves functional performance in patients with musculoskeletal pain. Simple and sequential finger movements [49,198] as well as fine motor control [50] performance are enhanced by AOMI. It is well-known that nociceptive stimulation and/or pain interfere with sensorimotor processes, and that M1 excitability is often strongly reduced during pain. These associated changes may be a protective mechanism [67] or an adaptation to painful movements [11]. The only study to date that has attempted to modulate the reduction in corticomotor excitability outside of non-invasive brain stimulation is that of Schabrun and colleagues [83], who reported that motor practice immediately after the resolution of pain, did not revert the pain-induced reduction in corticomotor excitability. This finding is supported by Study II, where the reduction in FDI corticomotor excitability was unaffected by the concurrent performance of a TBT [145]. Conversely, Study III provided the first evidence that performing AOMI during muscle pain, counterbalanced the reduction in FDI corticomotor excitability that would otherwise occur, and builds upon the body of evidence (see e.g. [49–51]) suggesting that AOMI can effectively modulate M1 excitability. These findings will be discussed more in-depth in the next Sections.

3.4. EVALUATING THE EFFECTS OF A TWO-BACK TASK ON PAIN-INDUCED REDUCTION IN CORTICOMOTOR EXCITABILITY – SUMMARY OF STUDY II

An increase in M1 excitability can be elicited by stimulating the PMC through cortico-cortical connections between M1 and subcortical [149] or cortical [55–57,200,201] regions. In this respect, as mentioned in Section 2.7, the rationale for employing the TBT in Study II will be elaborated.

As discussed in Section 3.3., Study II targeted the PMC-to-M1 link known from animals [52,53] and humans [54]. Civardi and colleagues [54] showed that conditioning subthreshold magnetic stimuli applied anteriorly and medially to the M1, suppressed suprathreshold magnetic stimuli to M1. Later, high-frequency rTMS [55] and low-frequency rTMS [56,57,200] were shown to concurrently facilitate and reduce corticomotor excitability, respectively. Therefore, PMC may drive M1 excitability, and shape corticomotor output, but this has never been tested in relation to pain-

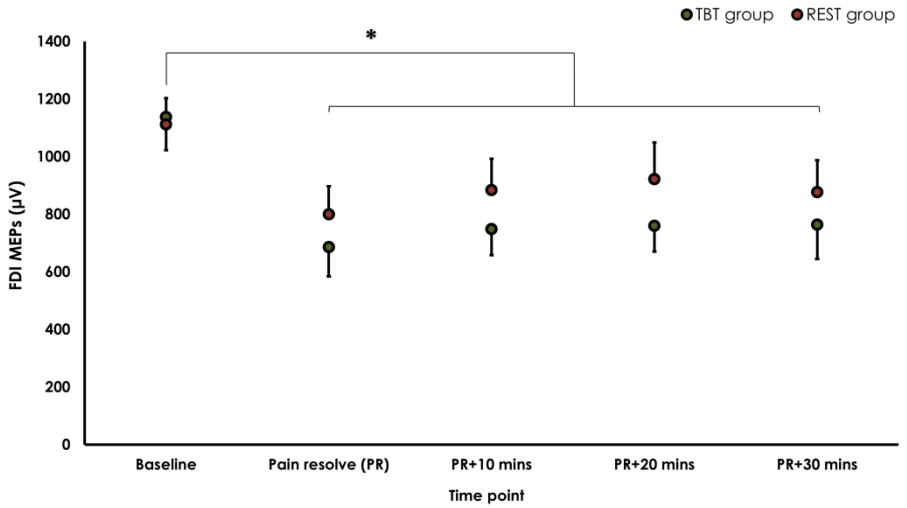

Figure 15. Study II: Motor-evoked potentials of the FDI muscle following injection of hypertonic saline injection (Mean ± SEM). *A significant main-effect of time showed that FDI MEP amplitudes were reduced at PR, PR+10, PR+20, and PR+30 mins. No difference in the reduction magnitude was found between the TBT group (green dots) or the REST group (red dots). *, $p < 0.05$*

induced reduction in corticomotor excitability. fMRI studies elucidated the activation of prefrontal and premotor areas during TBT performance [145]. Study II therefore employed the TBT to modulate M1 excitability indirectly through the PMC.

The reduction in corticomotor excitability was unaffected by the concurrent performance of the TBT (Fig. 15). Instead, both the TBT and the REST group both decreased at pain-resolve (PR) and remained so until PR + 30 mins (Fig. 15). The lasting reduction in corticomotor excitability of the FDI muscle is in line with earlier studies exploring hypertonic saline-induced pain-reduction in corticomotor excitability [12,83,115].

There are several possibilities as to why corticomotor excitability remained unaffected by the performance of the TBT. First, since the participants were asked to respond to the visual cues on the screen with their non-dominant left hand, it is possible that interhemispheric inhibition (transcallosal inhibition) [202,203] would counterbalance any changes to M1 excitability. Second, engagement of the contralateral PMC has been shown to directly influence ipsilateral M1 excitability [204]. Finally, other cortical and subcortical brain regions may have influenced the overall M1 excitability. For instance, prefrontal areas such as the DLPFC projects strongly to the basal ganglia, which is known to exert strong inhibitory influence over M1 excitability [205]. Since Study II did not include a no-pain group it is difficult to draw any conclusions with regards to the influence on M1 excitability. Taking

into consideration the limitations of Study II, further research is warranted to understand if the TBT can influence M1 excitability.

3.4.1. SAMPLE SIZE CONSIDERATIONS – STUDY II

In Study II, the sample size was calculated based on the same SMD as for Study I. Determining the sample size for a within-between factor interaction (two-way mixed model analysis of variance) with two groups and five repeated measurements, 80% power, and $\alpha = 0.05$, a total of 24 participants were needed (+6 for 20% dropout rate). This was based on a lower correlation among repeated measures (0.4), due to assessing two distinct groups who may show large intra- or interindividual differences in TMS response [97]. Since Study II was performed on 28 participants randomized into two different groups, there may be a sample size issue. However, when assessing the effect size of the missing interaction ($\eta^2_{partial} = 0.19$), it is unlikely to be the main factor. Instead, it is possible that since the sample size calculations were performed based on the SMD for pain effect on corticomotor excitability that the sample size needed to show a difference in corticomotor excitability due to TBT performance during pain was underestimated. This remains a speculation as of now but is worth considering when appraising the findings of Study II.

In summary, Study II showed that:

- The pain-induced reduction in corticomotor excitability remains unaffected by performing a TBT during pain
- Pain induces a long-lasting reduction in FDI corticomotor excitability, which is in line with earlier findings

3.5. MODULATING PAIN-INDUCED REDUCTION IN CORTICOMOTOR EXCITABILITY USING ACTION OBSERVATION AND MOTOR IMAGERY – SUMMARY OF STUDY III

Study III investigated the link between the PMC and M1 [52,53], by indirectly engaging the PMC and assessing M1 excitability changes during pain. The PMC was indirectly engaged by using an AOMI training paradigm, to assess if it could modulate M1 excitability changes [55–57] during pain.

Study III is the first to show that AOMI can attenuate the reduction in corticomotor excitability associated with acute experimental pain. Moreover, Study III is the first

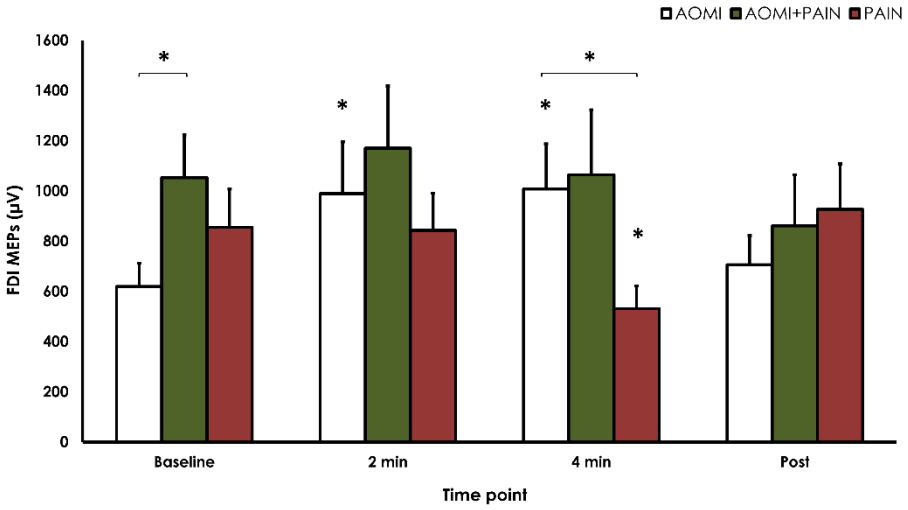

Figure 16. Study III: Motor-evoked potentials of the FDI muscle following injection of hypertonic saline injection (Mean + SEM). *Corticomotor excitability was increased after 2 mins and 4 mins, as compared to baseline, during the AOMI session. During the AOMI+PAIN session, no changes were detected throughout the experiment. Pain reduced corticomotor excitability at 4 mins, and returned to baseline values at post-measures. These findings suggest that pain reduces corticomotor excitability, and is countered by the facilitation in corticomotor excitability induced by AOMI (AOMI+PAIN). *, $p < 0.017$*

to demonstrate a non-invasive method outside of rTMS paradigms, that effectively attenuates the reduction in corticomotor excitability associated with acute experimental pain. Study I and earlier evidence showed that pain peaks between 2-4 mins and occurs concurrently with the maximum reduction in corticomotor excitability [12,115]. Therefore, in Study III the acute phase (2-4 mins) of pain and post-pain (PR and PR+10 combined) were assessed to determine if AOMI could counterbalance the reduction in FDI corticomotor excitability during peak-pain and after pain had resolved.

In Study III, participants performed, in a randomized manner, three sessions: The AOMI session (only AOMI performance), the AOMI+PAIN session (AOMI performance while being in pain), and the PAIN session (only pain). The AOMI session resulted in an increase in corticomotor excitability, as assessed using TMS (Fig. 16; white bars). Several studies have reported similar findings for AO [43–45] and MI [39–42] separately, and combined [49–51,198]. The facilitation in corticomotor excitability is mainly mediated at the cortical level, possibly through cortico-cortical connections [206]. Using paired-pulse TMS, it was shown that observing handwriting and arm movements, reduced intracortical inhibition [44]. A later study showed the same reduction in intracortical inhibition during observation of finger flexion [207]. These studies support the notion that facilitation of M1 excitability by the PMC is mainly mediated through facilitating cortico-cortical connections [208]. At present,

however, spinal contributions cannot be excluded due to the limitations of available techniques, such as H-reflex and F-waves (see Section 2.2.) and contradicting literature [207,209].

The results from the PAIN session are similar to earlier studies [12,21,109] (See also Study I) showing a reduction in corticomotor excitability at 4 mins after hypertonic saline injection (Fig. 16; red bars). This reduction is believed to be governed by GABA- and glutamate-mediated intracortical inhibition and facilitation [109]. Studies investigating the spinal and peripheral muscle excitability influence on the reduction in corticospinal excitability have reported that the H-reflex, F-waves, and M-waves are unaffected in the peak-pain phase [12,101–103] indicating a cortical site of origin. Furthermore, findings from Study I was replicated in that post-measures did not differ from baseline values, suggesting that the methodology employed in Studies I and III may have impacted the lasting reduction in corticomotor excitability as earlier reported [83,115] (see also Study II). One possibility is that due to the NRS ratings every 2 mins during pain (speech) may have facilitated corticomotor excitability [155]. This is, however, unlikely to be the main factor, since in Study II, participants still rated their pain every 2 mins, yet the pain-induced reduction in corticomotor excitability was observed at PR. Alternatively, attention is known to fluctuate, and having to attend to pain every 2 mins, may have influenced corticomotor excitability [82]. Finally, the 100 TMS stimulations over the 10 mins of pain may have caused a return-towards-baseline of the MEPs. Further research into the possible differences in methodologies on the lasting pain-induced reduction in corticomotor excitability is warranted.

During the AOMI+PAIN session there was no change in corticomotor excitability (Fig. 16; green bars). This indicates that AOMI may have offset the reduction in corticomotor excitability that would otherwise occur during experimental pain, as in the PAIN session (Fig. 16; red bars) and acute experimental pain may have offset the increase in corticomotor excitability that would normally occur during AOMI alone, as in the AOMI session (Fig. 16; white bars).

In this respect, AOMI appears to counterbalance the changes in cortical motor excitability associated with acute experimental pain. Age, gender, or pain intensity rating differences did not influence the lack of change in corticomotor excitability during the AOMI+PAIN session. Whereas AO and MI have been utilized for improving pain reports in phantom limb patients [210,211], cervical joint reposition in neck pain patients [212], and sensorimotor cortex activation and phantom limb motor recovery in stroke patients [58], little is known with respect to experimental pain. For example, Volz et al. [213] demonstrated that pressure pain thresholds increased following AO, possibly mediated through a reduction in intracortical inhibition, but found no facilitation in corticomotor excitability. Another study demonstrated that mental imagery did not affect pressure pain thresholds, but a reduction in corticomotor excitability was reported [81]. As such, Study III provides the first

evidence that pain-induced reduction in corticomotor excitability can be attenuated by performing AOMI. Further research is needed to establish the effectiveness of AOMI on pain induced by e.g. other pain models, the specific parameters needed to provide the largest neuroplastic potential, but most importantly, the functional relevance of the counterbalancing the reduction in corticomotor excitability.

An important limitation to Study III is that the baseline values for the AO session as compared to the AOMI+PAIN session differed. Several different factors influence corticomotor excitability on a day-to-day basis, including e.g. genetics (brain-derived neurotrophic factor particularly), metaplasticity, attention, or age [214]. Since each participant was scheduled at the same time of the day, circadian rhythm variation [215] is unlikely to explain the difference. Therefore, the issue of the difference at baseline is multifactorial and is not likely determined by only one. However, it is unlikely that the baseline difference influenced the overall result of Study III for several reasons: (1) corticomotor excitability returned-towards-baseline and was not different from baseline during all three sessions, suggesting that within-session, AOMI and PAIN only affected corticomotor excitability at 2-4 mins; (2) even if the AOMI session started at a lower average, the difference in baseline would only influence possible inferences made between the AOMI and AOMI+PAIN session where no differences were found; (3) a substantial body of evidence [48–50,198] has consistently demonstrated a facilitation of corticomotor excitability similar to Study III. Therefore, Study III supports earlier data on facilitation during AOMI performance, and adds that this facilitation counterbalances pain-induced reduction in corticomotor excitability.

3.5.1. SAMPLE SIZE CONSIDERATIONS – STUDY III

In Study III, the same sample size calculation was performed as for Study I, with high correlation among repeated measures (0.8), and 80% power to detect a difference at $\alpha = 0.05$. The inclusion of 12 participants in Study III therefore satisfied the sample size calculation.

3.6. MAIN FINDINGS FROM STUDY I-III (NEUROPHYSIOLOGICAL DATA)

In summary, Study III showed that:

- AOMI induces a large facilitation in corticomotor excitability
- Hypertonic saline-induced pain reduces corticomotor excitability of the FDI muscle

- Performing AOMI attenuates the reduction in corticomotor excitability associated with experimental muscle pain,

CHAPTER 4. DISCUSSION

The current PhD project has provided novel aspects to our current knowledge on pain-induced reduction in corticomotor excitability and confirmed earlier findings.

The hypertonic saline model is a valid model to explore early musculoskeletal pain and concurrent sensorimotor changes, given the highly reproducible pain intensity and qualities. Further, corroborating evidence on the strong hypertonic saline pain-induced reduction in corticomotor excitability was demonstrated in Study I-III. The reduction in corticomotor excitability seems to be more prominent for the FDI muscle than the ECR muscle [14] (Study I), despite sharing cortical motor representation The magnitude of pain intensity at peak-pain is not correlated with the reduction in corticomotor excitability for neither the ECR nor the FDI muscle, which is in line with other studies. As such, it is not the perception of the intensity of pain that drives the reduction in corticomotor excitability, but more likely the nociceptive input. State or trait anxiety, pain catastrophizing, mindwandering, age, or gender ratio could not explain any differences in pain intensity ratings between the TBT and REST groups in Study II. This may indicate that the group that performed the TBT during pain, experienced an enhanced perception of pain, but it remains inconclusive if other factors such as expectation or genetic predisposition [126] had an influence. Nonetheless, Study II provided the first evidence that even if pain perception increased during the performance of the TBT, the reduction in corticomotor excitability remained unaffected. Study III showed, for the first time, that the facilitation in corticomotor excitability by AOMI could counterbalance the pain-induced reduction that would otherwise occur.

When stimulating with TMS throughout the pain period, it seems that corticomotor excitability recovers at post-measures (Study I and III). Conversely, as has been reported earlier, when participants remain at rest throughout the pain period MEPs are reduced and remain so up until 30 mins post-PR as shown in Study II. The accuracy on correctly identifying targets in the TBT improved from baseline assessment to 30 mins post-PR, suggesting that a learning effect occurred. This learning effect was equal in the two groups, despite the TBT group performing the task for an additional 10 mins, compared to the REST group (Study II).

These findings suggest that the pain-induced reduction in corticomotor excitability can be modulated in the acute phase of muscle pain, but the engagement of the PMC and the influence on M1 excitability, may be task-dependent. Performing a TBT engages the PMC to provide spatial information and response selection [145]. Findings from Study II suggested that such engagement is unable to modulate M1 excitability. Conversely, AOMI influenced M1 excitability without any overt volitional movement, as demonstrated in Study III. Since AO, MI, and movement execution largely depends on the same neuroanatomical structures [186], it can be

speculated that subsequent pathways engaged through PMC activation by the TBT and AOMI, are different. One way to explore this would be to create a virtual lesion in the PMC by applying rTMS [56,57] preceding the performance of the TBT and AOMI tasks. This would allow relative influence of the PMC on M1 excitability to be extracted. However, as mentioned in Section 3.4., it would be needed to first establish whether the TBT indeed induces a facilitation of M1 excitability, and the limitations of Study II should be considered. It could be argued that since corticomotor excitability during pain in Study II, any transient facilitating effects of the TBT on M1 excitability may have been missed. This cannot be excluded, but findings from Study III suggest that if this was the case and accept the idea that M1 excitability is influenced by the PMC, the reduction in corticomotor excitability at PR should be absent.

There is a scarcity in studies designed to modulate the pain-induced reduction in corticomotor excitability. This is surprising given the potential and feasibility of using AOMI in the clinic. In this respect, several lines of evidence have highlighted the relevance of AO and MI in chronic pain populations such as patients recovering from stroke (see e.g. [58,216,217]) and phantom limb pain patients (see e.g. [211], [199], and [218] for meta-analysis). For instance, AO has been applied in stroke rehabilitation, and shown effective in improving function after consecutive training in moderate upper-limb deficiency [219]. Later studies confirmed this, by showing that four weeks of extensive AOMI increased sensorimotor cortex activation and improved motor function and muscle strength of the hand [58,220]. Therefore, emerging evidence for the applicability of AOMI in stroke patients support the use of AOMI in the acute-to-subacute phase but needs further exploration in chronic musculoskeletal pain populations.

Throughout the discussion on available literature on pain-induced reduction in corticomotor excitability, it is also clear that more research is warranted to understand if modulating this reduction yields improvements in motor function or other clinical outcomes. One possibility is that the reduction in corticomotor excitability is an important physiological process that must occur to bring back neuronal homeostasis after insult. Interfering with such process may prove to be maladaptive and should be cautiously investigated. It can be hypothesized that AOMI may prove beneficial in musculoskeletal pain conditions such as low-back pain or muscle soreness [16,17,23], since corticomotor representation reduces, as has also been described during limb immobilization [221]. In this respect, applying AO seemingly counterbalanced this reduction in corticomotor representation, suggesting that motor activity can be maintained in an immobilized limb [221]. It is important to highlight that MI was ineffective in reversing the reduction in corticomotor excitability due to limb immobilization, which is supported by an earlier kinematics study [196]. This indicates that AO is an important part of the beneficial outcomes seen when performing motor simulations and combined with MI, may prove beneficial in attenuating or reversing maladaptive motor neuroplasticity in response to pain.

Further research is needed to elucidate if AOMI as an intervention, can provide a readily applicable countermeasure to maladaptive motor neuroplasticity.

Some limitations to AO and MI (or AOMI) must be highlighted, and are related to the ability of performing AO, MI, or both combined. For instance, patients suffering from chronic musculoskeletal pain may have impaired MI [59], and its applicability in relation to patients suffering from e.g. low-back pain remains unknown. This has also been shown for MI, where MI of trunk movements may be reduced in patients with a history of back pain [222]. As such, it is necessary to establish parameters for the intervention to standardize delivery and improving the method to induce the greatest potential for inducing neuroplasticity at the sensorimotor level [26]. The findings from the current PhD suggest that muscle choice and task-dependency on PMC engagement are important to consider. Study III further supports the notion that combined AOMI is a powerful driver of motor neuroplasticity, however, research on its applicability in chronic musculoskeletal pain conditions is still in its infancy. It should also be mentioned that surprisingly little is known on the effects of rTMS in chronic musculoskeletal pain such as low-back pain, despite several lines of evidence suggest motor re-organizational changes in muscle representations of painful muscles [15,16], and may be related to pain severity [17]. Single-session tDCS did not affect experimentally-induced pain in chronic low-back pain patients [223] and tDCS as an adjunct to cognitive behavioral management in chronic low-back pain [166] did not affect pain intensity or disability. Novel evidence suggests that NGF-induced corticomotor and somatosensory excitability changes can be modulated by rTMS [172]. Nonetheless, it is clear that more research is warranted to determine the effectiveness of non-invasive brain stimulation on chronic musculoskeletal pain [157]

The current PhD project aimed to probe and modulate pain-induced reduction in corticomotor excitability. This was largely motivated by the findings that pain-induced reduction in corticomotor excitability is robust [12,67,101–103] and difficult to modulate [83]. Study I demonstrated eliciting pain in the FDI muscle yields a strong pain-induced reduction in FDI corticomotor excitability whereas the ECR muscle excitability remained unaffected. Consequently, Study II and Study III used the FDI muscle as primary target. Study II demonstrated that performance of a TBT was inefficient in counterbalancing the pain-induced reduction in corticomotor excitability. Study III demonstrated that performing AOMI effectively modulated pain-induced reduction in corticomotor excitability. These new findings open avenues to explore potential advantages of AOMI on musculoskeletal pain in larger cohort studies to clarify the role of pain-induced reduction in corticomotor excitability. This would further our understanding on how to optimize and provide the greatest neuroplastic potential [26] (see e.g. [224]) to target sensorimotor changes known to occur in relation to chronic musculoskeletal pain [11,16,17].

CHAPTER 5. CONCLUSION

The current PhD thesis addressed two specific objectives: (1) Probe pain-induced neuroplasticity of the motor system using a well-established acute experimental muscle pain model in two different upper limb muscles; and (2) modulate corticomotor excitability by paradigms known to engage the PMC.

Study I demonstrated that a more robust reduction in corticomotor excitability was achieved when targeting the FDI muscle, as compared to the ECR muscle (Study I; Fig. 17). Study II and Study III built upon the results of Study I, where modulation of pain-induced reduction in FDI corticomotor excitability was explored and tested. In Study II, the performance of the TBT during pain did not modulate the reduction in corticomotor excitability (Study II; Fig. 17). This study underscored the robustness of the hypertonic saline pain model in reducing MEPs, and further confirmed earlier findings with respect to pain intensity and quality. A peculiar finding was that the group, who performed the TBT during pain, also reported higher pain intensity than the group resting during pain (Study II; Fig. 17). Pain intensity was, however, unrelated to the magnitude of reduction in corticomotor excitability in response to the hypertonic saline injection. Performing an AOMI task during pain successfully counterbalanced the pain-induced reduction in FDI corticomotor excitability that would otherwise occur (Study III; Fig. 17). This novel finding opens new avenues of research such as exploring methods to reverse the extensive motor changes occurring in response to musculoskeletal pain, by tailoring

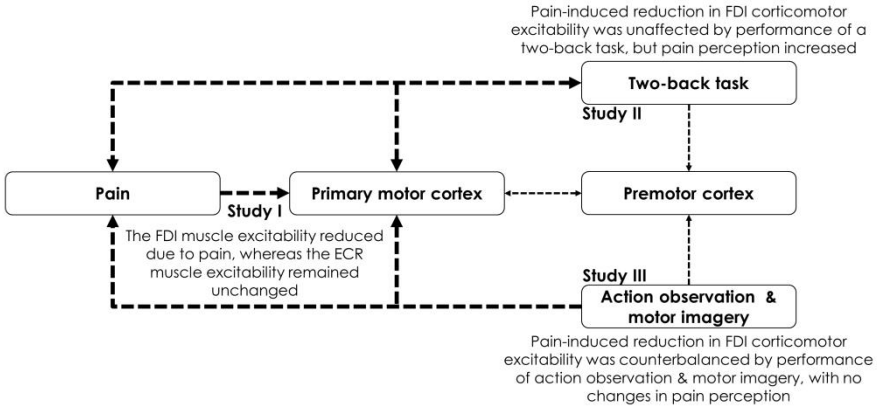

Figure 17. Main findings of the dissertation studies based on the conceptual overview presented in Chapter 1. *Study I showed that pain reduced FDI corticomotor excitability but not ECR corticomotor excitability. In Study II, pain-induced reduction in FDI corticomotor excitability was unaffected by the performance of a two-back task, but may have increased pain perception. In Study III, action observation & motor imagery were shown effective in counterbalancing the pain-induced reduction in FDI corticomotor excitability.*

motor rehabilitation paradigms to provide the greatest potential for altering maladaptive changes at the motor level. It will further allow for better understanding of the neurophysiological substrates involved in the interaction between adaptive and maladaptive neuroplasticity of the motor system and contribute to the basic understanding of brain neurophysiology during pain.

All three studies support that perceived intensity of pain is unlikely to be the main driver of the reduction in corticomotor excitability. Instead, the presence of a nociceptive input is a major contributor to the corticomotor changes, as there appears to be a uniform reduction in response to several different experimental pain models.

In summary, the three PhD studies presented novel approaches to modulate pain-induced reduction in corticomotor excitability. Using experimental pain models, as in the current PhD thesis, allow for testing and confirming the applicability of AOMI as a viable tool in motor rehabilitation for patients suffering from chronic musculoskeletal pain. These findings will serve as stepping stones to establish optimal parameters for inducing advantageous adaptive neuroplasticity.

LITERATURE LIST

1. Vos, T. *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* **388**, 1545–1602 (2016).

2. Vos, T. *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **390**, 1211–1259 (2017).

3. Pelletier, R., Higgins, J. & Bourbonnais, D. Is neuroplasticity in the central nervous system the missing link to our understanding of chronic musculoskeletal disorders? *BMC Musculoskelet. Disord.* **16**, 25 (2015).

4. Arendt-Nielsen, L. & Graven-Nielsen, T. Translational musculoskeletal pain research. *Best Pract. Res. Clin. Rheumatol.* **25**, 209–26 (2011).

5. Dieppe, P. A. & Lohmander, L. S. Pathogenesis and management of pain in osteoarthritis. *Lancet (London, England)* **365**, 965–73 (2016).

6. Garland, E. L. Pain Processing in the Human Nervous System. *Prim. Care Clin. Off. Pract.* **39**, 561–571 (2012).

7. Graven-Nielsen, T. & Arendt-Nielsen, L. Assessment of mechanisms in localized and widespread musculoskeletal pain. *Nat. Rev. Rheumatol.* **6**, 599–606 (2010).

8. Hanley, M. A. *et al.* Preamputation Pain and Acute Pain Predict Chronic Pain After Lower Extremity Amputation. *J. Pain* **8**, 102–109 (2007).

9. Katz, J. & Seltzer, Z. Transition from acute to chronic postsurgical pain: risk factors and protective factors. *Expert Rev. Neurother.* **9**, 723–744 (2009).

10. Kovacs, F. M., Abraira, V., Zamora, J. & Fern??ndez, C. The Transition from Acute to Subacute and Chronic Low Back Pain. *Spine (Phila. Pa. 1976).* **30**, 1786–1792 (2005).

11. Hodges, P. W. & Tucker, K. Moving differently in pain: A new theory to explain the adaptation to pain. *Pain* **152**, S90–S98 (2011).

12. Le Pera, D. *et al.* Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. *Clin. Neurophysiol.* **112**, 1633–1641 (2001).

13. Boudreau, S. *et al*. The effects of intra-oral pain on motor cortex neuroplasticity associated with short-term novel tongue-protrusion training in humans. *Pain* **132**, 169–178 (2007).

14. Larsen, D. B., Graven-Nielsen, T., Hirata, R. P. & Boudreau, S. A. Differential Corticomotor Excitability Responses to Hypertonic Saline-Induced Muscle Pain in Forearm and Hand Muscles. *Neural Plast.* **2018**, 1–9 (2018).

15. Tsao, H., Galea, M. P. & Hodges, P. W. Reorganization of the motor cortex is associated with postural control deficits in recurrent low back pain. *Brain* **131**, 2161–2171 (2008).

16. Tsao, H., Danneels, L. A. & Hodges, P. W. ISSLS Prize Winner: smudging the motor brain in young adults with recurrent low back pain. *Spine (Phila. Pa. 1976).* **36**, 1721–1727 (2011).

17. Schabrun, S. M., Elgueta-Cancino, E. L. & Hodges, P. W. Smudging of the Motor Cortex Is Related to the Severity of Low Back Pain. *Spine (Phila. Pa. 1976).* **42**, 1172–1178 (2017).

18. Burns, E., Chipchase, L. S. & Schabrun, S. M. Altered function of intracortical networks in chronic lateral epicondylalgia. *Eur. J. Pain* n/a-n/a (2016). doi:10.1002/ejp.841

19. Neige, C., Mavromatis, N., Gagné, M., Bouyer, L. J. & Mercier, C. Effect of movement-related pain on behaviour and corticospinal excitability changes associated with arm movement preparation. *J. Physiol.* **596**, 2917–2929 (2018).

20. Tsao, H., Tucker, K. J. & Hodges, P. W. Changes in excitability of corticomotor inputs to the trunk muscles during experimentally-induced acute low back pain. *Neuroscience* **181**, 127–133 (2011).

21. Schabrun, S. M., Jones, E., Kloster, J. & Hodges, P. W. Temporal association between changes in primary sensory cortex and corticomotor output during muscle pain. *Neuroscience* **235**, 159–64 (2013).

22. Dubois, J. *et al*. Effect of pain on deafferentation-induced modulation of somatosensory evoked potentials. *PLoS One* **13**, e0206141 (2018).

23. De Martino, E., Petrini, L., Schabrun, S. & Graven-Nielsen, T. Cortical Somatosensory Excitability Is Modulated in Response to Several Days of Muscle Soreness. *J. Pain* **19**, 1296–1307 (2018).

24. De Martino, E., Zandalasini, M., Schabrun, S., Petrini, L. & Graven-Nielsen,

T. Experimental Muscle Hyperalgesia Modulates Sensorimotor Cortical Excitability, Which is Partially Altered by Unaccustomed Exercise. *Pain* **00**, 1 (2018).

25. Bank, P. J. M., Peper, C. E., Marinus, J., Beek, P. J. & van Hilten, J. J. Motor consequences of experimentally induced limb pain: A systematic review. *Eur. J. Pain* **17**, 145–157 (2013).

26. Boudreau, S. A., Farina, D. & Falla, D. The role of motor learning and neuroplasticity in designing rehabilitation approaches for musculoskeletal pain disorders. *Man Ther* **15**, 410–414 (2010).

27. Rioult-Pedotti, M. S., Friedman, D., Hess, G. & Donoghue, J. P. Strengthening of horizontal cortical connections following skill learning. *Nat Neurosci* **1**, 230–234 (1998).

28. Kleim, J. A., Barbay, S. & Nudo, R. J. Functional reorganization of the rat motor cortex following motor skill learning. *J. Neurophysiol.* **80**, 3321–5 (1998).

29. Gallasch, E., Christova, M., Krenn, M., Kossev, A. & Rafolt, D. Changes in motor cortex excitability following training of a novel goal-directed motor task. *Eur. J. Appl. Physiol.* **105**, 47–54 (2009).

30. Ziemann, U., Muellbacher, W., Hallett, M. & Cohen, L. G. Modulation of practice-dependent plasticity in human motor cortex. *Brain* **124**, 1171–81. (2001).

31. Muellbacher, W., Ziemann, U., Boroojerdi, B., Cohen, L. & Hallett, M. Role of the human motor cortex in rapid motor learning. *Exp. Brain Res.* **136**, 431–438 (2001).

32. Elbert, T. *et al.* Increased Cortical Representation of the Fingers of the Left Hand in String Players Edward Taub Published by : American Association for the Advancement of Science Stable URL : https://www.jstor.org/stable/2888544 digitize , preserve and extend access to S. **270**, 305–307 (1995).

33. Tyč, F., Boyadjian, a. & Devanne, H. Motor cortex plasticity induced by extensive training revealed by transcranial magnetic stimulation in human. *Eur. J. Neurosci.* **21**, 259–266 (2005).

34. Cohen, G. *et al.* Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *J. Neurophysiol.* **74**, 1037–1045 (1995).

35. Hirano, M., Kubota, S., Tanabe, S., Koizume, Y. & Funase, K. Interactions among learning stage, retention, and primary motor cortex excitability in motor skill learning. *Brain Stimul.* **8**, 1195–1204 (2015).

36. Boudreau, S. A., Hennings, K., Svensson, P., Sessle, B. J. & Arendt-Nielsen, L. The effects of training time, sensory loss and pain on human motor learning. *J. Oral Rehabil.* **37**, 704–718 (2010).

37. Lamothe, M. *et al.* Effect of tonic pain on motor acquisition and retention while learning to reach in a force field. *PLoS One* **9**, e99159 (2014).

38. Bouffard, J., Bouyer, L. J., Roy, J. S. & Mercier, C. Tonic pain experienced during locomotor training impairs retention despite normal performance during acquisition. *J Neurosci* **34**, 9190–9195 (2014).

39. Fourkas, A. D., Avenanti, A., Urgesi, C. & Aglioti, S. M. Corticospinal facilitation during first and third person imagery. *Exp. Brain Res.* **168**, 143–151 (2006).

40. Stinear, C. M., Byblow, W. D., Steyvers, M., Levin, O. & Swinnen, S. P. Kinesthetic, but not visual, motor imagery modulates corticomotor excitability. *Exp. Brain Res.* **168**, 157–164 (2006).

41. Stinear, C. M., Fleming, M. K. & Byblow, W. D. Lateralization of unimanual and bimanual motor imagery. *Brain Res.* **1095**, 139–147 (2006).

42. Bufalari, I., Sforza, A., Cesari, P., Aglioti, S. M. & Fourkas, A. D. Motor imagery beyond the joint limits: A transcranial magnetic stimulation study. *Biol. Psychol.* **85**, 283–290 (2010).

43. Fadiga, L., Fogassi, L., Pavesi, G. & Rizzolatti, G. Motor facilitation during action observation: a magnetic stimulation study. *J. Neurophysiol.* **73**, 2608–2611 (1995).

44. Strafella, A. P. & Paus, T. Modulation of cortical excitability during action observation: a transcranial magnetic stimulation study. *Neuroreport* **11**, 2289–2292 (2000).

45. Koch, G. *et al.* Resonance of cortico-cortical connections of the motor system with the observation of goal directed grasping movements. *Neuropsychologia* **48**, 3513–3520 (2010).

46. Vogt, S., Rienzo, F. Di, Collet, C., Collins, A. & Guillot, A. Multiple roles of motor imagery during action observation. *Front. Hum. Neurosci.* **7**, 1–13 (2013).

47. Caspers, S., Zilles, K., Laird, A. R. & Eickhoff, S. B. ALE meta-analysis of action observation and imitation in the human brain. *Neuroimage* **50**, 1148–1167 (2010).

48. Eaves, D. L., Riach, M., Holmes, P. S. & Wright, D. J. Motor Imagery during Action Observation: A Brief Review of Evidence, Theory and Future Research Opportunities. *Front. Neurosci.* **10**, 1–10 (2016).

49. Wright, D. J., Williams, J. & Holmes, P. S. Combined action observation and imagery facilitates corticospinal excitability. *Front. Hum. Neurosci.* **8**, 1–9 (2014).

50. Ohno, K., Higashi, T., Phl, O. T. R., Sugawara, K. & Phl, R. P. T. Excitability changes in the human primary motor cortex during observation with motor imagery of chopstick use. *J. Phys. Ther. Sci.* **23**, 703–706 (2011).

51. Sakamoto, M., Muraoka, T., Mizuguchi, N. & Kanosue, K. Combining observation and imagery of an action enhances human corticospinal excitability. *Neurosci. Res.* **65**, 23–27 (2009).

52. Takada, M., Tokuno, H., Nambu, A. & Inase, M. Corticostriatal projections from the somatic motor areas of the frontal cortex in the macaque monkey: Segregation versus overlap of input zones from the primary motor cortex, the supplementary motor area, and the premotor cortex. *Exp. Brain Res.* **120**, 114–128 (1998).

53. Tokuno, H. & Tanji, J. Input organization of the distal and proximal forelimg areas in the monkey primary motor cortex: a retrograde labeling study. *J.Comp.Neurol.* **333**, 199–209 (1993).

54. Civardi, C., Cantello, R., Asselman, P. & Rothwell, J. C. Transcranial magnetic stimulation can be used to test connections to primary motor areas from frontal and medial cortex in humans. *Neuroimage* **14**, 1444–1453 (2001).

55. Rizzo, V. *et al.* Shaping the excitability of human motor cortex with premotor rTMS. *J. Physiol.* **554**, 483–495 (2004).

56. Gerschlager, W., Siebner, H. R. & Rothwell, J. C. Decreased corticospinal excitability after subthreshold 1 Hz rTMS over lateral premotor cortex. *Neurology* **57**, 449–455 (2001).

57. Münchau, A., Bloem, B. R., Irlbacher, K., Trimble, M. R. & Rothwell, J. C. Functional connectivity of human premotor and motor cortex explored with repetitive transcranial magnetic stimulation. *J. Neurosci.* **22**, 554–561

(2002).

58. Sun, Y., Wei, W., Luo, Z., Gan, H. & Hu, X. Improving motor imagery practice with synchronous action observation in stroke patients. *Top. Stroke Rehabil.* **23**, 245–253 (2016).

59. Breckenridge, J. D., Ginn, K. A., Wallwork, S. B. & McAuley, J. H. Do People With Chronic Musculoskeletal Pain Have Impaired Motor Imagery? A Meta-analytical Systematic Review of the Left/Right Judgment Task. *J. Pain* **00**, (2018).

60. Flor, H., Nikolajsen, L. & Staehelin Jensen, T. Phantom limb pain: a case of maladaptive CNS plasticity? *Nat Rev Neurosci* **7**, 873–881 (2006).

61. Flor, H. *et al.* Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. *Nature* **375**, 482–484 (1995).

62. Flor, H., Braun, C., Elbert, T. & Birbaumer, N. Extensive reorganization of primary somatosensory cortex in chronic back pain patients. *Neurosci. Lett.* **224**, 5–8 (1997).

63. Te, M., Baptista, A. F., Chipchase, L. S. & Schabrun, S. M. Primary motor cortex organization is altered in persistent patellofemoral pain. *Pain Med. (United States)* **18**, 2224–2234 (2017).

64. Vallence, A. M., Smith, A., Tabor, A., Rolan, P. E. & Ridding, M. C. Chronic tension-type headache is associated with impaired motor learning. *Cephalalgia* **33**, 1048–1054 (2013).

65. Bergin, M. J. G. *et al.* Movement Evoked Pain and Mechanical Hyperalgesia after Intramuscular Injection of Nerve Growth Factor: A Model of Sustained Elbow Pain. *Pain Med. (United States)* **16**, 2180–2191 (2015).

66. Schabrun, S. M., Christensen, S. W., Mrachacz-Kersting, N. & Graven-Nielsen, T. Motor Cortex Reorganization and Impaired Function in the Transition to Sustained Muscle Pain. *Cereb. Cortex* **26**, 1878–1890 (2016).

67. Burns, E., Chipchase, L. S. & Schabrun, S. M. Primary sensory and motor cortex function in response to acute muscle pain: A systematic review and meta-analysis. *Eur. J. Pain* **20**, 1203–1213 (2016).

68. Viaro, R., Budri, M., Parmiani, P. & Franchi, G. Adaptive changes in the motor cortex during and after longterm forelimb immobilization in adult rats. *J. Physiol.* **592**, 2137–52 (2014).

69. Zanette, G., Manganotti, P., Fiaschi, A. & Tamburin, S. Modulation of

motor cortex excitability after upper limb immobilization. *Clin. Neurophysiol.* **115**, 1264–1275 (2004).

70. Liepert, J., Tegenthoff, M. & Malin, J. P. Changes of cortical motor area size during immobilization. *Electroencephalogr. Clin. Neurophysiol. Electromyogr.* **97**, 382–386 (1995).

71. Avanzino, L., Bassolino, M., Pozzo, T. & Bove, M. Use-Dependent Hemispheric Balance. *J. Neurosci.* **31**, 3423–3428 (2011).

72. Huber, R. *et al.* Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. *Nat. Neurosci.* **9**, 1169–1176 (2006).

73. Liepert, J. *et al.* Motor cortex plasticity during constraint-induced movement therapy in stroke patients. *Neurosci. Lett.* **250**, 5–8 (1998).

74. Graven-Nielsen, T., Arendt-Nielsen, L., Svensson, P. & Jensen, T. S. Experimental Muscle Pain: A Quantitative Study of Local and Referred Pain in Humans Following Injection of Hypertonic Saline. *J. Musculoskelet. Pain* **5**, 49–69 (1997).

75. Ferguson, a. R., Crown, E. D. & Grau, J. W. Nociceptive plasticity inhibits adaptive learning in the spinal cord. *Neuroscience* **141**, 421–431 (2006).

76. Bilodeau, M. C., Roosink, M. & Mercier, C. Effect of local versus remote tonic heat pain during training on acquisition and retention of a finger-tapping sequence task. *Exp. Brain Res.* **234**, 475–482 (2016).

77. Dancey, E., Murphy, B., Srbely, J. & Yielder, P. The effect of experimental pain on motor training performance and sensorimotor integration. *Exp. Brain Res.* **232**, 2879–2889 (2014).

78. Dancey, E., Murphy, B. A., Andrew, D. & Yielder, P. The interactive effect of acute pain and motor learning acquisition on sensorimotor integration and motor learning outcomes. *J. Neurophysiol.* **2768**, jn.00337.2016 (2016).

79. Dancey, E., Murphy, B. A., Andrew, D. & Yielder, P. The effect of local versus remote experimental pain on motor learning and sensorimotor integration using a complex typing task. *Pain* **157**, 1682–1695 (2016).

80. Mavromatis, N., Gagné, M., Voisin, J. I. A. V., Reilly, K. T. & Mercier, C. Experimental tonic hand pain modulates the corticospinal plasticity induced by a subsequent hand deafferentation. *Neuroscience* **330**, 403–409 (2016).

81. Volz, M. S., Suarez-Contreras, V., Portilla, A. S. L. & Fregni, F. Mental

imagery-induced attention modulates pain perception and cortical excitability. *BMC Neurosci.* **16**, 1–10 (2015).

82. Stefan, K., Wycislo, M. & Classen, J. Modulation of associative human motor cortical plasticity by attention. *J. Neurophysiol.* **92**, 66–72 (2004).

83. Schabrun, S. M., Palsson, T. S., Thapa, T. & Graven-Nielsen, T. Movement Does Not Promote Recovery of Motor Output Following Acute Experimental Muscle Pain. *Pain Med.* 1–7 (2017). doi:10.1093/pm/pnx099

84. Barker, A. T., Jalinous, R. & Freeston, I. L. Non-Invasive Magnetic Stimulation of Human Motor Cortex. *Lancet* **325**, 1106–1107 (1985).

85. Sanes, J. N. & Donoghue, J. P. Plasticity and primary motor cortex. *Annu Rev Neurosci* **23**, 393–415 (2000).

86. Rothwell, J. C. *et al.* Magnetic stimulation : motor evoked potentials. The International Federation of Clinical Neuroohysiology, Electroencephalog Clin Neurophysiol Suppl. *Elsevier Sci.* **52**, 97–103 (1999).

87. Huang, Y. Z. *et al.* Plasticity induced by non-invasive transcranial brain stimulation: A position paper. *Clin. Neurophysiol.* **128**, 2318–2329 (2017).

88. Rossini, P. M. & Rossi, S. Clinical applications of motor evoked potentials. *Electroencephalogr. Clin. Neurophysiol.* **106**, 180–194 (1998).

89. Rossini, P. M. *et al.* Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin. Neurophysiol.* **126**, 1071–1107 (2015).

90. Carroll, T. J., Lee, M., Hsu, M. & Sayde, J. Unilateral practice of a ballistic movement causes bilateral increases in performance and corticospinal excitability. *J. Appl. Physiol.* **104**, 1656–1664 (2008).

91. Sommer, M., Classen, J., Cohen, L. G. & Hallett, M. Time course of determination of movement direction in the reaction time task in humans. *J. Neurophysiol.* **86**, 1195–201 (2001).

92. Pascual-Leone, A. *et al.* Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *J. Neurophysiol.* **74**, 1037–1045 (1995).

93. Wu, L., Goto, Y., Taniwaki, T., Kinukawa, N. & Tobimatsu, S. Different patterns of excitation and inhibition of the small hand and forearm muscles from magnetic brain stimulation in humans. *Clin. Neurophysiol.* **113**, 1286–

1294 (2002).

94. Rossini, P. M. *et al.* Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr. Cinical Neurophysiol.* **91**, 79–92 (1994).

95. Rossi, S., Hallett, M., Rossini, P. M. & Pascual-Leone, A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol* **120**, 2008–2039 (2009).

96. Liu, H. & Au-Yeung, S. S. Y. Reliability of transcranial magnetic stimulation induced corticomotor excitability measurements for a hand muscle in healthy and chronic stroke subjects. *J. Neurol. Sci.* **341**, 105–109 (2014).

97. Wassermann, E. M. Variation in the response to transcranial magnetic brain stimulation in the general population. *Clin. Neurophysiol.* **113**, 1165–1171 (2002).

98. Rodriguez-Falces, J. & Place, N. Determinants, analysis and interpretation of the muscle compound action potential (M wave) in humans: implications for the study of muscle fatigue. *Eur. J. Appl. Physiol.* **118**, 501–521 (2018).

99. Fisher, M. H reflexes and F waves: Physiology and clinical indications. *Muscle Nerve* **15**, 1222–1233 (1992).

100. Hoch, M. C. & Krause, B. A. Intersession reliability of H:M ratio is greater than the H-reflex at a percentage of M-max. *Int. J. Neurosci.* **119**, 345–352 (2009).

101. Farina, S. *et al.* Transient inhibition of the human motor cortex by capsaicin-induced pain. A study with transcranial magnetic stimulation. *Neurosci Lett* **314**, 97–101 (2001).

102. Svensson, P., Miles, T. S., McKay, D. & Ridding, M. C. Suppression of motor evoked potentials in a hand muscle following prolonged painful stimulation. *Eur. J. Pain* **7**, 55–62 (2003).

103. Tinazzi, M. *et al.* Long-lasting modulation of human motor cortex following prolonged transcutaneous electrical nerve stimulation (TENS) of forearm muscles: Evidence of reciprocal inhibition and facilitation. *Exp. Brain Res.* **161**, 457–464 (2005).

104. Mesrati, F. & Vecchierini, M. F. F-waves: Neurophysiology and clinical

value. *Neurophysiol. Clin.* **34**, 217–243 (2004).

105. Kellgren, J. H. A preliminary account of referred pains arising from muscle. *Br. Med. J.* 325–327 (1938).

106. Graven-Nielsen, T., Arendt-Nielsen, L., Svensson, P. & Jensen, T. S. Stimulus-response functions in areas with experimentally induced referred muscle pain - A psychophysical study. *Brain Res.* **744**, 121–128 (1997).

107. Arendt-Nielsen, L. & Graven-Nielsen, T. Muscle pain: sensory implications and interaction with motor control. *Clin. J. Pain* **24**, 291–8 (2008).

108. Graven-Nielsen, T. *et al.* In vivo model of muscle pain: Quantification of intramuscular chemical, electrical, and pressure changes associated with saline-induced muscle pain in humans. *Pain* **69**, 137–143 (1997).

109. Schabrun, S. M. & Hodges, P. W. Muscle pain differentially modulates short interval intracortical inhibition and intracortical facilitation in primary motor cortex. *J. Pain* **13**, 187–194 (2012).

110. Allen, D. G. Eccentric muscle damage: Mechanisms of early reduction of force. *Acta Physiol. Scand.* **171**, 311–319 (2001).

111. Hjermstad, M. J. *et al.* Studies comparing numerical rating scales, verbal rating scales, and visual analogue scales for assessment of pain intensity in adults: A systematic literature review. *J. Pain Symptom Manage.* **41**, 1073–1093 (2011).

112. Downie, W. W. *et al.* Studies with pain rating scales. *Ann. Rheum. Dis.* **37**, 378–381 (1978).

113. Bijur, P. E., Latimer, C. T. & Gallagher, E. J. Validation of a verbally administered numerical rating scale of acute pain for use in the emergency department. *Acad. Emerg. Med.* **10**, 390–392 (2003).

114. Salaffi, F., Stancati, A., Silvestri, C. A., Ciapetti, A. & Grassi, W. Minimal clinically important changes in chronic musculoskeletal pain intensity measured on a numerical rating scale. *Eur. J. Pain* **8**, 283–291 (2004).

115. Schabrun, S. M., Burns, E. & Hodges, P. W. New Insight into the Time-Course of Motor and Sensory System Changes in Pain. *PLoS One* **10**, e0142857 (2015).

116. Bantick, S. J. *et al.* Imaging how attention modulates pain in humans using functional MRI. *Brain* **125**, 310–319 (2002).

117. Seminowicz, D. A. & Davis, K. D. Interactions of pain intensity and cognitive load: The brain stays on task. *Cereb. Cortex* **17**, 1412–1422 (2007).

118. Buhle, J. & Wager, T. D. Performance-dependent inhibition of pain by an executive working memory task. *Pain* **149**, 19–26 (2010).

119. Bushnell, M. C., Duncan, G. H., Dubner, R., Jones, R. L. & Maixner, W. Attentional influences on noxious and innocuous cutaneous heat detection in humans and monkeys. *J. Neurosci.* **5**, 1103–1110 (1985).

120. Miron, D., Duncan, G. H. & Bushnell, M. C. Effects of attention on the unpleasantness and intensity of thermal pain. *Pain* **39**, 345–352 (1989).

121. Seminowicz, D. A., Mikulis, D. J. & Davis, K. D. Cognitive modulation of pain-related brain responses depends on behavioral strategy. *Pain* **112**, 48–58 (2004).

122. Petrovic, P., Petersson, K. M., Ghatan, P. H., Stone-Elander, S. & Ingvar, M. Pain-related cerebral activation is altered by a distracting cognitive task. *Pain* **85**, 19–30 (2000).

123. Wiech, K. *et al.* Modulation of pain processing in hyperalgesia by cognitive demand. *Neuroimage* **27**, 59–69 (2005).

124. Bingel, U., Rose, M., Gläscher, J. & Büchel, C. fMRI Reveals How Pain Modulates Visual Object Processing in the Ventral Visual Stream. *Neuron* **55**, 157–167 (2007).

125. Lorenz, J. *et al.* A unique representation of heat allodynia in the human brain. *Neuron* **35**, 383–393 (2002).

126. Coghill, R. C. Individual Differences in the Subjective Experience of Pain: New Insights Into Mechanisms and Models. *Headache J. Head Face Pain* **50**, 1531–1535 (2010).

127. Dworkin, R. H. *et al.* Development and initial validation of an expanded and revised version of the Short-form McGill Pain Questionnaire (SF-MPQ-2). *Pain* **144**, 35–42 (2009).

128. Burns, E., Chipchase, L. S., & Schabrun, S. M., Burns, E., Chipchase, L. S. & Schabrun, S. M. Reduced Short- and Long-Latency Afferent Inhibition Following Acute Muscle Pain: A Potential Role in the Recovery of Motor Output. *Pain Med.* **17**, 1343–1352 (2016).

129. Sullivan, M. J. L., Bishop, S. R. & Pivik, J. The Pain Catastrophizing Scale:

Development and Validation. *Psychol. Assess.* **7**, 524–532 (1995).

130. Spielbeger, C. Manual for the State-Trait Anxiety Inventory. rev. ed. *Consult. Psychol. Press* (1983).

131. Kucyi, A., Salomons, T. V & Davis, K. D. Mind wandering away from pain dynamically engages antinociceptive and default mode brain networks. *Proc. Natl. Acad. Sci.* **110**, 18692 LP – 18697 (2013).

132. Picavet, H. S. J., Vlaeyen, J. W. S. & Schouten, J. S. A. G. Pain catastrophizing and kinesiophobia: Predictors of chronic low back pain. *Am. J. Epidemiol.* **156**, 1028–1034 (2002).

133. Damme, S. Van, Crombez, G., Bijttebier, P., Goubert, L. & Houdenhove, B. Van. A confirmatory factor analysis of the Pain Catastrophizing Scale : invariant factor structure across clinical and non-clinical populations. **96**, 319–324 (2002).

134. Seminowicz, D. A. & Davis, K. D. Cortical responses to pain in healthy individuals depends on pain catastrophizing. *Pain* **120**, 297–306 (2006).

135. Julian, L. J. Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). *Arthritis Care Res.* **63**, 467–472 (2011).

136. Nouwen, A., Cloutier, C., Kappas, A., Warbrick, T. & Sheffield, D. Effects of focusing and distraction on cold pressor-induced pain in chronic back pain patients and control subjects. *J. Pain* **7**, 62–71 (2006).

137. Ruscheweyh, R. *et al.* Validation of the Pain Sensitivity Questionnaire in chronic pain patients. *Pain* **153**, 1210–1218 (2012).

138. Cioffi, I., Michelotti, A., Perrotta, S., Chiodini, P. & Ohrbach, R. Effect of somatosensory amplification and trait anxiety on experimentally induced orthodontic pain. *Eur. J. Oral Sci.* **124**, 127–134 (2016).

139. Tang, J. & Gibson, S. J. A psychophysical evaluation of the relationship between trait anxiety, pain perception, and induced state anxiety. *J. Pain* **6**, 612–619 (2005).

140. Kucyi, A. & Davis, K. D. The dynamic pain connectome. *Trends Neurosci.* **38**, 86–95 (2015).

141. Fox, K. C. R., Spreng, R. N., Ellamil, M., Andrews-Hanna, J. R. & Christoff, K. The wandering brain: Meta-analysis of functional neuroimaging studies of mind-wandering and related spontaneous thought

processes. *Neuroimage* **111**, 611–621 (2015).

142. Neige, C., Massé-Alarie, H. & Mercier, C. Stimulating the Healthy Brain to Investigate Neural Correlates of Motor Preparation: A Systematic Review. *Neural Plast.* **2018**, 1–14 (2018).

143. Brunoni, A. R. & Vanderhasselt, M. A. Working memory improvement with non-invasive brain stimulation of the dorsolateral prefrontal cortex: A systematic review and meta-analysis. *Brain Cogn.* **86**, 1–9 (2014).

144. Vermeij, A., van Beek, A. H. E. A., Olde Rikkert, M. G. M., Claassen, J. A. H. R. & Kessels, R. P. C. Effects of Aging on Cerebral Oxygenation during Working-Memory Performance: A Functional Near-Infrared Spectroscopy Study. *PLoS One* **7**, e46210 (2012).

145. Owen, A. M., McMillan, K. M., Laird, A. R. & Bullmore, E. N-back working memory paradigm: A meta-analysis of normative functional neuroimaging studies. *Hum. Brain Mapp.* **25**, 46–59 (2005).

146. Mulquiney, P. G., Hoy, K. E., Daskalakis, Z. J. & Fitzgerald, P. B. Improving working memory: Exploring the effect of transcranial random noise stimulation and transcranial direct current stimulation on the dorsolateral prefrontal cortex. *Clin. Neurophysiol.* **122**, 2384–2389 (2011).

147. Petrides, M. & Pandya, D. N. Dorsolateral prefrontal cortex: comparative cytoarchitectonic analysis in the human and macaque frontal cortex. *Handb. Neuropsychol. Vol IX* **11**, 17–58 (1999).

148. Seminowicz, D. A. & Moayedi, M. The Dorsolateral Prefrontal Cortex in Acute and Chronic Pain. *J. Pain* **18**, 1027–1035 (2017).

149. Hélie, S., Ell, S. W. & Ashby, F. G. Learning robust cortico-cortical associations with the basal ganglia: An integrative review. *Cortex.* **64C**, 123–135 (2015).

150. Bouffard, J., Bouyer, L. J., Roy, J. S. & Mercier, C. Pain Induced during Both the Acquisition and Retention Phases of Locomotor Adaptation Does Not Interfere with Improvements in Motor Performance. *Neural Plast.* **2016**, (2016).

151. Mavromatis, N., Neige, C., Gagné, M., Reilly, K. T. & Mercier, C. Effect of experimental hand pain on training-induced changes in motor performance and corticospinal excitability. *Brain Sci.* **7**, (2017).

152. Bastani, A. & Jaberzadeh, S. A Higher Number of TMS-Elicited MEP from a Combined Hotspot Improves Intra- and Inter-Session Reliability of the

Upper Limb Muscles in Healthy Individuals. *PLoS One* **7**, (2012).

153. Devanne, H. *et al.* The comparable size and overlapping nature of upper limb distal and proximal muscle representations in the human motor cortex. *Eur. J. Neurosci.* **23**, 2467–2476 (2006).

154. Romaniello, A., Cruccu, G., McMillan, A. S., Arendt-Nielsen, L. & Svensson, P. Effect of experimental pain from trigeminal muscle and skin on motor cortex excitability in humans. *Brain Res.* **882**, 120–127 (2000).

155. Sowman, P. F. *et al.* Asymmetric Activation of Motor Cortex Controlling Human Anterior Digastric Muscles During Speech and Target-Directed Jaw Movements. *J. Neurophysiol.* **102**, 159–166 (2009).

156. Martin, P. G., Weerakkody, N., Gandevia, S. C. & Taylor, J. L. Group III and IV muscle afferents differentially affect the motor cortex and motoneurones in humans. *J. Physiol.* **586**, 1277–1289 (2008).

157. O'Connell, N. E., Marston, L., Spencer, S., Desouza, L. H. & Wand, B. M. Non-invasive brain stimulation techniques for chronic pain. *Cochrane Database Syst. Rev.* **2018**, (2018).

158. Nitsche, M. A. *et al.* Transcranial direct current stimulation: State of the art 2008. *Brain Stimul.* **1**, 206–223 (2008).

159. Fregni, F. *et al.* A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. *Arthritis Rheum.* **54**, 3988–98 (2006).

160. Riberto, M. Efficacy of Transcranial Direct Current Stimulation Coupled with a Multidisciplinary Rehabilitation Program for the Treatment of Fibromyalgia. *Open Rheumatol. J.* **5**, 45–50 (2011).

161. Villamar, M. F. *et al.* Focal Modulation of the Primary Motor Cortex in Fibromyalgia Using 4×1-Ring High-Definition Transcranial Direct Current Stimulation (HD-tDCS): Immediate and Delayed Analgesic Effects of Cathodal and Anodal Stimulation. *J. Pain* **14**, 371–383 (2013).

162. Fagerlund, A. J., Hansen, O. A. & Aslaksen, P. M. Transcranial direct current stimulation as a treatment for patients with fibromyalgia. *Pain* **156**, 62–71 (2015).

163. Donnell, A. *et al.* High-definition and non-invasive brain modulation of pain and motor dysfunction in chronic TMD. *Brain Stimul.* **8**, 1085–1092 (2015).

164. Oliveira, L. B. *et al.* Transcranial direct current stimulation and exercises for

treatment of chronic temporomandibular disorders: A blind randomised-controlled trial. *J. Oral Rehabil.* **42**, 723–732 (2015).

165. Hazime, F. A. *et al.* Treating low back pain with combined cerebral and peripheral electrical stimulation: A randomized, double-blind, factorial clinical trial. *Eur. J. Pain (United Kingdom)* **21**, 1132–1143 (2017).

166. Luedtke, K. *et al.* Effectiveness of transcranial direct current stimulation preceding cognitive behavioural management for chronic low back pain: Sham controlled double blinded randomised controlled trial. *BMJ* **350**, 1–9 (2015).

167. O'Connell, N. E. *et al.* Transcranial Direct Current Stimulation of the Motor Cortex in the Treatment of Chronic Nonspecific Low Back Pain. *Clin. J. Pain* **29**, 26–34 (2013).

168. Kobayashi, M. & Pascual-Leone, A. Transcranial magnetic stimulation in neurology. *Lancet Neurol.* **2**, 145–156 (2003).

169. Fitzgerald, P. B., Fountain, S. & Daskalakis, Z. J. A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. *Clin. Neurophysiol.* **117**, 2584–2596 (2006).

170. Wassermann, E. M. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. **108**, 1–16 (1998).

171. Ziemann, U. *et al.* Consensus: Motor cortex plasticity protocols. *Brain Stimul* **1**, 164–182 (2008).

172. Seminowicz, D. A., de Martino, E., Schabrun, S. M. & Graven-Nielsen, T. Left dorsolateral prefrontal cortex repetitive transcranial magnetic stimulation reduces the development of long-term muscle pain. *Pain* **159**, 2486–2492 (2018).

173. André-Obadia, N. *et al.* Transcranial magnetic stimulation for pain control. Double-blind study of different frequencies against placebo, and correlation with motor cortex stimulation efficacy. *Clin. Neurophysiol.* **117**, 1536–1544 (2006).

174. Andre-Obadia, N., Mertens, P., Gueguen, A., Peyron, R. & Garcia-Larrea, L. Pain relief by rTMS: Differential effect of current flow but no specific action on pain subtypes. *Neurology* **71**, 833–840 (2008).

175. Lefaucheur, J. P. *et al.* Neurogenic pain relief by repetitive transcranial

magnetic cortical stimulation depends on the origin and the site of pain. *J. Neurol. Neurosurg. Psychiatry* **75**, 612–616 (2004).

176. Lefaucheur, J.-P., Drouot, X., Menard-Lefaucheur, I., Keravel, Y. & Nguyen, J.-P. Motor cortex rTMS in chronic neuropathic pain: Pain relief is associated with thermal sensory perception improvement. *J. Neurol. Neurosurg. Psychiatry* **79**, 1044–1049 (2008).

177. Mhalla, A. *et al.* Long-term maintenance of the analgesic effects of transcranial magnetic stimulation in fibromyalgia. *Pain* **152**, 1478–1485 (2011).

178. Passard, A. *et al.* Effects of unilateral repetitive transcranial magnetic stimulation of the motor cortex on chronic widespread pain in fibromyalgia. *Brain* **130**, 2661–2670 (2007).

179. Lima, M. C. & Fregni, F. Motor cortex stimulation for chronic pain: Systematic review and meta-analysis of the literature. *Neurology* **70**, 2329–2337 (2008).

180. Tsao, H., Galea, M. P. & Hodges, P. W. Concurrent excitation of the opposite motor cortex during transcranial magnetic stimulation to activate the abdominal muscles. *J. Neurosci. Methods* **171**, 132–139 (2008).

181. Rosenkranz, K., Kacar, A. & Rothwell, J. C. Differential Modulation of Motor Cortical Plasticity and Excitability in Early and Late Phases of Human Motor Learning. *J. Neurosci.* **27**, 12058–12066 (2007).

182. Jensen, J. L., Marstrand, P. C. D. & Nielsen, J. B. Motor skill training and strength training are associated with different plastic changes in the central nervous system. *J. Appl. Physiol.* **99**, 1558–1568 (2005).

183. Perez, M. A., Lungholt, B. K. S., Nyborg, K. & Nielsen, J. B. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. *Exp. Brain Res.* **159**, 197–205 (2004).

184. Chong, B. W. X. & Stinear, C. M. Modulation of motor cortex inhibition during motor imagery. *J. Neurophysiol.* **117**, 1776–1784 (2017).

185. Bruno, V., Fossataro, C. & Garbarini, F. Inhibition or facilitation? Modulation of corticospinal excitability during motor imagery. *Neuropsychologia* **111**, 360–368 (2018).

186. Jeannerod, M. Neural simulation of action: A unifying mechanism for motor cognition. *Neuroimage* **14**, 103–109 (2001).

187. Berends, H. I., Wolkorte, R., Ijzerman, M. J. & Van Putten, M. J. A. M. Differential cortical activation during observation and observation-and-imagination. *Exp. Brain Res.* **229**, 337–345 (2013).

188. Vogt, S. & Thomaschke, R. From visuo-motor interactions to imitation learning: Behavioural and brain imaging studies. *J. Sports Sci.* **25**, 497–517 (2007).

189. Munzert, J., Lorey, B. & Zentgraf, K. Cognitive motor processes: The role of motor imagery in the study of motor representations. *Brain Res. Rev.* **60**, 306–326 (2009).

190. van de Ruit, M. & Grey, M. J. The TMS Motor Map Does Not Change Following a Single Session of Mirror Training Either with Or without Motor Imagery. *Front. Hum. Neurosci.* **11**, 1–12 (2017).

191. Villiger, M. *et al*. Enhanced Activation of Motor Execution Networks Using Action Observation Combined with Imagination of Lower Limb Movements. *PLoS One* **8**, (2013).

192. Filimon, F., Nelson, J. D., Hagler, D. J. & Sereno, M. I. Human cortical representations for reaching: Mirror neurons for execution, observation, and imagery. *Neuroimage* **37**, 1315–1328 (2007).

193. Gallese, V., Fadiga, L., Fogassi, L. & Rizzolatti, G. Action recognition in the premotor cortex. *Brain* **119**, 593–609 (1996).

194. Rizzolatti, G., Fadiga, L., Gallese, V. & Fogassi, L. Premotor cortex and the recognition of motor actions. *Cogn. Brain Res.* **3**, 131–141 (1996).

195. Taube, W., Lorch, M., Zeiter, S. & Keller, M. Non-physical practice improves task performance in an unstable, perturbed environment: motor imagery and observational balance training. *Front. Hum. Neurosci.* **8**, 1–10 (2014).

196. Gatti, R. *et al*. Action observation versus motor imagery in learning a complex motor task: A short review of literature and a kinematics study. *Neurosci. Lett.* **540**, 37–42 (2013).

197. Stefan, K. *et al*. Formation of a motor memory by action observation. *J Neurosci* **25**, 9339–9346 (2005).

198. Wright, D. J., McCormick, S. A., Williams, J. & Holmes, P. S. Viewing Instructions Accompanying Action Observation Modulate Corticospinal Excitability. *Front. Hum. Neurosci.* **10**, 1–10 (2016).

199. Saruco, E. *et al.* Motor imagery ability of patients with lower-limb amputation : Exploring the course of rehabilitation effects. (2017). doi:10.23736/S1973-9087.17.04776-1

200. Huang, Y. Z. *et al.* The effect of continuous theta burst stimulation over premotor cortex on circuits in primary motor cortex and spinal cord. *Clin. Neurophysiol.* **120**, 796–801 (2009).

201. Chouinard, P. A. & Paus, T. The Primary Motor and Premotor Areas of the Human Cerebral Cortex. *Neurosci.* **12**, 143–152 (2006).

202. Ferbert, A. *et al.* Interhemispheric inhibition of the human motor cortex. *J. Physiol.* **453**, 525–546 (1992).

203. Ziemann, U. & Hallett, M. Hemispheric asymmetry of ipsilateral motor cortex activation during unimanual motor tasks: Further evidence for motor dominance. *Clin. Neurophysiol.* **112**, 107–113 (2001).

204. Mochizuki, H., Huang, Y.-Z. & Rothwell, J. C. Interhemispheric interaction between human dorsal premotor and contralateral primary motor cortex. *J. Physiol.* **561**, 331–338 (2004).

205. Mink, J. W. The basal ganglia: Focused selection and inhibition of competing motor programs. *Prog. Neurobiol.* **50**, 381–425 (1996).

206. Fadiga, L., Craighero, L. & Olivier, E. Human motor cortex excitability during the perception of others' action. *Curr. Opin. Neurobiol.* **15**, 213–218 (2005).

207. Patuzzo, S., Fiaschi, A. & Manganotti, P. Modulation of motor cortex excitability in the left hemisphere during action observation: A single- and paired-pulse transcranial magnetic stimulation study of self- and non-self-action observation. *Neuropsychologia* **41**, 1272–1278 (2003).

208. Fadiga, L. & Craighero, L. Electrophysiology of Action Representation Motor Properties of Monkey Ventral Premotor. *J. Clin. Neurophysiol.* **21**, 157–169 (2004).

209. Baldissera, F. *et al.* Excitability changes occurring in the human spinal cord while looking at video recorded hand movements. *Pflugers Arch. J. Physiol.* (1998).

210. Beaumont, G., Mercier, C., Michon, P. E., Malouin, F. & Jackson, P. L. Decreasing Phantom Limb Pain Through Observation of Action and Imagery: A Case Series. *Pain Med.* **12**, 289–299 (2011).

211. MacIver, K., Lloyd, D. M., Kelly, S., Roberts, N. & Nurmikko, T. Phantom limb pain, cortical reorganization and the therapeutic effect of mental imagery. *Brain* **131**, 2181–2191 (2008).

212. Beinert, K., Preiss, S., Huber, M. & Taube, W. Cervical joint position sense in neck pain. Immediate effects of muscle vibration versus mental training interventions: a {RCT.}. *Eur J Phys Rehabil Med* **51**, 825–832 (2015).

213. Volz, M. S. *et al.* Movement observation-induced modulation of pain perception and motor cortex excitability. *Clin. Neurophysiol.* **126**, 1204–1211 (2015).

214. Ridding, M. C. & Ziemann, U. Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J. Physiol.* **588**, 2291–2304 (2010).

215. Sale, M. V., Ridding, M. C. & Nordstrom, M. A. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. *Exp. Brain Res.* **181**, 615–626 (2007).

216. Brunner, I. C., Skouen, J. S., Ersland, L. & Grüner, R. Plasticity and response to action observation: A longitudinal fMRI study of potential mirror neurons in patients with subacute stroke. *Neurorehabil. Neural Repair* **28**, 874–884 (2014).

217. Takeuchi, N. & Izumi, S. I. Rehabilitation with poststroke motor recovery: A review with a focus on neural plasticity. *Stroke Res. Treat.* **2013**, (2013).

218. Thieme, H., Morkisch, N., Rietz, C., Dohle, C. & Borgetto, B. The efficacy of movement representation techniques for treatment of limb pain - A systematic review and meta-analysis. *J. Pain* **17**, 167–180 (2016).

219. Ertelt, D. *et al.* Action observation has a positive impact on rehabilitation of motor deficits after stroke. *Neuroimage* **36**, 164–173 (2007).

220. Franceschini, M. *et al.* Clinical relevance of action observation in upper-limb stroke rehabilitation: A possible role in recovery of functional dexterity. A randomized clinical trial. *Neurorehabil. Neural Repair* **26**, 456–462 (2012).

221. Bassolino, M., Campanella, M., Bove, M., Pozzo, T. & Fadiga, L. Training the motor cortex by observing the actions of others during immobilization. *Cereb. Cortex* **24**, 3268–3276 (2014).

222. Bowering, K. J., Butler, D. S., Fulton, I. J. & Moseley, G. L. Motor imagery in people with a history of back pain, current back pain, both, or neither.

Clin. J. Pain **30**, 1070–1075 (2014).

223. Luedtke, K., May, A. & Jürgens, T. P. No Effect of a Single Session of Transcranial Direct Current Stimulation on Experimentally Induced Pain in Patients with Chronic Low Back Pain - An Exploratory Study. *PLoS One* **7**, (2012).

224. Christiansen, L., Larsen, M. N., Grey, M. J., Nielsen, J. B. & Lundbye-Jensen, J. Long-term progressive motor skill training enhances corticospinal excitability for the ipsilateral hemisphere and motor performance of the untrained hand. *Eur. J. Neurosci.* **45**, 1490–1500 (2017).

APPENDICES

Appendix A. Overview of studies investigating the effects of acute pain of different modalities on motor-evoked potentials of the upper limbs

Authors	Year	Pain model	Main findings
Valeriani et al.	1999	Phasic heat stimulation by CO_2 laser stimulation to the right hand dorsum	↓ MEPs of the right FDI muscle evoked by TMS
Romaniello et al.*	2000	Hypertonic saline infusion into the left masseter muscle Capsaicin cream applied to the skin of the left cheek	Neither muscle or skin pain affected MEPs of the masseter muscle
Le Pera et al.	2001	Hypertonic saline infusion into the right ADM, right FDI, left ADM, or subcutaneous injection into the region around the right ADM	↓ MEPs of the right ADM muscle evoked by TMS during right ADM or FDI pain No change in MEPs of the right ADM muscle evoked by TMS during left ADM pain or right subcutaneous ADM injection ↓ H-reflex 1 minute after peak-pain
Valeriani et al.	2001	Phasic heat stimulation by CO_2 laser stimulation to the right hand dorsum or lateral surface of the right arm	↓ MEPs of the biceps brachii muscle evoked by TMS
Farina et al.	2001	Capsaicin cream on the skin overlying the right FDI and FCR	↓ MEPs of the right FDI and FCR muscle evoked by TMS H/M ratio for the FCR muscle did not change suggesting a cortical mechanism
Svensson et al.	2003	Hypertonic saline injection into the FDI muscle Painful electrical stimulation of the FDI muscle	↓ MEPs of the right FDI muscle evoked by TMS

Del Santo et al.*	2007	Ascorbic acid injection in the ADM and BIC muscles during constant force	↑ MEPs of the right ADM and BIC muscles evoked by TMS
Boudreau et al.*	2007	Capsaicin cream application to the tongue	MEPs of the tongue increased in vehicle session but not in the pain session
Martin et al.	2008	Hypertonic saline infusion into the biceps brachii muscle	No change in MEPs, but relative to CMEPs, they ↓ ↑ CMEPs (corticospinal tract measure) of the biceps brachii and triceps Hypertonic saline infusion facilitated motoneurones innervating elbow flexor and extensor muscles, but depressed corticomotor cells projecting to the muscles
Schabrun et al.*	2012	Hypertonic saline infusion into the right FDI	↓ MEPs of the FDI and ADM muscles at pain-resolve compared to baseline
Schabrun et al.*	2013	Hypertonic saline infusion into the right FDI	↓ MEPs of the right FDI at pain-resolve compared to pre-pain
Rittig-Rasmussen et al.*	2014	Hypertonic saline infusion in the right side of the neck (2 cm lateral to the spinous process of the third cervical vertebra)	↓ MEPs of the right trapezius muscle 30 mins, 1 hour, and 7 days after infusion ↑ MEPs of the right trapezius muscle after training
Schabrun et al.*	2016	Hypertonic saline infusion four days after NGF-injection	↑ MEPs of the right ECRB
Schabrun et al.*	2017	Injection of hypertonic saline into the right ECRB	↓ MEPs of the right ECRB
Mavromatis et al.*	2017	Capsaicin cream applied to the lateral border of the first metacarpal prior to performing a motor task	During training, the control group increased in corticospinal excitability, whereas the pain group did not
Martel et al.	2017	Capsaicin cream application on the middle volar part of the left forearm (4x4 cm)	MEPs of the left FDI remained unaffected by the capsaicin-induced pain

Larsen et al.	2018	Injection of hypertonic saline into the right ECR or FDI muscle	↓ MEPs of the right FDI muscle when hypertonic saline was injected into the FDI muscle No change in MEPs for the ECR muscle neither during FDI injection nor ECR injection

* **Included other outcomes that were not accounted for such (e.g. motor task performance, pre-contraction, constant force production, or SICI/ICF measures)**

Appendix B. Examples of the effects of the effects of action-observation and motor imagery on motor-evoked potentials of the upper limb

Authors	Year	Action observation (AO) or motor imagery (MI)	Main findings
Fadiga et al.	1995	AO	↑ MEPs of hand muscles during observation
Strafella & Paus	2000	AO	↑ MEPs of FDI and BIC during hand writing and arm movement, respectively – specificity
Patuzzo et al.	2003	AO & MI	↑ MEPs of FDS during AO and MI, ↓ ICI, H-reflex and F-waves remained unaffected
Stefan et al.	2005	AO	↑ MEPs of the EPB and FPB during physical practice but not AO Affected thumb movement towards the direction of the observed movement
Stinear et al.	2006	MI	↑ MEPs of the APB during kinesthetic MI but not visual MI (thumb movement) – specific to the involved muscle
Stinear et al.	2006	MI	↑ MEPs of the APB during MI of right and both hands No effect of MI on F-waves
Sakamoto et al.	2009	AO+MI	↑ MEPs of the BB during observation of elbow movements was higher for AOMI than MI and AO alone

Caspers et al.	2010	AO & MI	Meta-analysis of AO and MI and activation of specific brain areas
Bufalari et al.	2010	MI	↑ MEPs of the right FDI independent of the biomechanical possibility of performing the action
Ohno et al.	2011	AO+MI	Combining AO and MI ↑ MEPs of the FDI and thenar muscles compared to AO and control
Vogt et al.	2013	AO+MI	Seminal paper on the integration of MI during AO
Wright et al.	2014	AO+MI	↑ MEPs of the right FDI – AOMI produced stronger facilitation than control conditions and passive observation but not MI
Wright et al.	2016	AO+MI	↑ MEPs of the right FDI , OP, and ADM higher during AOMI than passive observation and static alone – no difference to observation with intention to imitate
Chong et al.	2017	MI	↑ MEPs of the right APB during imagery compared to voluntary contraction and rest – GABA-mediated inhibition and disinhibition
Bruno et al.	2018	MI	↑ MEPs of the FDI increased when asked to imagine finger-thumb opposition – when explicitly asked to avoid unwanted finger movements, ↓ MEPs of the FDI was demonstrated

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