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# An investigation of cortical inhibition in acute and chronic pain

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I dedicate this thesis to my daughters.

Little girls can achieve anything xx

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The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Emma Burns

# Publications, abstracts and presentations arising from this thesis

## *Peer-reviewed journal articles*

**Burns E**, Chipchase LS, Schabrun SM (2017). Temporal and spatial characteristics of post silent period electromyographic bursting in low back muscles: comparisons between persons with and without low back pain. International Journal of Neuroscience. 1-23.

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**Burns E**, Chipchase LS, Schabrun SM (2016). Altered function of intracortical networks in chronic lateral epicondylalgia. European Journal of Pain. 20(7): 1166-75.

## *Conference presentations*

**Burns E**, Chipchase LS, Schabrun SM (2015). Short- and long-latency afferent inhibition are reduced following acute muscle pain. Australasian Neuroscience Society (ANS) Sensorimotor Control Satellite Meeting. Brisbane 20th-21st February.

**Jones E** (2014). Novel cortical therapies for acute pain. School of Science and Health with the School Medicine: Mid-year Higher-Degree Research Symposium. Western Sydney University, Kensington 5-6th June.

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## Other related publications and abstracts

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Schabrun SM, **Burns E**, Hodges PW (2015). New insight into the time-course of motor and sensory system changes in pain. PLoS One. 10(11): e0142857

Schabrun SM, Hodges PW, Vicenzino B, **Jones E**, Chipchase LS (2014). Novel adaptations in motor cortical maps: the relation to persistent elbow pain. Medicine & Science in Sports & Exercise. 47(4): 681-90.

Schabrun SM, **Jones E**, Elgueta Cancino EL, Hodges PW (2014). Targeting chronic recurrent low back pain from the top-down and the bottom-up: a combined transcranial direct current stimulation and peripheral electrical stimulation intervention. Brain Stimulation. 7(3): 451-9.

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### *Conference abstracts*

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Schabrun SM, **Jones E**, El Gueta Cancino E, Hodges PW (2013). Combined back and brain stimulation improves pain and function in chronic low back pain. Australian Physiotherapy Association Conference. Melbourne, Australia 17-20th October.

Schabrun SM, **Jones E**, El Gueta Cancino E, Hodges PW (2013). Understanding the brain in the transition from acute to chronic low back pain. Australian Physiotherapy Association Conference. Melbourne, Australia 17-20th October.

## Authors note

This thesis has been prepared in the format of 'Thesis by Publication'. The content of each publication has been preserved. However, minor editorial changes have been made to maintain standard formatting throughout this document. References are presented as a continuous list at the end of the thesis to minimise repetition and improve readability. A copy of each publication in original format is provided in the Appendices (Appendix A-D).

# Table of contents

## Chapter 5: Temporal and spatial characteristics of post silent period electromyographic bursting in low back muscles: comparison between persons with and without low back pain

## Chapter 6: General discussion



## References



## Appendices

# List of Tables

## Chapter 1



## Chapter 2



## Chapter 4



## Chapter 5

# List of Figures

## Chapter 1



## Chapter 2



## Chapter 3

## Chapter 4



## Chapter 5



## Chapter 6

# List of Appendix Tables

# List of Appendix Figures

## Abbreviations

| | |
|---|---|
| ACh | acetylcholine |
| AMT | active motor threshold |
| ASL | arterial spin labelling |
| BOLD | blood-oxygen-level dependent |
| CBF | cerebral blood flow |
| CMO | corticomotor output |
| CoG | centre of gravity |
| CS | conditioning stimulus |
| ECRB | extensor carpi radialis brevis |
| ED | extensor digitorum |
| EDB | extensor digitorum brevis |
| EEG | electroencephalography |
| EMG | electromyography |
| FDI | first dorsal interosseous |
| fMRI | functional magnetic resonance imaging |
| GABA | gamma-aminobutyric acid |
| ICF | intracortical facilitation |
| IO | internal obliques |
| ISI | inter stimulus interval |
| LAI | long latency afferent inhibition |

| | |
|---|---|
| LBP | low back pain |
| LE | lateral epicondylalgia |
| LES | longissimus erector spinae |
| LICI | long interval intracortical inhibition |
| M1 | primary motor cortex |
| MEP | motor evoked potential |
| MVC | maximum voluntary contraction |
| NGF | nerve growth factor |
| NRS | numerical rating scale |
| PBR | percentage burst ratio |
| PET | positron emission tomography |
| PNS | peripheral nerve stimulation |
| PPT | pressure pain threshold |
| PRTEE | patient rated tennis elbow evaluation |
| rCBF | regional cerebral blood flow |
| RF | rectus femoris |
| RMS | root mean square |
| RMT | resting motor threshold |
| rTMS | repetitive transcranial magnetic stimulation |
| S1 | primary somatosensory cortex |
| SAI | short latency afferent inhibition |
| SEP | somatosensory evoked potential |

| | |
|---|---|
| sICF | short interval intracortical facilitation |
| SICI | short interval intracortical inhibition |
| SMD | standardized mean differences |
| TA | tibialis anterior |
| tDCS | transcranial direct current stimulation |
| TMS | transcranial magnetic stimulation |
| TrA | transverse abdominus |
| TS | test stimulus |
| VAS | visual analogue scale |
| VL | vastus lateralis |
| VM | vastus medialis |
| VMO | vastus medialis oblique |

## Abstract

Musculoskeletal disorders are a leading cause of disease burden worldwide. Alarmingly, the prevalence and socioeconomic impact of these conditions is rising. Pain is a common and disabling feature of many musculoskeletal disorders. While significant individual burden is evident at all stages of pain, socioeconomic costs increase dramatically when pain becomes chronic (> 3 months). Therapies that reduce the duration and severity of chronic pain are needed to lessen the individual and socioeconomic burden of these conditions. However, current interventions achieve limited success. The lack of effective therapies is hypothesised to be caused, in part, by a limited understanding of the neurophysiology of chronic musculoskeletal pain and the mechanisms driving the transition to chronicity.

Recent advances in technology provide evidence of structural and functional changes in brain regions, including the primary motor cortex (M1), in response to musculoskeletal pain. Lasting changes in the excitability, topography and organisation of M1 have been hypothesized to underpin symptom chronicity in musculoskeletal disorders, however the mechanisms underlying these changes remain unclear. Inhibitory intracortical networks modulate the excitability, organisation and output of extrinsically projecting M1 neurons, and are well positioned to influence the cortical response to pain. Yet, the effect of acute and chronic muscle pain on inhibitory intracortical networks has not been fully elucidated. Thus, the overall aim of this thesis was to contribute to the body of knowledge on the intracortical response to musculoskeletal pain using i) acute

experimental pain models in healthy individuals and ii) clinical populations living with chronic pain.

This aim was addressed via one secondary and three primary research studies. First, a systematic review and meta-analyses was performed to establish the effect of experimental muscle pain on the primary motor (M1) and somatosensory cortices (S1). Following a detailed and reproducible systematic search of the literature, 25 studies (15 neuroimaging and 10 electrophysiological studies) were included. Systematic evaluation of functional magnetic resonance imaging (fMRI) data revealed consistent evidence of increased S1 activation in response to acute muscle pain. As meta-analyses of electrophysiological recordings at S1 (n = 3) revealed strong evidence of reduced intracortical processing during pain [1.99 (0.64, 3.34)] and moderate evidence following the resolution of pain [0.65 (0.15, 1.16)], increased activity in cortical networks with inhibitory functionality may underpin increased S1 activation. The systematic review also identified evidence of increased M1 activation using fMRI during muscle pain. However, this effect was not consistent across studies. These inconsistencies may relate to the temporal profile of the M1 response since meta-analyses of transcranial magnetic stimulation data (TMS; n = 10) revealed strong evidence of reduced M1 excitability post pain [0.97 (0.59, 1.35)], and moderate evidence during pain [0.52 (-0.01, 1.06)]. Evidence of reduced intracortical facilitation and increased intracortical inhibition was also noted in M1. However, this finding was derived from a single study, such that no definitive conclusion could be made as to whether these mechanisms underpin the M1 response to pain. Thus,

three primary research studies were performed to further evaluate the effect of muscle pain on intracortical activity.

In studies 2, 3 and 4, TMS was used to evaluate forms of intracortical inhibition previously unexplored in: healthy persons with acute experimental muscle pain (study 2) and persons with specific chronic musculoskeletal pain conditions (study 3 and 4). The results of study 2 confirmed the findings of study 1, while also providing the first description of the temporal effect of muscle pain on intracortical networks engaged in sensorimotor integration. Consistent with the findings of the systematic review, corticomotor output (M1 excitability) was reduced during ($p$ = 0.001) and following an episode of acute experimental muscle pain ($p$ = 0.003). Concurrent measurements of intracortical inhibition in these participants revealed that this effect was accompanied by reduced short- and long latency afferent inhibition in the post pain period ($p$ = 0.039, $p$ = 0.035). These mechanisms reflect the integration of somatosensory afferent input at M1, thus, this response may reflect altered intracortical activity within S1, M1, or both regions. As an additional form of inhibition, long interval intracortical inhibition, was unaffected by acute muscle pain, this study also provides evidence of a differential effect of acute muscle pain on distinct inhibitory intracortical networks.

Study 3 provides the first description of intracortical activity in persons experiencing chronic musculoskeletal pain due to lateral epicondylalgia (LE, 'tennis elbow'). Participants with LE (n = 14) displayed reduced short interval intracortical inhibition ($p$ = 0.005), long interval intracortical inhibition ($p$ = 0.046) and intracortical

facilitation ($p$ = 0.026), compared to pain-free controls. There was no direct relationship between reduced intracortical activity and pain or disability in LE (p > 0.16). These findings support previous observations of reduced intracortical inhibition in other musculoskeletal conditions and suggest a shift towards cortical disinhibition in response to chronic pain.

Study 4 provides the first description of the depth and magnitude of cortical disinhibition in chronic musculoskeletal pain. The temporal and spatial profile of post-silent period electromyographic (EMG) bursting in persons with low back pain (LBP; n = 11) was significantly different to that observed in pain-free controls. In these individuals, EMG bursts were smaller ($p$ = 0.050) and occurred earlier ($p$ = 0.009) implying reduced corticomotor disinhibition in this condition. Similar to the findings of study 3, no direct relationship was found between burst characteristics and pain severity ($p$ > 0.24).

The findings from the four studies conducted in this thesis provide novel insight into the intracortical response to acute and chronic musculoskeletal pain. As each of the mechanisms investigated are thought to be mediated by distinct neuronal populations, these findings suggest that the intracortical response to musculoskeletal pain is extensive and diverse. Observations of opposing changes in short- and long interval intracortical inhibition in the acute (increased inhibition) and chronic (decreased inhibition) stages of pain suggest that these forms of inhibition could play a role in the transition to chronicity. However, study 3 and 4 did not detect a correlation between intracortical inhibition and measures of pain and disability in LE

and LBP, respectively. While this suggest that changes in intracortical activity may not contribute directly to symptoms of chronic pain and motor dysfunction, there is the possibility of a non-linear relationship between these variables.

When the findings are taken together, changes in intracortical inhibition in pain suggest the enactment of a cortically driven motor strategy to protect painful tissues from further insult. While beneficial in the short term when the need to protect the tissues is high, failure to return to normal intracortical function in the long-term could lead to pain persistence through altered tissue loading. Large, longitudinal trials examining the transition from acute to chronic pain are necessary to confirm these hypotheses. If confirmed, future therapies which target maladaptive intracortical change could lead to greater improvements in pain and function for individuals experiencing chronic musculoskeletal pain.

## Thesis overview

Chronic musculoskeletal pain is a leading cause of disease burden worldwide (Vos et al. 2012). Despite the enormity of the problem, most therapies have moderate effect sizes at best (Moulin 2001, Nielson et al. 2001, Menke 2014). This is not surprising given that many treatments, such as pharmacotherapy, target generic symptoms not underlying mechanisms (Moulin 2001). An area of increasing research and therapeutic interest is the effect that pain, both acute and chronic, has on the brain. Transient and lasting changes in cortical organisation and function have been documented in the primary motor cortex (M1) using methodologies including functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS). This body of work has led to the suggestion that structural and functional changes within M1 contribute to symptom chronicity in musculoskeletal pain conditions (Wand et al. 2011, Moseley et al. 2012). However, while observations of altered M1 excitability, topography and organisation have been demonstrated, the physiological processes driving these effects remain unclear (Tsao et al. 2008, Tsao et al. 2011b, Schabrun et al. 2014, Schabrun et al. 2015a, Schabrun et al. 2015b, Te et al. 2017). As inhibitory interneuronal activity is a key determinant of cortical plasticity (Liepert et al. 1998a, Murakami et al. 2012), it is possible that evaluation of intracortical networks could provide valuable insight into the mechanisms underpinning short and long-term M1 adaptation to musculoskeletal pain. Thus, the overarching objective of this thesis was to explore the effect of musculoskeletal pain on intracortical networks acting within M1.

To answer this objective, one secondary research study and three primary research studies were conducted. Specific study aims and hypotheses were as follows:

Study 1

**Aim:** To examine current evidence regarding the effect (direction, strength, duration) of acute experimental muscle pain on the excitability of the primary motor (M1) and primary somatosensory cortices (S1).

**Hypothesis:** That M1 and S1 excitability is reduced during and following the resolution of, acute muscle pain.

Study 2

**Aim:** To determine the effect of acute experimental muscle pain on corticomotor output, long interval intracortical inhibition, short latency afferent inhibition and long latency afferent inhibition.

**Hypothesis:** That corticomotor output is decreased and intracortical/afferent inhibition are increased in response to acute experimental muscle pain.

Study 3

**Aim:** To investigate short interval intracortical inhibition, long interval intracortical inhibition and intracortical facilitation in persons with and without chronic musculoskeletal pain due to lateral epicondylalgia ('tennis elbow').

**Hypothesis:** That intracortical inhibition is reduced and intracortical facilitation is increased in persons with lateral epicondylalgia compared to pain free controls.

Study 4

**Aim:** To investigate the temporal and spatial characteristics of post silent period electromyographic bursting in persons with and without chronic low back pain.

**Hypothesis:** That post silent period electromyographic bursting is both decreased and widespread in persons with low back pain compared to pain free controls.

Each research study included in this thesis has been published as a stand-alone article in a peer reviewed journal: European Journal of Pain (Study 1 and 3), Pain Medicine (Study 2) and the International Journal of Neuroscience (Study 4). These journal articles are presented (subject to minor editorial changes) in Chapters 2 to 5. The findings from the four studies are then synthesized and discussed '*in toto*' in Chapter 6 to provide an overarching theory on the role of cortical inhibition in musculoskeletal pain. In this final chapter, the limitations, clinical implications and future directions of the research are also discussed.

The next chapter (Chapter 1) establishes a framework for the research undertaken by providing a detailed, focused review of the literature surrounding M1 adaptations in musculoskeletal pain conditions. The contribution of cortical inhibition is discussed and avenues available to study intracortical mechanisms are described. The chapter then concludes with a detailed summary of the research rationale behind each study.

# Chapter 1: General Introduction

This chapter provides an overview of the literature surrounding musculoskeletal pain and cortical inhibition. The chapter focuses on the role of the brain in acute and chronic musculoskeletal pain and the methodologies available to study inhibitory cortical mechanisms in the primary motor cortex (M1). Critical review of specific literature relevant to each research study is provided in the Introduction and Discussion sections of Chapters 2 to 5.

### *1.1 Musculoskeletal pain is a significant health problem*

Pain is a normal physiological response to noxious stimuli. In the period following injury, pain supports the healing process by encouraging rest and recovery. However, pain that persists beyond the normal scope of healing (typically three months or longer) neither protects nor supports this process and may reflect an underlying pathology (Peyron 2013). Persistent or 'chronic' musculoskeletal pain states (i.e. pain arising from muscles, tendons, ligaments, bones, joints and associated tissues) have been estimated to affect 1.3 billion people worldwide (Vos et al. 2016). Chronic low back pain (LBP) is the most prevalent form of musculoskeletal pain and has been the leading cause of disability globally since 1990 (Vos et al. 2012, Vos et al. 2016).

Musculoskeletal pain conditions have, and continue to be, a costly problem for Australia. While significant burden is evident at all stages of pain, many costs associated with these conditions increase dramatically when pain becomes chronic. In 2012, the direct cost of caring for persons with chronic musculoskeletal pain, combined with indirect costs associated with loss of worker participation and productivity, totaled 55.1 billion Australian dollars (Arthritis and Osteoporosis Victoria 2013). Since back complaints remain the eighth most common reason for presenting to a general practitioner and up to 75% of these presentations require ongoing care and management (i.e. are chronic), the cost of care is expected to remain high well into the future (Britt et al. 2015). Alarmingly, epidemiological studies suggest this may be the reality for nearly all populations around the world (Murray et al. 2012, Vos et al. 2016).

Findings from the most recent Global Burden of Disease Study indicate that the global burden of painful musculoskeletal disorders (measured as 'years lived with disability'- a disability weighted measure of prevalence) rose by 18.6-20.5% in the last decade (Vos et al. 2016). The rate of burden also increased over this time. However, this was modest (4.9-6.6%) and suggests that the majority of increase in burden could be explained by an increase in the severity or duration of these conditions, rather than the emergence of a significant number of new cases. Recent data from the Australian Institute of Health and Welfare (AIHW) indicate that the health impact of chronic musculoskeletal pain is considerable. For example, in an AIHW 2016 bulletin, it was noted that a significant proportion of individuals with chronic back problems suffered high levels of disability and poor quality of life due to reductions in mobility, selfcare, employment and social participation (AIHW 2016). Of these individuals, 13% reported their pain as 'severe', and 3.7%, 'very severe'. The level of pain and disability experienced by persons with chronic musculoskeletal conditions is perhaps unsurprising given that many treatments are only moderately efficacious (Moulin 2001, Nielson et al. 2001, Menke 2014). The lack of effective therapies may be caused, in part, by a limited understanding of the neurophysiology of chronic musculoskeletal pain. Prioritization of research into musculoskeletal pain is therefore essential to lessen the burden of these conditions on the individual, community and economy.

## *1.2 Musculoskeletal pain in the laboratory*

### 1.2.1 Acute muscle pain

Research into the impact of acute musculoskeletal pain on cortical plasticity in clinical populations is challenging due to the narrow window in which participants must be identified and recruited. Additionally, in these situations there is likely to be a lack of baseline data prior to the pain incident. This has led researchers to develop a number of experimental models to study muscle pain in otherwise pain-free volunteers. Endogenous and exogenous methods of inducing acute muscle pain are detailed in Table 1.1. Endogenous methods based on ischemia or exercise are suitable for studying general pain states as these interventions typically induce pain in entire muscle groups or body segments, whereas exogenous methods based on thermal, electrical or chemical stimulation are more appropriate for investigations of local and/or referred pains (Arendt-Nielsen et al. 2001).

Experimental pain models have advantages and disadvantages. An advantage shared by all is that they permit investigation of the temporal effects of acute pain in a controlled environment, without the threat of lasting tissue damage and independent of comorbidities which often accompany clinical pain (Graven-Nielsen 2006). However, a common disadvantage is their lack of specificity. For example, although mechanical models have a large array of research applications (e.g. assessment of hyperalgesia, pain thresholds and pain tolerance) and can be used to target individual muscles, in actuality this methodology is not muscle specific as skin and deeper structures are also impacted upon by mechanical pressure. Furthermore,

models which do demonstrate tissue specificity do not always demonstrate nociceptive specificity. This is best evidenced by chemical models of acute pain, which, despite being considered muscle specific, have been shown to activate both low (non-nociceptive) and high threshold (nociceptive) mechanosensitive group IV afferents (Cairns et al. 2003, Hoheisel et al. 2004).

***Table 1.1*** *Experimental models of acute musculoskeletal pain.*

| MODE | METHODOLOGY | STIMULUS DURATION | TISSUE SPECIFIC? | NOCICEPTOR SPECIFIC? | PAIN DISTRIBUTION | PROPOSED MECHANISM OF ACTION | TECHNICAL REFERENCES |
|---|---|---|---|---|---|---|---|
| **Ischemic** | Tourniquet is applied to limb to restrict blood flow at rest or during volitional contraction | ≤2 hours | No | No | Diffuse | Restricted blood flow leads to the accumulation of endogenous substances which activate nociceptive afferents | Mills et al. (1982), Graven-Nielsen et al. (2003), Segerdahl et al. (2004) |
| **Exercise** | Participant performs concentric muscle contractions usually to fatigue | Minutes | No | No | Diffuse | Restricted blood flow leads to the accumulation of endogenous substances which activate nociceptive afferents | Vecchiet et al. (1983), Cook et al. (1997), O'Connor et al. (2001) |
| **Mechanical** | A hand-held or automated pressure algometer is applied over muscle tissue | Seconds - minutes | No | No | Local | Downward pressure activates group III and IV nociceptive afferents | Kosek et al. (1999), Schubert et al. (2004), Finocchietti et al. (2011) |
| **Thermal** | Intramuscular injection of heated (48°C) or cooled (8°C) isotonic saline | Bolus | Yes | Yes | Local | Activation of heat-sensitive nociceptors | (Graven-Nielsen et al. 2002a) |
| **Electrical** | Current delivered to muscle tissue via needle electrodes with uninsulated tips | Milliseconds | Yes | No | Local and referred | Intensities corresponding to pain threshold activate group III nociceptive afferents | Svensson et al. (1997b, 1997a), Niddam et al. (2001) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Chemical** | Intramuscular infusion/injection of hypertonic saline | Bolus - 30 minutes | Yes | No | Local and referred | Increased intramuscular sodium activates group III and IV nociceptive afferents | Jensen et al. (1992), Graven-Nielsen et al. (1997a), Coppieters et al. (2006) |
| | Intramuscular injection of glutamate | Bolus | Yes | No | Local and referred | Activation of peripheral NMDA receptors on group III and IV afferents | Cairns et al. (2003), Svensson et al. (2003b) |
| | Intramuscular injection/infusion of capsaicin | Bolus - 13 minutes | Yes | No | Local and referred | Activation of vanilloid receptor 1 on group III and IV afferents | Arima et al. (2000), Witting et al. (2000) |

The most common method of inducing acute muscle pain is via hypertonic saline (Graven-Nielsen 2006). This model induces pain of clinical quality (deep, sharp ache, local and referred) by artificially raising intramuscular sodium to a concentration capable of triggering nociceptive afferents in muscle tissue (Graven-Nielsen et al. 1997c). Two variations of the model exist, the original model consisting of a manual bolus dosage, and the contemporary model, a prolonged computer-controlled infusion (Graven-Nielsen et al. 1997a, Coppieters et al. 2006). Compared to bolus delivery, infusion protocols can induce pain of consistent intensity and predictable duration (Figure 1.1). As these variables can be carefully managed by altering the rate, volume and timespan of infusion (Graven-Nielsen et al. 1997a), this technique is particularly advantageous for experiments featuring lengthy protocols.

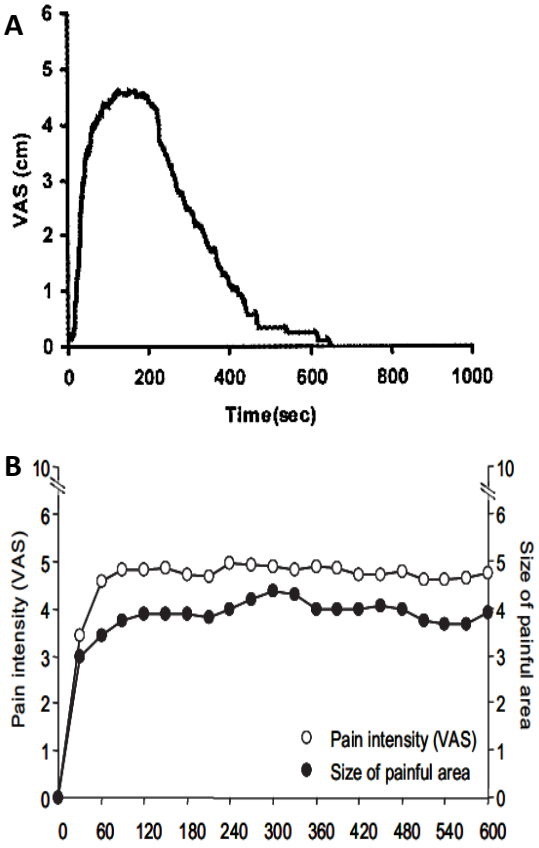


***Figure 1.1*** *Hypertonic saline induced muscle pain. Typical muscle pain intensity profiles after A) bolus injection of 5% hypertonic saline (0.2ml over 20s) and B) continuous infusion of 5% hypertonic saline (0.2ml over 20s, then 6-9mL/hr).*

*A) Reprinted from Clinical Neurophysiology, 112 /9, Domenica Le Pera, Thomas Graven-Nielsen, Massimiliano Valeriani, Antonio Oliviero, Vincenzo Di Lazzaro, Pietro Attilio Tonali, Lars Arendt-Nielsen, Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain, 1633-1641, Copyright (2001), with permission from Elsevier*

*B) Reprinted from Archives of Physical Medicine and Rehabilitation, 87 /10, Michel W. Coppieters, Ali M. Alshami, Paul W. Hodges, An experimental pain model to investigate the specificity of the neurodynamic test for the median verve in the differential diagnosis of hand symptoms, 1412-1417, Copyright (2006), with permission from Elsevier*

The typical experimental set up for infusion involves sterile preparation of 4-6% saline solution. Concentrations within this range are most common as they show no evidence of in vitro or in vivo toxicity and have been demonstrated to be safe for human use (Graven-Nielsen et al. 1997b, Graven-Nielsen et al. 1997a, Graven-Nielsen et al. 1997c, Svendsen et al. 2005). Once loaded into a syringe, the solution is delivered via a computer controlled syringe pump through a small gauge stainless steel needle inserted into muscle tissue at a volume/rate appropriate for the experimental conditions (e.g. 6ml/hour for 10 minutes) (Schabrun et al. 2012). Additions to this protocol can include anaesthetization of cutaneous tissue surrounding the injection site (to negate any local skin pain arising from needle insertion) or a small (0.2ml) bolus injection prior to infusion (to accelerate pain onset)(Graven-Nielsen et al. 1997a, Coppieters et al. 2006). Although infusion parameters can be varied to accommodate different study designs, it is rare for infusion time to extend beyond 30 minutes (Graven-Nielsen 2006).

### 1.2.2 Transitional muscle pain

Until recently, avenues for studying acute muscle pain beyond the 30-minute timeframe were limited to models of ischemia-induced pain which are neither tissue specific, long-lasting or well understood (Graven-Nielsen 2006). However, there is now increasing use of Recombinant Human β-nerve Growth Factor (NGF), a neuropeptide involved in the development and reconstruction of nerves, as a proxy for studying muscle pain of moderate duration (i.e. between the acute and chronic stages) (Schabrun et al. 2016). Unlike hypertonic saline, intramuscular injection of NGF does not result in spontaneous pain, but rather a progressively developing

muscle soreness that spreads from the site of injection into surrounding tissues (Svensson et al. 2003a, Andersen et al. 2008). Although symptoms are not typically present at rest, muscle ache and discomfort may be provoked by palpation and functional tasks (Nie et al. 2009, Hayashi et al. 2013). As these effects have been demonstrated to last around 14 days (Svensson et al. 2003a, Andersen et al. 2008), the NGF model of muscle pain is thought to be a valuable method of investigating cortical responses to acute muscle pain for up to two weeks.

### 1.2.3 Chronic muscle pain

Investigations of muscle pain beyond two weeks duration is confined mainly to clinical studies of chronic pain as an experimental model for human use has yet to be developed. While animal models of chronic musculoskeletal pain are available, they are limited, imperfect and somewhat controversial due to ethical concerns associated with inflicting long-term pain and suffering (Coderre et al. 1987, Kehl et al. 2000, Nagakura et al. 2009, Sharma et al. 2010). Hence, muscle pain of longer duration is usually studied using cross-sectional study designs where individuals with chronic pain are compared with healthy, pain free controls (Schabrun et al. 2015a, Schabrun et al. 2015b). Although persons with chronic musculoskeletal pain are easier to access than those experiencing an acute episode, subjective and quantitative assessments in these individuals may be confounded by the presence of other comorbid symptoms (physical and psychological), treatment history and medication use. While quality studies attempt to control these confounders, either via rigorous pre-screening and exclusion or post-hoc subgroup analyses, this can restrict the generalisability of findings. Differences in pain severity, symptom

duration and movement dysfunction between individuals can also make it difficult to draw definitive conclusions. In summary, despite the limitations of clinical pain populations and acute experimental models, both are used widely to generate valuable information on motor adaptation and cortical responses to muscle pain.

### *1.3 Movement dysfunction in pain*

Movement dysfunction is a key symptom of acute and chronic musculoskeletal pain. While some motor consequences are outwardly obvious (i.e. altered gait and posture), less obvious effects such as diminished proprioception, reduced fine motor performance, altered muscle activation and disturbed muscle synergies are also a frequent occurrence (Graven-Nielsen et al. 1997d, Radebold et al. 2000, Graven-Nielsen et al. 2002b, Matre et al. 2002, Rossi et al. 2003, Slater et al. 2003, Hortobagyi et al. 2004, Alizadehkhaiyat et al. 2007, Skinner et al. 2007, Juul-Kristensen et al. 2008, Henriksen et al. 2011). Although traditional hypotheses advocate a uniform effect of muscle pain on motor physiology (i.e. increased or decreased muscle activity; Travell et al. 1942, Lund et al. 1991), current opinion is that the motor adaptation selected by any one individual is likely to be unique, and dependent upon the anatomical and functional complexity of the body part involved (Peck et al. 2008, Hodges et al. 2011).

Contemporary theories of motor changes in pain are supported by a wealth of evidence demonstrating variable patterns of adaptation to musculoskeletal pain (Hodges et al. 2011). For example, EMG responses of an acutely painful muscle have been demonstrated to increase or decrease depending on the individual being

examined, even under identical experimental conditions (Hodges et al. 2013, van den Hoorn et al. 2015). Acute muscle pain has also been demonstrated to differentially affect the activity of distal (increased) and proximal (decreased) muscles within the same body segment (Del Santo et al. 2007), as well as the movement characteristics of simple and complex multi joint systems (e.g. decreased vs. increased variability re joint excursions) (Madeleine et al. 2008, Bergin et al. 2014). There is also evidence of variable responses in chronic pain, as muscle activity has been demonstrated to be increased (Kaigle et al. 1998, Ambroz et al. 2000), decreased (Ahern et al. 1988, Watson et al. 1997) and unchanged (Ahern et al. 1988, Watson et al. 1997) in individuals with LBP, compared to pain-free controls. The wide range of adaptations evident between individuals and within conditions is testament to the flexible nature of the motor system at both the micro and macro level.

The ultimate goal of motor adaptation to pain is thought to be that of protecting injured tissues from further pain or insult. While this concept is not new (Travell et al. 1942, Lund et al. 1991), the idea that this can be achieved regardless of the type of adaptation or direction of change is a fairly recent development (Hodges et al. 2011). According to Hodges et al. (2011), seemingly contrary adaptations such as those described above are hypothesised to be equally as effective at providing protection to an injured body part. For instance, decreased EMG at the site of pain may prevent symptom aggravation by restricting the movement capabilities of the affected body part, while increased EMG may act to splint the affected body part, thus unloading painful tissues and structures. While these adaptations may be beneficial in the short term, Hodges et al. (2011) hypothesised that such changes

could be detrimental if maintained long term. There has also been the suggestion that variable responses between individuals could explain why some develop chronic pain and others do not (van den Hoorn et al. 2015). Although the physiological basis of motor adaptations to muscle pain is not fully understood, a growing body of evidence points towards altered central nervous system function (Coderre et al. 1993, Hodges et al. 2011, Bank et al. 2013, Pelletier et al. 2015). As the source of movement planning and execution is the motor cortex, changes to the structure and function of the primary motor cortex (M1) provides a likely substrate.

### *1.4 Primary motor cortex anatomy and physiology*

The primary motor cortex (Brodmann's area 4) is a subdivision of the greater cerebral motor area located in the dorsal portion of the frontal lobe, between the premotor cortex (anterior) and primary somatosensory cortex (posterior). Direct electrical stimulation of M1 in humans indicates that this region is concerned primarily with coordinating and directing physical movement (Penfield et al. 1950). The cortical surface of M1 is arranged medial-lateral to depict anatomical divisions of the body, with the amount of cortex devoted to each body region being reflective of the movement capabilities of that part, rather than the physical size (Figure 1.2; Penfield et al. 1937). In this way, muscles required for fine motor control (e.g. intrinsic hand muscles) occupy a disproportionately larger area of the cortex than those necessary for gross motor activities (e.g. leg muscles) (Rasmussen et al. 1947). Importantly, brain imaging studies demonstrate that these divisions demonstrate considerable

overlap and plasticity, especially in response to pain and injury (Roricht et al. 1999, Sanes et al. 2000, Karl et al. 2001, Schwenkreis et al. 2001, Meier et al. 2008).

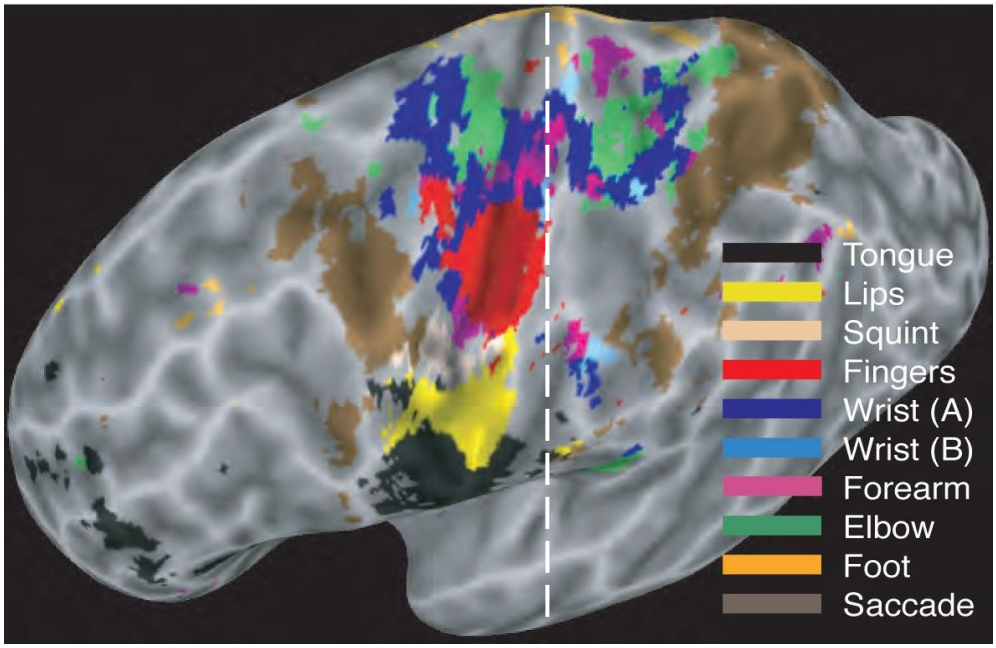


***Figure 1.2*** *Somatotopic representation of movement in the human brain. This surface map represents hemodynamic responses identified by high resolution fMRI during a range of motor tasks (tongue protrusion, squinting, finger flexion/extension, wrist flexion/extension [A], wrist adduction/abduction [B], forearm pronation/supination, elbow flexion/extension, curl/uncurl of toes and rapid eye movement [saccade]). Movement of different body parts is shown to activate overlapping areas of the cortex in both M1 (left of central sulcus, indicated by the dashed line) and primary somatosensory cortex (S1; right of the central sulcus). Source: Michael Graziano [CC BY-SA 1.0 (http://creativecommons.org/licenses/by-sa/1.0)], via Wikimedia Commons.*

Below the cortical surface exists a complex cytoarchitecture. Here, neurons demonstrate a vertical columnar arrangement subdivided into six distinct horizontal layers, the most distinctive of which is the descending output layer five. This layer is characterized by the presence of a small but significant population (~10%) of giant pyramidal neurons known as Betz cells (Rivara et al. 2003). These excitatory long

projection neurons, together with other layer five pyramidal cells, project extrinsically from the cortex to form 30% of the corticospinal tract (Hall 2015). Pyramidal neurons of the corticospinal pathway are the main means by which M1 controls the force, direction, extent and speed of movement (Byrne et al. 1997).

The functional properties of pyramidal neurons are modulated by local circuit neurons. These 'interneurons' project exclusively within the cortex and are morphologically and electrophysiologically diverse (Markram et al. 2004). Up to 50 subtypes of interneuron have been described, the majority of which are classed as inhibitory (Markram et al. 2004). While most inhibitory cells use gamma-amino-butyric-acid (GABA) as their main neurotransmitter, cholinergic (excitatory/inhibitory) and glutamatergic (excitatory) interneurons have also been described (Okhotin et al. 1999). GABAergic neurons can be broadly defined based on receptor subtype; type A mediate fast inhibitory post synaptic potentials (~2.5ms), while type B demonstrate slower temporal characteristics (~45ms) (Benardo 1994). Although it remains to be confirmed whether these responses are due to independent interneuron populations or differential receptor positioning on the same cell (i.e. $GABA_B$ receptors situated external from the synapse), there is increasing evidence to suggest that separate classes of interneuron may be involved (Benardo 1994, Sanger et al. 2001). In addition to interactions with excitatory pyramidal neurons, there is also evidence that interneuronal networks are interconnected (Tamas et al. 1998), and that interactions between different inhibitory populations may be complementary or competitive (Sanger et al. 2001, Sailer et al. 2002, Stefan et al. 2002, Sailer et al. 2003, Alle et al. 2009).

GABAergic interneurons contribute to motor output through extensive connections with M1 pyramidal neurons. Findings in animal and human studies suggest that subtle modifications to the strength of inhibitory networks function to regulate the excitatory drive to muscles as well as excitatory connections within and between cortical motor representations (Jacobs et al. 1991, Liepert et al. 1998a). For example, there is evidence in humans that GABAergic inhibition is decreased in actively contracting muscles, but increased for muscles not immediately engaged in a motor task, presumably to prevent unwanted movements, muscle overflow and co-contraction (Ridding et al. 1995, Liepert et al. 1998a, Zoghi et al. 2003, Hammond et al. 2007, McNeil et al. 2009). In a seminal study by Jacobs et al. (1991), pharmacological blockade of GABAergic inhibition in the rodent brain led to an expansion of motor maps, presumably due to the unmasking of existing, but latent, excitatory connections between adjacent cortical representations. Since then, altered intracortical inhibition has also been proposed as a substrate for altered corticomotor output and M1 reorganisation in humans experiencing musculoskeletal pain (Schabrun et al. 2012, Schabrun et al. 2015b).

### *1.5 The corticomotor response to musculoskeletal pain*

Musculoskeletal pain has been demonstrated to affect the haemodynamic (Henderson et al. 2006, Nash et al. 2010b, Takahashi et al. 2011, Loggia et al. 2012), metabolic (Svensson et al. 1997c, Kupers et al. 2004), and neuroelectric properties (Del Santo et al. 2007, Hoeger Bement et al. 2009, Tsao et al. 2011c, Schabrun et al. 2012, Rittig-Rasmussen et al. 2014) of M1. While haemodynamic and metabolic responses can be examined via brain imaging technologies such as functional

magnetic resonance imaging (fMRI) and positron emission tomography (PET), the most accessible method of assessing the neuroelectric properties of M1 is via transcranial magnetic stimulation (TMS).

### 1.5.1 Transcranial magnetic stimulation

Transcranial magnetic stimulation is a safe, and non-invasive technique used widely to examine the corticomotor pathway in conscious humans (Rossini et al. 1998, Chen 2000). When applied to the scalp overlying M1, TMS produces a corticomotor effect (motor evoked potential: MEP; Figure 1.3) via electromagnetic induction of currents in the underlying neural tissue (Barker et al. 1985). The strength of this effect is dependent upon methodological factors such as coil orientation and stimulation intensity, as well as intrinsic factors such as the excitability of cortical, spinal and peripheral components of the pathway (Edgley et al. 1997, Di Lazzaro et al. 1998a, Di Lazzaro et al. 2004, Di Lazzaro et al. 2007b, Groppa et al. 2012).

Responses evoked by TMS have been demonstrated to be due to the direct and/or indirect (i.e. interneuronal) activation of pyramidal neurons (Edgley et al. 1997). Direct epidural recordings in conscious humans show that the resultant descending activity is comprised of a series of high frequency waves (Di Lazzaro et al. 1998b, Di Lazzaro et al. 2001a, Di Lazzaro et al. 2001b, Di Lazzaro et al. 2002b). These findings indicate that TMS preferentially activates “I” (indirect) waves. However, “D” (direct) waves can also be recruited when higher stimulus intensities are used (Di Lazzaro et al. 1998b). Since similar patterns of activity have never been recorded during natural movement, it is important to recognise that TMS is a means of *artificially* activating

the motor system and that the evoked responses do not necessarily reflect natural motor physiology (Lazzaro et al. 2008).

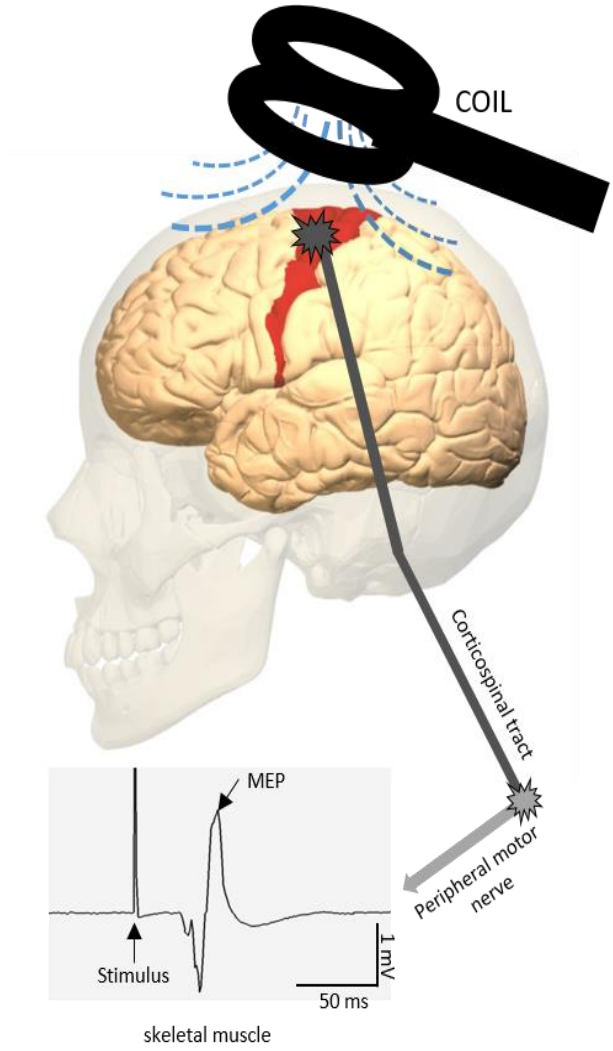


***Figure 1.3*** *TMS of the motor cortex. TMS is performed using a stimulation device consisting of an electromagnetic coil attached to a high-voltage discharge system. TMS capacitors discharge a large electric pulse which causes current flow in the coil. The resultant brief (≤ 1ms) and powerful (1 - 2.5 Tesla) magnetic field can, if sufficiently intense, penetrate the scalp and skull and induce eddy currents within the underlying neural tissue causing depolarisation of cell membranes. TMS evoked volleys from M1 (red), descend via the corticospinal tract and peripheral motor nerve, producing a motor response from skeletal muscle known as a motor evoked potential (MEP).*

Quantification of MEPs in the periphery via electromyography provides information on the excitability and conductivity of the corticomotor pathway. Basic outcome measures of single pulse TMS include MEP peak-to-peak amplitude (reflective of excitability), MEP latency (reflective of signal conduction time from cortex to periphery) and 'motor threshold'. Motor threshold provides a relative measure of the resting membrane potential of pyramidal neurons and is defined as the average stimulation intensity required to generate a small MEP in a target muscle (typically between 50-200uV peak-to-peak amplitude)(Rossini et al. 1994, Pascual-Leone et al. 2002). As responses to TMS are highly variable, it is common practice to report average values for such outcomes. Trials consisting of five stimuli demonstrate excellent within-session reliability for measures of MEP amplitude (trials of ten stimuli are ideal between-session) (Cavaleri et al. 2017b), however under certain conditions as little as two stimuli may be required (Cavaleri et al. 2017a).

A scan of electronic databases (PubMed, MEDLINE) indicates that over 1000 TMS studies are now published each year. Despite this popularity, there are still a number of concerns regarding the reliability of TMS generated data. Of most concern is the variable nature of the MEP response. Exploratory investigations of normative data indicate that this variability may have biological and technical sources (Boroojerdi et al. 2000, Wassermann 2002, Cueva et al. 2016). Between subject factors are thought to be the main source of variation, accounting for as much as 67% of the total variability of MEP measurements (Boroojerdi et al. 2000). Indeed, in addition to individual differences in the excitability of the corticomotor pathway, evidence suggests that inter-subject factors such as participant age, sex and handedness may

also influence study outcomes (Wassermann 2002, Cahn et al. 2003, Pitcher et al. 2003, Cueva et al. 2016). Although the precise impact of these factors remains unclear, it is now expected that these variables are reported and controlled in TMS studies (Chipchase et al. 2012). Within subject factors such as menstrual phase, caffeine use and time of day are also demonstrated to negatively impact the reliability of TMS data, and thus are also increasingly accounted for in TMS study designs (Smith et al. 1999, Smith et al. 2002, Cerqueira et al. 2006, Sale et al. 2007).

Another determinant of the quality and reliability of TMS data is the proficiency of the examiner. Alarmingly, in 2002 it was estimated that up to 50% of the between subject variability for motor threshold measurements could be due to experimental error caused by incorrect electrode/coil placement and inappropriate trial frequency (Wassermann 2002). However, results from a more recent study of inter- and intra-examiner variability provide a more conservative estimate, especially for single pulse measures of cortical excitability (Cueva et al. 2016). Improvements in examiner reliability over time may reflect increased methodological quality of studies using TMS and/or advances in technical competency due to the advent of computer guided neuronavigation systems. However, despite this progress, the impact of biological and technical factors on TMS evoked responses still remains a significant concern. This has led an international panel of experts to develop a TMS methodological checklist to inform researchers as to which factors should be reported and/or controlled in TMS studies (Chipchase et al. 2012). Adoption of the recommendations outlined by this checklist will hopefully lead to improvements in the quality and transparency of TMS research.

### 1.5.2 Corticomotor adaptations to acute pain

Studies using TMS provide evidence of altered corticomotor output during and following short lasting experimentally induced muscle pain. Although these effects generally manifest as reduced MEP amplitudes (decreased corticomotor excitability) (Le Pera et al. 2001, Svensson et al. 2003c, Martin et al. 2008, Schabrun et al. 2012, Schabrun et al. 2013, Rittig-Rasmussen et al. 2014), the occasional instance of increased (Del Santo et al. 2007) or unchanged excitability (Romaniello et al. 2000) indicates a degree of variability in this response. As previously discussed, it is possible that inter-subject (and potentially inter-investigator) variability may account for differences between technically similar studies. However, as the method for threshold determination, coil position, trial frequency and stimulation parameters differ between most studies (Appendix A.4), there is a high likelihood that much of this variability is due to methodological factors. For example, the same muscle can display evidence of increased or decreased excitability depending on whether it is examined at rest or during active contraction (Le Pera et al. 2001, Del Santo et al. 2007). It is also possible that variable responses to pain reflect differences in anatomical location since MEPs are consistently reduced in painful muscles of the hand, forearm and arm (Le Pera et al. 2001, Svensson et al. 2003c, Martin et al. 2008, Schabrun et al. 2012, Schabrun et al. 2013), but increased in superficial abdominal and low back muscles (Tsao et al. 2011c). However, as opposing responses have also been demonstrated between muscles within a body segment under similar experimental conditions (Tsao et al. 2011c), a comprehensive review of these data is necessary to clarify the effect of acute muscle pain on corticomotor output, as well as the methodological quality of these studies.

As mentioned in section 1.3.1, when reviewing TMS data, it is important to recognize that the MEP is an aggregate measure of corticospinal excitability, rather than a direct reflection of M1 output. Thus, it also remains unclear whether the findings described above specifically reflect altered cortical output or simply a net change in the excitability of the corticomotor pathway. Although a small number of TMS studies have endeavored to control for changes occurring downstream from the cortex by including measures of spinal excitability, these investigations remain inconclusive since reductions in MEPs were accompanied by decreased spinal excitability in some studies (Le Pera et al. 2001, Svensson et al. 2003c), but increased excitability in others (Martin et al. 2008). In contrast, joint investigations of MEPs and peripheral nerve excitability (M-waves) demonstrate stability of the peripheral element over time (Svensson et al. 2003c, Schabrun et al. 2013), thus ruling out any contribution of peripheral mechanisms to the MEP response. As peripheral changes have also been excluded by non-TMS studies (Svensson et al. 1998, Graven-Nielsen et al. 2002b, Farina et al. 2004, Farina et al. 2005), future work should focus on discerning the relative contribution of cortical and spinal mechanisms to the corticomotor response to acute muscle pain.

### 1.5.3 Corticomotor adaptations in chronic pain

#### *1.5.3.1 Corticomotor excitability*

A variety of corticomotor adaptations have also been documented for chronic musculoskeletal pain, however, as this is a relatively new area of research, the range of conditions investigated, as well as data available, is limited. Despite this,

preliminary evidence indicates that corticomotor excitability is altered in persons with chronic musculoskeletal pain originating from various anatomical regions and structures (Table 1.2). For example, compared to pain-free controls, motor threshold is increased (indicative of decreased excitability) in painful muscles at the site of LBP (Strutton et al. 2005), the infraspinatus muscle in chronic shoulder pain (Bradnam et al. 2016) and in muscles of the hand and leg in persons with diffuse pain due to fibromyalgia (Salerno et al. 2000, Mhalla et al. 2010). A lack of change in spinal excitability in fibromyalgia (Salerno et al. 2000) indicates that this response may be of cortical origin, although this remains to be confirmed for other conditions. However, observations of increased motor threshold are directly contrasted by evidence of hyperexcitability (increased MEP amplitudes) in persons with chronic pain due to rotator cuff injury (Berth et al. 2009), osteoarthritis (Caumo et al. 2016), myofascial pain syndrome (Caumo et al. 2016), lateral epicondylalgia (Schabrun et al. 2015b) and patellofemoral pain (On et al. 2004). Interestingly, numerous studies have failed to detect significant differences in motor threshold or corticomotor output between individuals with chronic musculoskeletal pain and pain free controls (Tsao et al. 2008, Schwenkreis et al. 2010, Schwenkreis et al. 2011, Masse-Alarie et al. 2012, Kittelson et al. 2014, Marker et al. 2014, Vidor et al. 2014, Masse-Alarie et al. 2016, Parker et al. 2017). However, as many chronic conditions have only been examined on a single occasion and in a sub-set of individuals with chronic pain (those with co-morbidities or taking medications are usually excluded), it is difficult to draw definitive conclusions regarding the effect of chronic pain on corticomotor excitability.

The generalisability of these findings is also uncertain since the majority of data regarding corticomotor excitability in chronic musculoskeletal pain is drawn from investigations involving small cohorts of participants (Table 1.2). The main issue with small studies like these is that they are typically underpowered and thus predisposed to type I (false positive) and type II (false negative) errors (Button et al. 2013). Indeed, since the ability of a study to detect an association between a predictor/outcome variable is dependent upon the magnitude of association being investigated (Banerjee et al. 2009), it is quite possible that a lack of adequate power precluded the detection of subtle differences in corticomotor excitability between some patient/control groups (Tsao et al. 2008, Schwenkreis et al. 2010, Schwenkreis et al. 2011, Masse-Alarie et al. 2012, Kittelson et al. 2014, Marker et al. 2014, Vidor et al. 2014, Masse-Alarie et al. 2016, Parker et al. 2017). However, as few authors provide sample size calculations or effect size estimates in text, it remains unclear whether inadequate power contributed to these null observations. Furthermore, in the rare instance that such details are provided (Schabrun et al. 2015b, Parker et al. 2017), it is evident that sample sizes were based on effects described in other small (and potentially underpowered) studies (Schwenkreis et al. 2010, Tsao et al. 2011b). Although technically correct, this practice is less than ideal as it has to potential to lead to the generation of an unreliable body of literature (Button et al. 2013).

***Table 1.2*** *Description of studies examining corticomotor excitability in chronic musculoskeletal pain*

| **STUDY** *(year)* | **N** *(patient, control)* | **CONDITION** | **TARGET MUSCLE** | **MEASURE** | **RESULT** *(patient vs. control)* |
|---|---|---|---|---|---|
| ***Berth (2009)*** | 10, 13 | Chronic rotator cuff tear | Deltoid | CMO (rest)<br>CMO (active) | ↑<br>↓ |
| ***Berth (2010)*** | 10, 11 | Chronic rotator cuff tear | FDI | RMT | ↓ |
| ***Bradnam (2016)*** | 8, 18 | Shoulder pain | Infraspinatus | AMT<br>CMO | ↑<br>• |
| ***Caumo (2016)*** | 19, 14<br>27, 14<br>54, 14 | Fibromyalgia<br>Osteoarthritis<br>Myofascial pain | FDI | CMO | ↑<br>↑<br>↑ |
| ***Kittelson (2014)*** | 17, 20 | Osteoarthritis | VL | RMT | • |
| ***Marker (2014)*** | 9,8 | Neck pain | Trapezius | RMT<br>AMT<br>CMO | •<br>•<br>• |
| ***Masse-alarie (2012)*** | 13, 9 | Low back pain | TRA/IO | AMT<br>CMO | •<br>• |
| ***Masse-Alarie (2016)*** | 11, 13 | Low back pain | Multifidus | AMT<br>CMO | •<br>• |
| ***Mhalla (2010)*** | 46, 21 | Fibromyalgia | FDI | RMT<br>CMO | ↑<br>↓ |

| | | | | | |
|---|---|---|---|---|---|
| ***On (2004)*** | 13, 13 | Patellofemoral pain | VMO, VL<br>EDB | CMO | ↑<br>• |
| ***Parker (2017)*** | 23, 20 | Arthritis | FDI | RMT<br>CMO | •<br>• |
| ***Salerno (2000)*** | 13, 13 | Fibromyalgia | FDI, TA | RMT<br>CMO | ↑<br>• |
| ***Schabrun (2015)*** | 11, 11 | Lateral epicondylalgia | ECRB, ED | RMT<br>CMO | •<br>↑ |
| ***Schwenkreis (2010)*** | 20, 14 | Hand osteoarthritis | FDI | RMT | • |
| ***Schwenkreis (2011)*** | 16, 23 | Fibromyalgia | Superficial flexor of forearm | RMT<br>CMO | •<br>• |
| ***Strutton (2005)*** | 24, 11 | Low back pain | LES | AMT<br>CMO | ↑<br>• |
| ***Tsao (2008)*** | 11, 11 | Low back pain | TrA | RMT<br>AMT | •<br>• |
| ***Vidor (2014)*** | 47, 11 | Myofascial pain | FDI | CMO | • |

Tabulated results refer to the hemisphere contralateral to the side of pain. RMT, resting motor threshold; AMT, active motor threshold; CMO, corticomotor output; ↓ decrease MT/CME; ↑ increase RMT/CME; • no difference RMT/CME; FDI, first dorsal interosseous; VL, vastus lateralis; TrA/IO, transverse abdominus/internal obliques; VM, vastus medialis oblique; EDB, extensor digitorum brevis; TA, tibialis anterior; ECRB, extensor carpi radialis brevis; ED, extensor digitorum; LES; longissimus erector spinae; TrA, transverse abdominus

*1.5.3.2 M1 organisation*

A small number of studies also provide evidence of cortical reorganisation in chronic musculoskeletal conditions (Tsao et al. 2008, Tsao et al. 2011b, Schabrun et al. 2015a, Schabrun et al. 2015b, Te et al. 2017). These studies used TMS mapping protocols to construct a visual representation of the excitability and organisation of neurons projecting to painful muscles and/or muscles within the vicinity of a painful joint (Wassermann et al. 1992). This technique, which involves recording the MEP over a range of scalp sites (Figure 1.4), is a common and reliable method of studying the topographical plasticity of M1 (Uy et al. 2002).

Similar to observations of motor threshold and corticomotor output, the effect of chronic pain on map excitability varies between studies and muscles (Table 1.3). For example, map volume (the sum of the MEP amplitude at all map sites) is reduced for deep paraspinal muscles in LBP (Tsao et al. 2011b) and quadriceps muscles in patellofemoral pain (indicative of decreased excitability) (Te et al. 2017), but increased for wrist extensor muscles in lateral epicondylalgia (increased excitability) and deep abdominal muscles in LBP (Tsao et al. 2008), compared to pain free controls (Schabrun et al. 2015b). There is also evidence of both reduced and unchanged map volume for superficial paraspinal muscles, however these discrepancies may be attributable to methodological differences between studies (surface vs. fine wire EMG) (Tsao et al. 2011b, Schabrun et al. 2015a). Taken together, data for map volume in chronic musculoskeletal conditions imply a differential effect of pain on superficial and deep musculature of the trunk, and opposing effects in muscles of the upper and lower limb. However, as these data are derived from small cohorts of participants,

further work is needed to confirm these findings. It is also important to note that changes in map volume, although informative, are not necessarily evidence of M1 reorganisation. Instead, reorganisation can be inferred based on variations in the amplitude weighted centre of the map, or centre of gravity (CoG). As CoG is located close to the motor hotspot of the target muscle (Wilson et al. 1993, Thickbroom et al. 1998), changes in its location are interpreted to reflect reorganisation of the spatial territory and location of the most excitable projections corresponding to a given muscle.

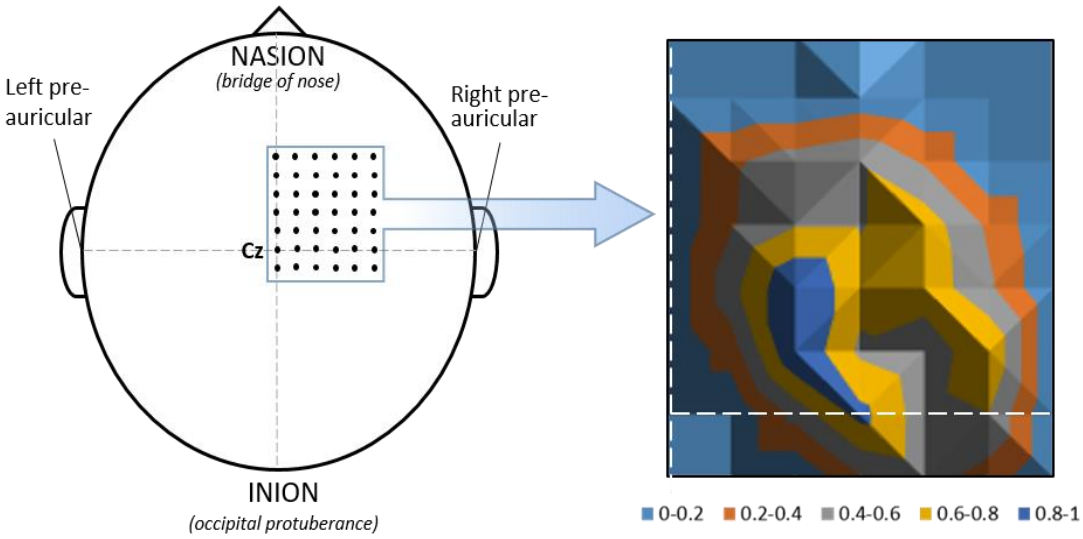


***Figure 1.4*** *<u>Mapping the excitability and topography of M1 with TMS.</u> Single pulse TMS can be used to construct a representation of the excitability and organisation of neurons that project to a target muscle. TMS is delivered to each site on a grid orientated to the vertex (Cz) with the target muscle at rest or during low-level contraction (left). The average amplitude of MEPs evoked at each site is used to create a map of the cortical representation of the target muscle. The example provided here was constructed from MEP responses recorded from the left vastus medialis oblique muscle of a healthy individual. Starting at the vertex, five magnetic stimuli were delivered at 1cm intervals on a 6 x 7cm grid with the aid of a neuronavigation instrument. Stimuli were applied at 100% TMS output at 6s intervals during low-level volitional contraction. Note: This map is generated from data that has been normalised to maximal MEP amplitude (1mV). Colour scale denotes increments of 0.02mV.*

In contrast to map excitability changes, observations of CoG in chronic musculoskeletal pain conditions are remarkably similar. For example, both individuals with LBP and patellofemoral pain demonstrate significant relocation of the CoG compared to pain-free controls (Tsao et al. 2008, Tsao et al. 2011b, Schabrun et al. 2015a, Te et al. 2017). Although the direction of change differed in studies of LBP (posterior-lateral shift vs. anterior shift), it is possible that this variability may be due to differences in the spatial resolution of fine wire and surface recordings (Tsao et al. 2011b, Schabrun et al. 2015a). A reduction in the distance between CoGs of muscles at the site of pain was also consistent across conditions (Tsao et al. 2011b, Schabrun et al. 2015b, Te et al. 2017). Reduced CoG distance between discrete muscles suggest that their cortical territories exhibit disproportionate overlap. This may indicate that there are fewer discrete corticospinal projections and a greater sharing of neural resources between the muscles investigated. Although it has been hypothesised that such changes have the potential to negatively impact coordination and independent muscle control (Schabrun et al. 2007, Schabrun et al. 2015b), the mechanisms underpinning M1 reorganisation remain unclear.

***Table 1.3*** *Description of studies examining the excitability and topography of M1 in chronic musculoskeletal pain*

| **STUDY** *(year)* | **N** *(patient, control)* | **CONDITION** | **TARGET MUSCLE**(s) | **MAP VOLUME** *(patient vs. control)* | **COG LOCATION** *(patient vs. control)* | **COG DISTANCE BETWEEN MUSCLES** *(patient vs. control)* |
|---|---|---|---|---|---|---|
| ***Schabrun (2015)*** | 11, 11 | Lateral epicondylalgia | ECRB<br>ED | ↑<br>↑ | •<br>• | ↓ |
| ***Schabrun (2015)*** | 27, 23 | Low back pain | Paraspinal at L3<br>Paraspinal at L5 | •<br>• | Anterior<br>Anterior | NR |
| ***Te (2017)*** | 11, 11 | Patellofemoral pain | RF<br>VL<br>VM | ↓<br>↓<br>↓ | Anterior<br>Anterior<br>Anterior | ↓<br>↓<br>↓ |
| ***Tsao (2008)*** | 11, 11 | Low back pain | TrA | ↑ | Posterior and lateral | NA |
| ***Tsao (2011)*** | 9, 11 | Low back pain | Multifidus<br>LES | ↓<br>↓ | •<br>Posterior | ↓<br>↓ |

Tabulated results refer to the hemisphere contralateral to the side of pain. COG, centre of gravity; ↓ decrease volume/COG; ↑ increase volume/COG; • no difference volume/COG; NR, not reported; NA, not assessed; VL, vastus lateralis; TrA, transverse abdominus; VM, vastus medialis; ED, extensor digitorum; ECRB, extensor carpi radialis brevis; LES; longissimus erector spinae; RF, rectus femoris

### *1.6 Could intracortical inhibition underpin observations of altered corticomotor excitability and M1 reorganisation in muscle pain?*

The anatomical arrangement and functional properties of inhibitory interneurons place them in a prime position to influence, if not drive, the corticomotor response to pain. Indeed, it is estimated that pyramidal neurons receive inputs from approximately 70 inhibitory interneurons, each of which may form as many as 30 synaptic connections. As these synapses are usually distributed across multiple sites, inhibitory cells can modulate crucial neural functions such as the generation, timing, propagation and discharge of action potentials, as well as functions related to synaptic plasticity such as dendritic processing and the integration of other synaptic inputs (see Markram et al. 2004 for review). Thus, it is somewhat surprising that few studies have examined the effect of muscle pain on cortical inhibition specifically. Indeed, despite the availability of several validated methods for assessing intracortical activity, there have been less than a dozen investigations of cortical inhibition in chronic pain and only a single study in acute pain (Salerno et al. 2000, Mhalla et al. 2010, Schwenkreis et al. 2010, Schwenkreis et al. 2011, Masse-Alarie et al. 2012, Schabrun et al. 2012, Kittelson et al. 2014, Marker et al. 2014, Bradnam et al. 2016, Caumo et al. 2016, Masse-Alarie et al. 2016, Parker et al. 2017). Fortunately, each of these studies examined intracortical activity via TMS, thus permitting direct comparisons between studies and conditions.

### 1.6.1 Short interval intracortical inhibition

The majority of investigations of intracortical activity in musculoskeletal pain report an index of GABAergic inhibition known as short interval intracortical inhibition (SICI). This form of inhibition is evoked following the delivery of two TMS pulses through the same stimulating coil, 1-5ms apart (Kujirai et al. 1993). If the conditioning (first) pulse is of subthreshold intensity, inhibition of the MEP generated by the test (second) pulse is observed (Figure 1.5). This inhibition (SICI) is expressed as a percentage, calculated by dividing the MEP amplitude of the conditioned-test pulse by the MEP amplitude of the test pulse alone.

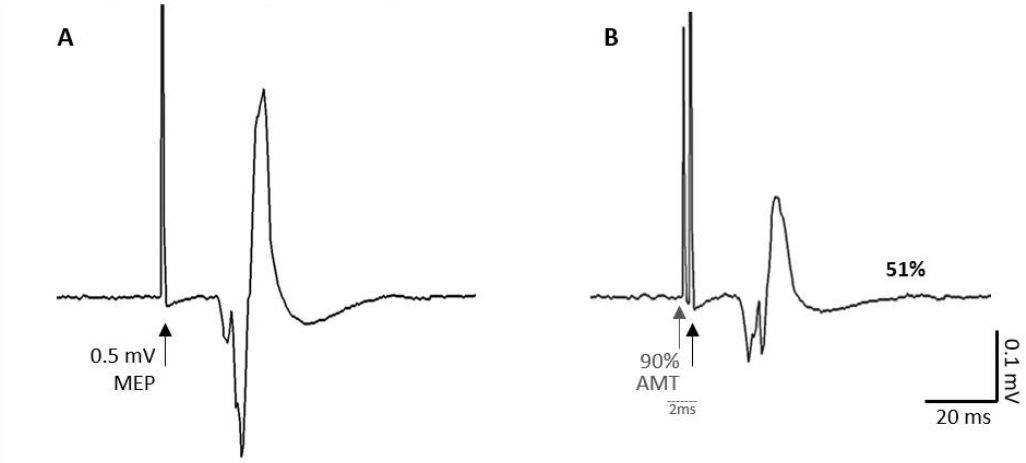


***Figure 1.5*** *Short interval intracortical inhibition recorded from the resting extensor carpi radials brevis muscle of a healthy individual. SICI is calculated by comparing the MEP amplitude following single pulse TMS (A) with the MEP amplitude following paired pulse TMS (B). This example is the outcome of 12 trials recorded in random order at an inter-stimulus interval of 2ms (B) and for the test stimulus alone (A; rate of 1 every 6s, total of 24 trials). Test intensity (black arrows) was set to produce a 0.5mV MEP and conditioning intensity was set at 90% active motor threshold (AMT; grey arrow). Under these conditions, this individual demonstrated 51% SICI (≥100% represents no inhibition).*

Direct cervical epidural recordings of descending corticospinal activity confirm the cortical origin of SICI by demonstrating that the conditioned-test pulse evokes efferent volleys of a latency consistent with indirect (interneuronal) activation of M1 neurons (Di Lazzaro et al. 1998c). Pharmacological evidence indicates this measure reflects GABAergic activity mediated by type A receptors (α2 and α3 subtypes), as SICI is increased by drug formulations which enhance GABA neurotransmission through these channels (Ziemann et al. 1996a, Di Lazzaro et al. 2000a, Ilic et al. 2002, Di Lazzaro et al. 2005c, Di Lazzaro et al. 2006a, Di Lazzaro et al. 2007a, Florian et al. 2008, Teo et al. 2009). Based on this receptor profile, it likely that SICI reflects post-synaptic activity of inhibitory interneurons at the somatic and/or dendritic membrane of pyramidal cells (Markram et al. 2004).

*1.6.1.2 Short interval intracortical inhibition and musculoskeletal pain*

The results of a recent systematic review and meta-analysis suggest that cortical inhibition is unaffected by musculoskeletal pain as pooled analyses failed to identify a statistically significant effect size for SICI measures in several chronic pain conditions (Parker et al. 2016). This result is most likely an accurate representation of these data, as only one of the five studies reviewed reported significant differences between patients and controls (Mhalla et al. 2010). However, it could be argued that the conclusion from the systematic review does not truly reflect the state of cortical inhibition in chronic pain as an additional five studies, either not identified, excluded or published since Parker et al. (2016), demonstrate significant differences between patients and controls (Table 1.4). These studies provide consistent evidence of reduced SICI (cortical disinhibition) in fibromyalgia (Schwenkreis et al. 2011, Caumo

et al. 2016), chronic LBP (Masse-Alarie et al. 2012, Masse-Alarie et al. 2016) and arthritis (Caumo et al. 2016, Parker et al. 2017), as well as preliminary evidence of reduced SICI in myofascial pain syndrome (Caumo et al. 2016). These observations suggest that cortical inhibition is *reduced* (disinhibition) in chronic pain, and that this effect is the same regardless of the body region or tissue affected. In contrast, evidence suggests cortical inhibition is *increased* by acute pain. In a study by Schabrun et al. (2012), an increase in SICI was observed immediately following the resolution of pain due to intramuscular infusion of hypertonic saline. However, as this effect was only present post intervention, a linear relationship between SICI and pain severity in this study appears unlikely. A significant correlation between SICI and pain severity has also yet to be identified in studies of chronic pain, thus it is possible that this non-linearity exists regardless of the direction of change in SICI or duration of symptoms (acute vs. chronic). Furthermore, since the change in SICI does not follow the temporal profile of MEP depression in acute pain (Schabrun et al. 2012), and reductions in SICI are rarely accompanied by increased corticomotor excitability in chronic pain (Caumo et al. 2016), the relationship between SICI and corticomotor adaptations to muscle pain may also be non-linear in nature.

***Table 1.4*** *Description of studies examining SICI in chronic musculoskeletal pain*

| **STUDY** *(year)* | **N** *(patient, control)* | **CONDITION** | **TARGET MUSCLE** | **CS INTENSITY** | **TS INTENSITY** | **ISI** *(ms)* | **SICI** *(patient vs. control)* |
|---|---|---|---|---|---|---|---|
| ***Caumo (2016)*** | 19, 14<br>27, 14<br>54, 14 | Fibromyalgia<br>Osteoarthritis<br>Myofascial pain | FDI | 80% RMT | 130% RMT | 2 | ↓<br>↓<br>↓ |
| ***Kittelson (2014)*** | 17, 20 | Osteoarthritis | VL | 80% RMT | 120% RMT | 3 | • |
| ***Marker (2014)*** | 9, 8 | Neck pain | Trapezius | 70% AMT | 120% AMT | 2.5 | • |
| ***Masse-Alarie (2012)*** | 10, 9 | Low back pain | TrA/IO | 70% AMT | 120% AMT | 2 | ↓ |
| ***Masse-Alarie (2016)*** | 8, 8 | Low back pain | Multifidus | 70% AMT | 0.1mV MEP | 2 | ↓ |
| ***Mhalla (2010)*** | 46, 21 | Fibromyalgia | FDI | 80% RMT | 120% RMT | 2, 4 | ↓ |
| ***Parker (2017)*** | 23, 20 | Arthritis | FDI | 70%, 80%* AMT | 1mV MEP | 2 | ↓* |
| ***Salerno (2000)*** | 13, 13 | Fibromyalgia | FDI, TA | 80% RMT | 150% RMT | 4 | • |
| ***Schwenkreis (2010)*** | 20, 14 | Osteoarthritis | FDI | 80% RMT | 1mV MEP | 2, 4 | • |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Schwenkreis (2011)*** | 16, 23 | Fibromyalgia | Superficial flexor of forearm | 80% RMT | 0.5mV MEP | 2, 4 | ↓ |

CS, conditioning stimulus; TS, test stimulus; ISI, inter stimulus interval; FDI, first dorsal interosseous; TA, tibialis anterior; RMT, resting motor threshold; AMT, active motor threshold, MEP, motor evoked potential; TrA/IO, transverse abdominus/internal obliques; VL, vastus lateralis; ↓ decrease SICI; • no difference SICI

Different SICI responses in the acute and chronic stages of pain may reflect fundamental differences in motor strategy at these times (Schabrun et al. 2016). In the acute phase, increased cortical inhibition may reduce excitatory drive to muscles and restrict movement, while chronic disinhibition may support cortical reorganisation and the development of (mal)adaptive motor strategies that facilitate movement (Hodges et al. 2011, Schabrun et al. 2012). Although the precise relationship between the corticomotor and SICI response to pain has yet to be determined, it has been hypothesised that altered SICI may contribute to neuroplastic change in chronic pain (Schabrun et al. 2015b). For example, since a key function of cortical inhibition is the maintenance of cortical representations (Liepert et al. 1998a), reduced SICI in paraspinal and abdominal muscles in LBP (Masse-Alarie et al. 2012, Masse-Alarie et al. 2016) could plausibly underpin observations of posterior-lateral or anterior shifts in the motor maps of these muscles (Tsao et al. 2008, Tsao et al. 2011b, Schabrun et al. 2015a). Differential effects for SICI also indicate an important role for intracortical inhibition in the transition from acute to chronic pain. Indeed, although SICI is increased immediately following an episode of acute pain (Schabrun et al. 2012), a shift towards cortical disinhibition (reduced SICI) has been demonstrated to occur as early as four days into a study of progressively developing muscle pain (Schabrun et al. 2016). While the authors of that study speculate that this may reflect the transition from a restrictive to adaptive motor strategy (Schabrun et al. 2016), the trigger for this change and the cause of its persistence in chronic conditions remains unclear.

### 1.6.2 Other forms of cortical inhibition in musculoskeletal pain

Two other forms of TMS-evoked cortical inhibition have been investigated in musculoskeletal pain. Although these measures are thought to represent distinct and competitive inhibitory circuits to those mediating SICI, early data suggests that these inhibitions are similarly affected by chronic pain.

#### *1.6.2.1 Long interval intracortical inhibition*

Long interval intracortical inhibition (LICI) is an alternate measure of GABAergic inhibition evoked by paired pulse TMS. In contrast to SICI, which reflects short-lasting inhibition evoked by subthreshold conditioning stimulation, LICI reflects the induction of slow inhibitory postsynaptic potentials by identical suprathreshold stimuli delivered 50-250ms apart (Claus et al. 1992, Valls-Sole et al. 1992). Similar to SICI, LICI is expressed as a percentage, calculated by dividing the MEP amplitude of the conditioned-test pulse by the MEP amplitude of the test pulse alone (Figure 1.6).

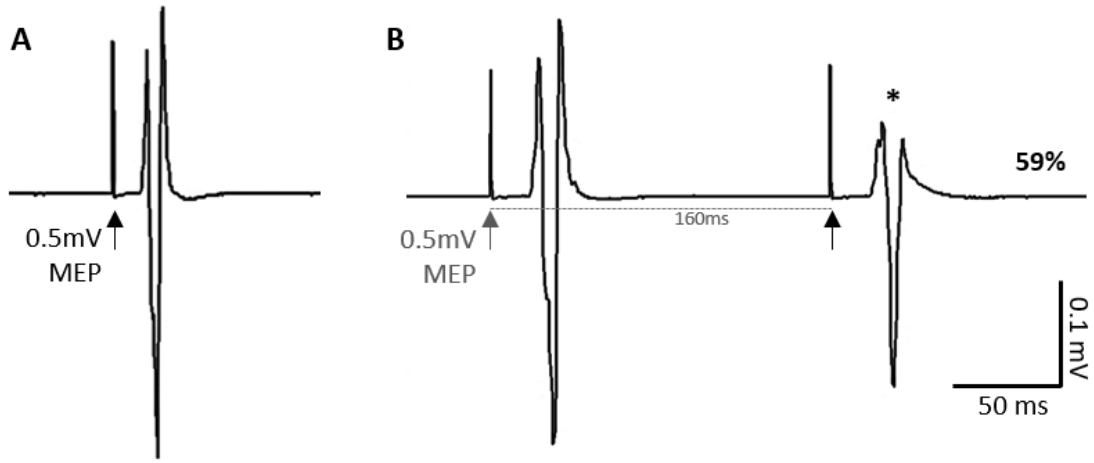


***Figure 1.6*** *Long interval intracortical inhibition recorded from the resting extensor carpi radials brevis muscle of a healthy individual. LICI is calculated by comparing the MEP amplitude following single pulse TMS (A) with the MEP amplitude following paired pulse TMS (B, * denotes the conditioned MEP). This example is the outcome of 12 trials recorded in random order at an inter-stimulus interval of 160ms (B) and for the test stimulus alone (A; rate of 1 every 6s, total of 24 trials). Test intensity (black arrows) and conditioning intensity (grey arrow) were both set to produce a 0.5mV MEP. Under these conditions, this individual demonstrated 59% LICI (≥100% represents no inhibition).*

The range of intervals over which LICI can be evoked suggests that this measure may reflect long lasting $GABA_B$ mediated cortical inhibition (Werhahn et al. 1999, Sanger et al. 2001). This origin is supported by neuropharmacological evidence of enhanced LICI in the presence of the $GABA_B$ receptor agonist drug baclofen (McDonnell et al. 2006) and direct cervical epidural recordings of descending corticospinal activity (Nakamura et al. 1997, Di Lazzaro et al. 2002a). However, since both LICI and cervicomedullary evoked potential amplitude are decreased during volitional contraction (McNeil et al. 2009, 2011), a potential spinal contribution cannot be ruled out. As these interactions have thus far only been documented at $LICI_{(100ms)}$, and two distinct phases of inhibition have been demonstrated at 100ms and 150ms (Chu et

al. 2008, Vallence et al. 2012, Vallence et al. 2014), this contribution, particularly at later inter-stimulus intervals, requires further investigation.

Current knowledge of the effect of musculoskeletal pain on LICI is derived from two studies performed in clinical pain populations. The first, provides evidence of reduced LICI in persons with fibromyalgia (Salerno et al. 2000), while the second reports no difference between arthritic individuals and pain-free controls (Parker et al. 2017). Aside from obvious differences in pain populations, these discrepancies may be explained by differences in methodology since LICI in the first instance was investigated at 155/200ms and the second, 99ms. Thus, as discussed above, it is possible that these studies examined distinct GABAergic processes. As it is hypothesised that $LICI_{(100ms)}$ reflects presynaptic $GABA_B$ mediated inhibition, while $LICI_{(150ms)}$ reflects post synaptic $GABA_B$ mediated inhibition (Chu et al. 2008), these preliminary findings suggest that chronic musculoskeletal pain may specifically affect $GABA_B$ mediated inhibition occurring at the post-synaptic terminal.

*1.6.2.2 Short latency afferent inhibition*

The final form of cortical inhibition investigated in chronic musculoskeletal pain thus far is short latency afferent inhibition (SAI). In contrast to other forms of TMS evoked inhibition, evocation of afferent inhibition requires a multimodal condition-test technique. Although the test stimulus for afferent inhibition is identical to that evoking SICI/LICI (suprathreshold TMS pulse at M1), the conditioning stimulus typically involves transcutaneous electrical stimulation of a peripheral nerve at the wrist (median or ulnar) (Tokimura et al. 2000). Two time dependent phases of

inhibition have been described using this method, the first 20ms (SAI), and the second 200ms (long latency afferent inhibition, LAI), post conditioning stimulus. Although the exact pathways travelled by the afferent volley prior to reaching M1 are still being elucidated, there is good evidence to suggest that MEP suppression resulting from this protocol is of cortical origin as direct epidural recordings demonstrate a reduction in the magnitude and number of descending corticospinal volleys to TMS following peripheral nerve conditioning stimulation (Tokimura et al. 2000). As is the case for other TMS evoked inhibitions, it is standard practice to describe SAI as a percentage, calculated by dividing the MEP amplitude of the conditioned-test pulse by the MEP amplitude of the test pulse alone (Figure 1.7).

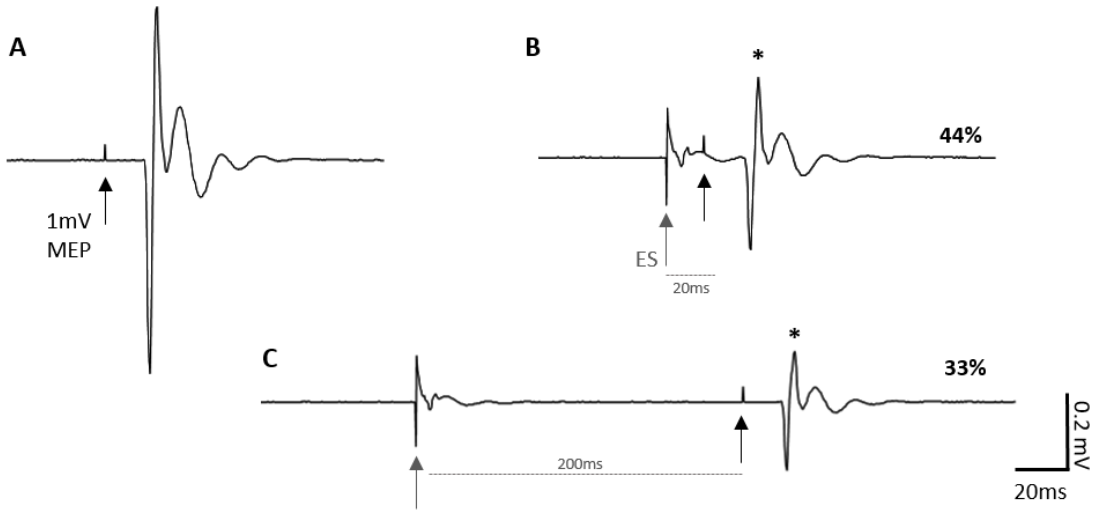


***Figure 1.7*** *Afferent inhibition recorded from the resting first dorsal interosseous muscle of a healthy individual. Afferent inhibition is calculated by comparing the MEP amplitude following single pulse TMS (A) with the MEP amplitude following peripheral nerve stimulation (B, C; * denotes the conditioned MEP). This example is the outcome of 12 trials recorded in random order at an inter-stimulus interval of 20ms (SAI; B), 200ms (LAI; C) and for the test stimulus alone (A; rate of 1 every 6s, total of 36 trials). Test intensity (black arrows) was set to produce a 1mV MEP. Conditioning stimulation consisted of an electrical pulse (ES, grey arrow) delivered to the ulnar nerve at an intensity sufficient to elicit a visible muscle contraction. Under these conditions, this individual demonstrated 44% SAI and 33% LAI (≥100% represents no inhibition).*

Short latency afferent inhibition is thought to be mediated by acetylcholine (ACh) as it is reduced in patients with degeneration of the central cholinergic system (Di Lazzaro et al. 2002c, Di Lazzaro et al. 2005a). Pharmaco-TMS studies support this hypothesis as SAI is reduced by ACh antagonists (Di Lazzaro et al. 2000b), but increased by neuroactive drugs that enhance cholinergic neurotransmission (Di Lazzaro et al. 2005a). However, there is also pharmacological evidence to indicate that this measure is sensitive to $GABA_A$ergic neurotransmission (Di Lazzaro et al. 2005c). This observation is corroborated by findings of triple pulse TMS studies which

demonstrate mutual inhibition between SAI and SICI ($GABA_A$ mediated) when one mechanism is activated in the presence of the other (Alle et al. 2009).

Thus far, SAI has only been examined in a small number of individuals with chronic shoulder pain (n = 8), where it was reported to be reduced in the infraspinatus muscle, compared to pain free controls (Bradnam et al. 2016). Not only is this the first example of altered SAI in chronic pain, but it is also one of the first descriptions of SAI in a proximal upper limb muscle. However, as these data are derived from a small sample of convenience, the reliability and generalisability of these observations is unclear. A degree of uncertainty also surrounds these findings since the methodology and manifestation of SAI in the upper limb differs to that described for the hand (Hendy et al. 2014). For example, since all previous investigations of SAI have focused on the effect of somatosensory input on the hand motor cortex, it is unclear whether the afferent volley from suprascapular nerve stimulation (conditioning stimulus for infraspinatus) traverses the same pathways and engages the same neural processes (Tokimura et al. 2000, Tsang et al. 2014). Even if confirmed, it remains possible that these findings could be partially spinally mediated, since data were recorded from preactivated infraspinatus and muscle contraction attenuates hand SAI via both cortical and spinal mechanisms (Asmussen et al. 2014). Furthermore, in addition to observing SAI at 20ms inter-stimulus intervals, Bradnam et al. (2016) also reported evidence of MEP inhibition at 30 and 40ms in all participants. This is in direct contrast to what is known to occur in the hand, as SAI is typically absent or replaced with MEP facilitation at these intervals (Tokimura et al. 2000, Fischer et al. 2011).

Reduced SAI in painful infraspinatus may be interpreted several ways. For example, as SAI is linked to cholinergic neurotransmission (Di Lazzaro et al. 2000b), it is possible that this change reflects impaired cholinergic modulation in this condition. Alternatively, as this measure is thought to reflect the effect of sensory input on the excitability of M1, reduced SAI may signal a deficiency in processes related to sensorimotor integration. Possible contributors to this deficiency include reduced activity of interneurons providing direct connections between S1 and M1, and/or increased activity of GABAergic interneurons (as SAI is sensitive to GABA) (Udupa et al. 2009, Tsang et al. 2014, Udupa et al. 2014). However, as both M1 and S1 excitability is reduced by musculoskeletal pain (Schabrun et al. 2013), the exact source of this deficit remains unclear.

### *1.7 Study rationale*

The studies presented in this introduction, provide initial evidence of altered cortical inhibition in acute and chronic musculoskeletal pain. While pain duration appears to be a key determinant of the intracortical response to pain (inhibition is increased in acute pain, but decreased in chronic pain), our understanding of these changes is restricted to the conditions and mechanisms investigated in these studies. Thus, the overarching aim of this thesis was to develop our understanding of the intracortical response to musculoskeletal pain by expanding on the conditions and/or mechanisms already described in the literature. This aim was achieved by four studies that each addressed a key knowledge gap outlined in the introduction.

### 1.7.1 Study 1

The aim of study 1 (Chapter 2) was to investigate the variable nature of the corticomotor response to acute musculoskeletal pain (section 1.5.2). This was achieved via a systematic review and meta-analysis of TMS studies examining the effect of experimental-induced acute muscle pain on the excitability of the primary motor cortex (M1). Somatosensory afference (e.g. nociceptive input) is likely to influence M1 via the primary somatosensory cortex (S1), thus, the excitability of this brain region was also included. As the hemodynamic and metabolic properties of M1/S1 during acute muscle pain are also discussed in this review, this chapter provides the first comprehensive summary of the M1/S1 response to acute muscle pain.

### 1.7.2 Study 2

As outlined in section 1.6, research regarding the effect of acute muscle pain on intracortical mechanisms is lacking. Subsequently, it remains unclear which mechanisms, if any, underpin the corticomotor response to acute pain. Thus, the aim of study 2 (Chapter 3) was to expand on this research by investigating three measures of cortical inhibition previously unexamined in acute musculoskeletal pain; LICI, SAI and LAI. The hypertonic saline model of acute pain was selected for this study in order to control the location, intensity and duration of pain throughout the experimental procedure. As the painful effects of saline are immediate, but quickly resolving, this selection enabled the timely completion of data collection, thus limiting the potential influence of patient fatigue/attention on neurophysiological outcomes. Additional

recommendations outlined by the TMS methodological checklist were also observed in order to easily facilitate comparison of these findings in future studies.

### 1.7.3 Study 3

Although altered cortical inhibition is a likely candidate underpinning M1 reorganisation and motor dysfunction in chronic musculoskeletal pain, our understanding of cortical inhibition in conditions displaying these traits is derived entirely from studies conducted in LBP. Thus, the aim of study 3 (Chapter 4) was to provide further evidence for this hypothesis by examining intracortical mechanisms in lateral epicondylalgia (LE). This condition was selected for study as, similar to LBP, persons with LE demonstrate significant reorganisation of the motor representation of painful muscles, compared to pain-free controls (Table 1.3). In this study, paired-pulse TMS was used to investigate SICI and LICI in persons with and without LE. An additional measure known as intracortical facilitation (ICF) was also included for investigation as preliminary data suggest that the activity of glutamatergic (excitatory) interneurons may also be affected by musculoskeletal pain (Schabrun et al. 2012). In order to minimize the impact of external and internal confounders on these findings and to account for the lack of baseline data from patients, each LE participant was subject to strict inclusion/exclusion criteria and was age and sex matched to a pain-free control participant.

### 1.7.4 Study 4

With evidence pointing towards reduced intracortical inhibition in chronic pain (section 1.6), it is surprising that there has yet to be a discrete investigation of cortical disinhibition in chronic musculoskeletal pain. Thus, the objective of study 4 (Chapter 5) was to examine a single pulse TMS measure known as post-silent period electromyographic bursting (EMG bursting) in persons with and without chronic LBP. The latency and duration of EMG bursting in the hand, as well as its affiliation with LICI and the cortical silent period (both $GABA_B$ mediated), suggests that this phenomena may be a measure of the depth and magnitude of disinhibition in M1 due to the action of pre-synaptic $GABA_B$ receptors (Chin et al. 2012). As EMG bursting is a relatively new measure of cortical inhibition, the first aim of this study was to confirm the presence of this phenomena in the low back muscles of pain free individuals. The temporal and spatial characteristics of EMG bursting throughout the motor representation of the paraspinal muscles of these individuals were then compared with those of individuals with LBP (aim 2).

In the following chapters (Chapter 2 to 5) each study is discussed in detail. Following this, the findings from the four studies are synthesized to provide an overarching theory on the role of cortical inhibition in musculoskeletal pain (Chapter 6).

# Chapter 2:

## Primary sensory and motor cortex function in response to acute muscle pain: a systematic review and meta-analysis

As discussed in detail in Chapter 1, there is increasing evidence to suggest that acute muscle pain affects the excitability and output of M1. The aim of this chapter is to systematically review and meta-analyse these data to determine the direction, strength and temporal profile of the cortical response to experimental pain. The content of this chapter has been published in Burns E, Chipchase LS, Schabrun SM (2016). Primary sensory and motor cortex function in response to acute muscle pain: A systematic review and meta-analysis. European Journal of Pain. 20(8): 1203-13. A copy of this publication is provided in Appendix A.

### *2.1 Abstract*

Acute muscle pain has both motor and sensory consequences, yet the effect of muscle pain on the primary sensory (S1) and motor cortices (M1) has yet to be systematically evaluated. Here we aimed to determine the strength of the evidence for (1) altered activation of S1/M1 during and after pain, (2) the temporal profile of any change in activation and (3) the relationship between S1/M1 activity and the symptoms of pain. In September 2015, five electronic databases were systematically searched for neuroimaging and electrophysiological studies investigating the effect of acute experimental muscle pain on S1/M1 in healthy volunteers. Demographic data, methodological characteristics and primary outcomes for each study were extracted for critical appraisal. Meta-analyses were performed where appropriate. Twenty-five studies satisfied the inclusion criteria. There was consistent evidence from fMRI for increased S1 activation in the contralateral hemisphere during pain, but insufficient evidence to determine the effect at M1. Meta-analyses of TMS and EEG data revealed moderate to strong evidence of reduced S1 and corticomotor excitability during and following the resolution of muscle pain. A comprehensive understanding of the temporal profile of altered activity in S1/M1, and the relationship to symptoms of pain, is hampered by differences in methodological design, pain modality and pain severity between studies. Overall, the findings of this review indicate reduced S1 and corticomotor activity during and after resolution of acute muscle pain, mechanisms that could plausibly underpin altered sensorimotor function in pain.

### *2.2 Introduction*

It is well accepted that acute muscle pain alters sensory and motor function. Yet, the mechanisms that underpin these changes are poorly understood. Current theories on sensorimotor adaptation in pain hypothesize that the primary sensory (S1) and motor (M1) cortex contribute to altered sensorimotor function. For instance, reduced S1 activity is hypothesized to underpin reduced kinaesthesia and position sense (Rossi et al. 2003), whereas reduced M1 activity is hypothesised to underpin restriction of motor output and afford protection from further pain and injury (Hodges et al. 2011).

Numerous studies using a range of methodological tools, including positron emission tomography (PET), functional magnetic resonance imaging (fMRI), transcranial magnetic stimulation (TMS) and electroencephalography (EEG), have investigated how and when S1 and M1 activity are altered in response to acute muscle pain (Svensson et al. 1997c, Niddam et al. 2002, Schabrun et al. 2013). These studies use similar in vivo experimental pain models to induce short-lasting muscle pain that is of a clinical quality (deep, constant, dull or a sharp ache) which allow collection of pre-pain baseline data that cannot be obtained in clinical pain populations (Graven-Nielsen et al. 1997c). However, despite similarities in pain models and brain regions under investigation, there has been no systemic evaluation of S1 or M1 data in acute muscle pain. Integration of data obtained from studies using different methodologies is essential to drive a comprehensive understanding of the nature and time-course of altered S1 and M1 activity in response to acute muscle pain and to elucidate the relationship between S1/M1 and the symptoms of pain.

Here, we synthesised and critically evaluated data corresponding to activity in S1 and M1 cortical regions in order to: (1) examine S1/M1 activation in response to acute muscle pain, (2) quantify the direction and temporal profile of change and (3) determine the evidence for a relationship between altered S1/ M1 activity and symptoms of pain.

## *2.3 Literature Search Methods*

### 2.3.1 Search strategy

In line with the methodology outlined by the Cochrane Handbook for Systematic Reviews of Interventions, the initial search strategy involved examination of major biomedical science databases EMBASE and MEDLINE (Higgins et al. 2011). Pubmed, Scopus and Web of Science were also examined to ensure comprehensive coverage of the literature and minimize selection bias (5 electronic databases in total). Together, these databases are a valuable source of both publisher controlled- and grey literature (such as conference proceedings). Relevant studies were identified using MeSH terms and free text terms including motor cortex, somatosensory cortex, muscle pain, acute pain, and experimental pain. The most recent search was performed in September 2015. Studies were first screened for relevance by title and abstract before analysis of full text. Inclusion was dependent upon the following criteria: (1) English language, (2) original, primary research, (3) healthy adult human subjects, (4) acute experimental pain was induced in a muscle, (5) acute muscle pain was induced in the absence of another stimulation or intervention, and (6) outcome measures included full brain image analysis of regional cerebral blood flow (rCBF;

measured using PET or fMRI) or blood-oxygen-level dependent contrast imaging (BOLD; measured using fMRI), corticomotor excitability (motor evoked potentials; measured using TMS) and/or sensory cortex excitability (somatosensory evoked potentials; measured using EEG). To ensure the reliability of the process for inclusion/exclusion of studies, 10 abstracts were selected at random and independently reviewed by three assessors.

### 2.3.2 Data extraction and assessment of methodological quality

A standard form was used to extract subject demographics, methodological parameters (techniques, outcome measures) and pain characteristics (modality, location, intensity) for each study. Additional technical information specific to EEG (electrode orientation, conditioning location and intensity) and TMS (target muscle, muscles state, TMS intensity, coil type and position) methodologies was also recorded. Primary outcome measures included MEP amplitude/area to single and/or paired-pulse TMS, the amplitude/area of SEP components/complexes corresponding to S1 activation/processing and the direction of change (increase or decrease) in rCBF or BOLD-contrast.

Methodological quality was appraised using a modified version of the Downs and Black's checklist (Downs et al. 1998) (Appendix A.1). A maximum score of 17 points were awarded based on reporting within the text and external and internal validity. Studies involving TMS were further appraised using the TMS methodological checklist (Chipchase et al. 2012). The maximum score for reported and controlled items was 26 or 30 points, depending on methodology (single or paired-pulse TMS). The

summed score for reported items (r) as a percentage of the maximum score [r/(26 or 30) x 100] to provide an indication of adherence to the checklist. The summed score for controlled items (c) was expressed as a percentage of reported items [(c/r) x 100] to determine the extent to which reported items were controlled.

### 2.3.4 Meta-analyses

Meta-analyses were performed on MEP and SEP data but not for rCBF or BOLD-contrast due to the heterogeneity of the analysis approaches used between studies and inconsistent reporting of non-significant findings. Mean ± standard deviation for MEP and SEP amplitude/area were extracted at time points 'baseline', 'during pain' and 'post pain', where available. When data was not reported within the text, an email was sent to the corresponding author to request missing values. If authors were no longer contactable, did not respond, or declined requests for data, means ± standard deviation/standard error were estimated by handfrom illustrations or calculated from available t values, p values or F statistics. Standardized mean differences (SMD) and 95% confidence intervals were calculated using a random-effects model in RevMan 5.2 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012) and heterogeneity determined using calculations of I2. Effect estimates ≤ 0.2 were considered small, 0.5 moderate and ≥ 0.8 large.

## *2.4. Results*

### 2.4.1 Search results

The search strategy retrieved 257 studies, minus duplicates (Figure 2.1). Screening of title and abstract and evaluation of full text identified 20 suitable studies. Examination of reference lists revealed an additional five studies, thus, a total of 25 studies were included in the systematic review.

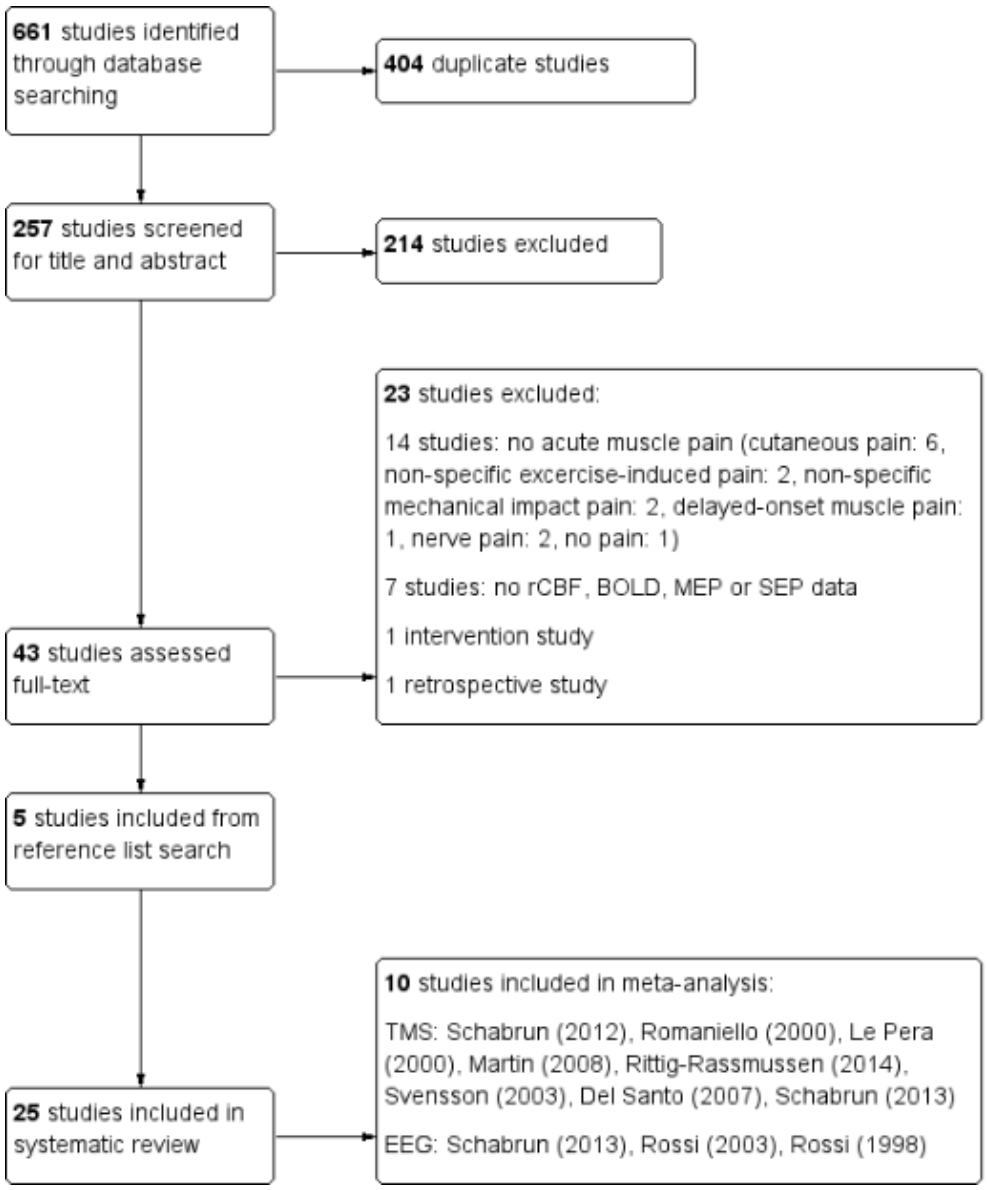


***Figure 2.1*** *Search strategy flow diagram.*

### 2.4.2. Study characteristics

Of the 25 included studies, four examined rCBF with PET (participants, n = 56), two examined rCBF with fMRI (n = 32), eight examined BOLD responses with fMRI (n = 147), eight examined corticomotor excitability (MEPs) with TMS (n = 80) and three examined sensory cortex excitability (SEPs) with EEG (n = 25). One study reported on both corticomotor and sensory cortex excitability (Schabrun et al. 2013). Hypertonic saline was the most common method of inducing experimental muscle pain (n = 17) and most studies (n = 13) induced pain into muscles of the upper extremity (hand, forearm or arm). Other sites included muscles of the leg (studies, n = 7), jaw (n = 4), neck (n = 1) and low back (n = 1). Demographic information and pain characteristics of included studies are presented in Table 2.1.

***Table 2.1*** *Characteristics of included studies (n = 25). Pain is described as mild, moderate, severe based on reported pain score (mild: < 3.0, moderate: 3.0 - 5.4, severe: > 5.4).*

| | DEMOGRAPHICS | | | METHODOLOGY | | PAIN CHARACTERISTICS | | | METHODOLOGICAL QUALITY | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **STUDY** *(year)* | *N* | *male, female* | *Age (years, mean ± SD)* | *Technique* | *Outcome measures* | *Model* | *Location* | *Intensity* | *Downs and Black Checklist /17* | *TMS Checklist Reported %* | *TMS Checklist Controlled %* |
| ***Kupers (2004)*** | 10 | 6,4 | 21-25 (range) | PET | rCBF, $^{15}O$ labelled water | hypertonic saline | masseter | severe [p] | 14 | - | - |
| ***Svensson (1997)*** | 11 | 11, 0 | 30.4 ± 3.5 | PET | rCBF, $^{15}O$ labelled water | electrical stimulation | brachioradialis | mild [p] | 14 | - | - |
| ***Thunberg (2005)*** | 19 | 19, 0 | 26.7 ± 7.6 | PET | rCBF, $^{15}O$ labelled water | hypertonic saline | erector spinae at L3 | moderate p | 14 | - | - |
| ***Korotkov (2002)*** | 16 | 16, 0 | 24.3 ± 7.7 | PET | rCBF, $^{15}O$ labelled water | hypertonic saline | triceps brachii | moderate [a] | 13 | - | - |
| ***Owen (2010)*** | 13 | 13, 0 | 29.5 ± 5 | fMRI | CBF via ASL at 3T | hypertonic saline | brachioradialis | severe [a] | 14 | - | - |
| ***Owen (2012)*** | 19 | 19, 0 | 26 ± 5 | fMRI | CBF via ASL at 3T | hypertonic saline | brachioradialis | moderate a | 13 | - | - |
| ***Nash (2010a)*** | 25 | NR | NR | fMRI | BOLD at 3T | hypertonic saline | masseter | moderate [p] | 12 | - | - |
| ***Uematsu (2011)*** | 17 | 10, 7 | 23-33 (range) | fMRI | BOLD at 1.5T | pressure (skin anaesthetised), 10mm probe | gastrocnemius | moderate [a] | 12 | - | - |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Henderson (2006)*** | 15 | NR | NR | fMRI | BOLD at 3T | hypertonic saline | tibialis anterior | moderate [p] | 11 | - | - |
| ***Loggia (2012)*** | 16 | 11, 5 | 28.8 ± 9.7 | fMRI | BOLD at 3T | pressure, 13.5cm cuff | gastrocnemius | 7 increments: mild [p] - severe [p] | 14 | - | - |
| ***Macefield (2007)*** | 22 | 11, 11 | 22-49 (range) | fMRI | BOLD at 3T | hypertonic saline | tibialis anterior, flexor carpi radialis | severe [p] | 13 | - | - |
| ***Nash (2010b)*** | 17 | NR | NR | fMRI | BOLD at 3T | hypertonic saline | masseter | moderate [p] | 13 | - | - |
| ***Maeda (2011)*** | 12 | 7, 5 | 24-56 (range) | fMRI | BOLD at 1.5T | pressure, 10mm probe | gastrocnemius | moderate [a], severe [a] | 14 | - | - |
| ***Niddam (2002)*** | 10 | 10, 0 | 26.8 ± NR | Event-related fMRI | BOLD at 3T | electrical stimulation | abductor pollicis brevis | NR | 13 | - | - |
| ***Takahashi (2011)*** | 13 | 13, 0 | 20-36 (range) | Event-related fMRI | BOLD at 3T | electrical stimulation | tibialis anterior | mild [p], moderate [p], severe [p] | 13 | - | - |
| ***Schabrun (2012)*** | 11 | 7, 4 | 23.3 ± 6.5 | TMS | MEP amp, SICI, ICF. | hypertonic saline | first dorsal interosseous | severe [a] | 13 | 87 | 91 |
| ***Svensson (2003)*** | 10 | 8, 2 | 34.3 ± 4.0 | TMS | MEP amp | hypertonic saline | first dorsal interosseous | moderate [p] | 13 | 81 | 81 |
| ***Del Santo (2007)*** | 8 | 5, 3 | 33.9 ± 11.5 | TMS | MEP area | ascorbic acid | abductor digiti minimi, biceps brachii | severe [p] | 13 | 66 | 95 |
| ***Martin (2008)*** | 6 | NR | NR | TMS | MEP amp | hypertonic saline | biceps brachii | moderate [p] | 11 | 66 | 83 |
| ***Rittig-Rasmussen (2014)*** | 12 | NR | 23 ± 2 | TMS | MEP amp | hypertonic saline | neck muscular tissue 2cm lateral to C3 | moderate [a] | 13 | 74 | 90 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Romaniello (2000)*** | 10 | NR | NR | TMS | MEP amp | hypertonic saline | masseter | moderate a | 9 | 81 | 96 |
| ***Le Pera (2001)*** | 10<br>11 | 7, 3<br>8, 3 | 23.5 ± 2.7<br>26.1 ± 4.8 | TMS | MEP amp | hypertonic saline | abductor digiti minimi, first dorsal interosseous; flexor carpi radialis | severe [p] & moderate [p]; moderate [p] | 12 | 85 | 91 |
| ***Schabrun (2013)*** | 12 | 5, 7 | 28 ± 9 | TMS, EEG | MEP amp, SEP area | hypertonic saline | first dorsal interosseous | moderate a | 13 | 81 | 77 |
| ***Rossi (2003)*** | 6 | NR | NR | EEG | SEP area | levo-ascorbic acid | first dorsal interosseous | severe p | 12 | - | - |
| ***Rossi (1998)*** | 7 | 5, 2 | 22-40 (range) | EEG | SEP area | levo-ascorbic acid | extensor digitor brevis | severe p | 9 | - | - |

TMS, transcranial magnetic stimulation; EEG, electroencephalography; PET, positron emission tomography; fMRI, functional magnetic resonance imaging; rCBF, regional cerebral blood flow; ASL, arterial spin labelling; BOLD, blood-oxygen-dependent level contrast imaging; MEP, motor evoked potential; SEP, somatosensory evoked potential; [a], average pain score; [p] , peak pain score; NR, not reported.

### 2.4.3 Methodological quality

Average scores for methodological quality were 12.1 ± 1.5 (out of 17) for MEP studies, 11.3 ± 2.1 for SEP studies, 13.8 ± 0.5 for rCBF studies and 12.9 ± 0.9 for BOLD studies. Items consistently unmet by reviewed studies related to internal and external validity. For example, male and female participants were unequally represented in the majority of study samples (Macefield et al. 2007) and sample size calculations were rarely performed a priori (Rittig-Rasmussen et al. 2014). No study blinded the investigator during data analysis and all recruited a sample of convenience.

The mean score for reported items of the TMS checklist was 78 ± 8%. Compliance was high for methodological items, however, items relating to participant characteristics, were less well reported. This may be because authors may not have considered it necessary to collect or report detailed data on health characteristics for participants considered 'healthy'. All studies reported on subject age and gender, position and contact of EMG electrodes, amount of relaxation/contraction of muscles, prior motor activity of the tested muscle, coil location and stability, type of stimulator, stimulation intensity, subject attention, number of MEP recordings and the method for determining MEP size in analysis. The mean score for controlled items was 88 ± 7%. Items reported but poorly controlled included subject gender (bias towards male participants), prior activity of the muscle being tested, level of relaxation of muscles not being directly tested, and coil stability. Individual study scores are presented in Table 2.1.

### 2.4.4 The effect of experimental muscle pain at the primary sensory cortex (S1).

#### *2.4.4.1 Regional cerebral blood flow (rCBF)*

Four studies investigated rCBF using PET and two studies investigated rCBF using fMRI and arterial spin labelling (ASL) (Table S1; Appendix A.2). Full brain image analysis using PET (studies, n = 4) revealed no effect of muscle pain on rCBF at S1. There was also no effect on ASL-derived rCBF (n = 2) when pain was of moderate intensity (Owen et al. 2012), however a bilateral reduction at S1 was observed in response to severe pain (Owen et al. 2010). These conflicting findings may be explained by pain severity as reduced rCBF at S1 was found to negatively correlate with subjective pain ratings for the pain (hypertonic saline) and control groups (isotonic saline) in the latter study (contralateral S1: R2 = 0.75, $p$ = 0.000; ipsilateral S1: R2 = 0.69, $p$ = 0.000) (Owen et al. 2010).

#### *2.4.4.2 Blood-oxygen-level dependent (BOLD)-contrast imaging*

Seven of the nine studies that investigated BOLD responses using fMRI reported increased S1 activation in response to muscle pain (Table S1; Appendix A.2). Three studies reported increased BOLD-contrast bilaterally at S1 (Niddam et al. 2002, Nash et al. 2010a, b), while three studies reported increases exclusive to the hemisphere contralateral to the side of hypertonic saline- or electrically induced muscle pain (Henderson et al. 2006, Macefield et al. 2007, Takahashi et al. 2011). In contrast, results for mechanically induced pain were conflicting. For example, Loggia et al (Loggia et al. 2012) reported an increased BOLD response at S1 in the contralateral hemisphere and a decreased response in the ipsilateral hemisphere following painful

pressure stimulation, whereas Uematsu et al and Maeda et al (Maeda et al. 2011, Uematsu et al. 2011) reported no change for either hemisphere. These discrepancies could be explained by differences in the spatial resolution of the pressure stimulation between these studies (pressure cuff vs. pressure probe).

*2.4.4.3 Somatosensory evoked potentials (SEPs)*

Three studies used EEG to investigate the effect of acute muscle pain on S1 excitability following electrical stimulation of the ulnar (Rossi et al. 2003, Schabrun et al. 2013) or peroneal nerve (Rossi et al. 1998) (Table S2; Appendix A.3). Ulnar SEPs were recorded using surface electrodes placed on the scalp 2cm posterior to C3 using the 10-20 International EEG system, while peroneal SEPs were recorded subcutaneously at a location 2cm posterior to Cz on the midline. All studies reported on components thought to reflect S1 activation ($P_{14}$-$N_{20}$, $P_{40}$-$N_{50}$) and processing ($N_{20}$-$P_{25}$-$N_{33}$, $P_{60}$-$N_{75}$). As pooled analyses revealed that the area of components corresponding to S1 activation was unchanged both during (Figure S1; Appendix A.5) and following (Figure S2; Appendix A.6) the resolution of muscle pain [-0.36 (-0.86, 0.14), -0.23 (-0.72, 0.26)], this suggests that input to S1 remains stable over time. In contrast, pooled effect estimates during pain show a strong reduction in the area of components corresponding to S1 processing [1.99 (0.64, 3.34)]. A moderate reduction [0.65 (0.15, 1.16)] is also present post pain. Such findings suggest that acute muscle pain may exert a lasting inhibitory effect on S1 excitability.

### 2.4.5 The effect of experimental muscle pain on the primary motor cortex (M1) and corticomotor pathway.

#### *2.4.5.1 Regional cerebral blood flow (rCBF)*

Full brain image analysis (PET n = 4, fMRI n = 2) revealed no change in rCBF at M1 in any study (Table S1; Appendix A.2).

#### *2.4.5.2 Blood-oxygen-level dependent (BOLD)-contrast imaging*

At the location of M1, findings for BOLD contrast were mixed (Table S1; Appendix A.2). For example, four studies reported increased BOLD responses in the hemisphere contralateral to the side of pain (Henderson et al. 2006, Nash et al. 2010b, Takahashi et al. 2011, Loggia et al. 2012), while five studies observed no change (Niddam et al. 2002, Macefield et al. 2007, Nash et al. 2010a, Maeda et al. 2011, Uematsu et al. 2011). These discrepancies do not appear to be related to the use of different pain models between studies but may be related to pain severity since a positive relationship between increased BOLD and pain was observed in one study (Loggia et al. 2012). However, as the results from two additional studies indicate that increased BOLD outlasts peak pain and persists during waning pain (Henderson et al. 2006, Takahashi et al. 2011), the nature of this relationship remains uncertain. Interpretation of these findings is further complicated by observations from Nash et al, who reported that initial increases in BOLD-contrast were followed by lasting decreases in signal intensity (Nash et al. 2010b). With the exception of one study (Henderson et al. 2006), there is no evidence of an effect of muscle pain on ipsilateral BOLD responses.

*2.4.5.3 Transcranial magnetic stimulation: assessment of corticomotor excitability*

Eight studies investigated corticomotor excitability using TMS (Table S3; Appendix A.4). Separate meta-analyses were performed for data collected with the muscle at rest and during active contraction and sub group analysis was performed for 'target' or 'non-target' muscles. 'Target' refers to the muscle where experimental pain was induced, and was reported in all eight studies. The term 'non-target' refers to muscles remote, synergistic or antagonistic to the 'target muscle' and was reported in three studies (Le Pera et al. 2001, Svensson et al. 2003c, Martin et al. 2008).

The size of the MEP to single-pulse TMS was reported by five studies 'during pain' (Figure S3; Appendix A.7). Pooled effect estimates revealed a moderate reduction in corticomotor excitability (MEP amplitude/area) from 'baseline' for target and non-target muscles at rest [0.52 (-0.01, 1.06), 0.72 (0.01, 1.42)], but not for target muscles during active contraction [-0.13 (-0.61, 0.35)]. 'Post pain' data collected either 0 (immediately post), 20 or 30 minutes following the cessation of pain or painful stimulation was available from all eight studies (Figure S4; Appendix A.8). Compared to 'baseline' there was a strong reduction in MEP amplitude/area for target muscles at rest [0.97 (0.59, 1.35)] and a similar trend for non-target muscles at rest [0.37 (-0.02, 0.76)}. A moderate reduction in MEP amplitude/area was also detected for actively contracting target muscles [0.44 (0.05, 0.83)].

*2.4.5.4 Transcranial magnetic stimulation: intracortical inhibitory and facilitatory networks*

The MEP response to paired-pulse TMS was examined by one study (Schabrun et al. 2012). Short-interval intracortical inhibition (at 2 and 3ms inter-stimulus intervals) was increased immediately following (2ms: $p = 0.012$, 3ms: $p = 0.007$), but not during muscle pain (2ms: $p = 0.24$; 3ms: $p = 0.61$). In contrast, intracortical facilitation (13ms inter-stimulus interval) was reduced compared to baseline both during ($p = 0.009$) and immediately post ($p = 0.001$) muscle pain.

## *2.5 Discussion*

The aim of this review was to synthesize and critically evaluate evidence for altered S1 and M1 activity in response to acute muscle pain. The findings provide evidence of reduced excitability in the contralateral S1 both during and following pain and moderate-strong evidence of reduced corticomotor output to the painful muscle. Currently, there is insufficient evidence to draw conclusions regarding effects at ipsilateral S1/M1.

### 2.5.1 The effect of acute muscle pain on S1

Eight of nine studies using fMRI provide evidence of altered activation (increased BOLD-contrast) at S1 in the hemisphere contralateral to the side of induced pain, during pain. As BOLD-contrast is a measure of cerebral metabolism based on the level deoxyhaemoglobin content, and oxygen demands are assumed to be higher at areas of increased cortical function (Howseman et al. 1999), such observations imply

increased synaptic activation. However, as fMRI detects hemodynamic and not electrophysiological changes, it is unclear whether increased synaptic activation reflects increased or decreased S1 excitability. The inclusion of studies that record SEPs in response to pain provides insight into the direction of the effects observed with fMRI. The area of the $N_{20}$-$P_{25}$-$N_{33}$ and $P_{60}$-$N_{75}$ potential is thought to reflect early cortical processing of low-threshold somatosensory afferent information related to kinaesthesia and position sense (Rossi et al. 1998, Rossi et al. 2003) and is commonly used to infer changes in S1 excitability. Pooled-analysis of the three EEG studies provides strong evidence that acute muscle pain activates synaptic processes that reduce S1 excitability. Thus, taken together, data from fMRI and SEP studies suggest that S1 is altered during acute muscle pain and that this change is in the direction of reduced S1 excitability. However, as the relationship between SEP amplitude and BOLD contrast has not been characterized at stimulation intensities at or above pain threshold, evidence to support a correlation between these methodologies under pain conditions is limited.

Although the functional relevance of altered S1 activity during pain was not directly investigated in this review, previous studies have suggested that reduced S1 excitability may reflect a defensive adaptation designed to orient cortical attention towards stimuli that threaten the body's integrity (Legrain et al. 2011). Such a mechanism may reduce processing of non-painful afferent information and thus contribute to reduced sensorimotor performance when pain is present (Rossi et al. 1998, Rossi et al. 2003). However, further work is needed to determine the

relationship between reduced S1 excitability during pain and altered sensorimotor function.

The present review provides moderate evidence of reduced S1 excitability following the resolution of pain. However, this evidence is drawn exclusively from SEP data collected immediately post pain (Rossi et al. 2003, Schabrun et al. 2013) and further research is required to determine the duration and reliability of these findings. The effect of pain on the ipsilateral S1 also remains unclear as synthesis of fMRI data was largely inconclusive and SEPs were not investigated in this hemisphere. The results of all five PET studies show no S1 activation during pain. Since previous studies have shown that fMRI and PET activations typically correlate under identical stimulus conditions (Dettmers et al. 1996, Sadato et al. 1998), these findings were unexpected. One explanation may be that the lower temporal resolution of PET precluded the identification of rapid and/or transient pain-related neural activity. fMRI images are typically sampled between 0.3 and 3s, whereas PET image acquisition is performed over a longer time period (50 - 120s). If pain related changes in S1 occurred prior to 50s they may not have been detected using PET methodologies. Alternatively, as analyses are typically performed on data averaged over the entire scanning period, it is possible that changes were not detected due to a dynamic, fluctuating pattern of cortical activation during acute muscle pain.

### 2.5.2 The effect of acute muscle pain on M1

The effect of acute muscle pain on BOLD-contrast at M1 in the hemisphere contralateral to the side of induced pain varied between studies with some reporting increased activation (n = 4), some decreased activation (n = 1) and some no change (n = 5) during pain. With the exception of one study (Henderson et al. 2006), there was no evidence for an effect of acute muscle pain on BOLD-contrast in the ipsilateral M1. In contrast, meta-analyses of MEP data show moderate evidence of reduced corticomotor output to painful muscles when pain is present. Inconsistent findings between fMRI and TMS could be explained by differences in temporal resolution between methodologies or alternatively, by changes occurring at spinal and/or peripheral, rather than cortical, level. As the MEP is a summative measure of cortico-cortical, cortico-motoneuronal and spinal motoneuron synaptic excitability, changes occurring at cortical, spinal and/or peripheral level may have contributed to the reduction in corticomotor output observed during pain. Few TMS studies controlled for changes in peripheral (n = 3) or spinal excitability (n = 3) and only one study examined mechanisms thought to directly reflect activity in M1. That study reported enhanced activity of M1 intra-cortical circuits mediated by the inhibitory neurotransmitter GABA, and decreased activity of facilitatory circuits acting through NMDA receptors on glutamatergic interneurons (Schabrun et al. 2012). Therefore, further research is required to determine whether reduced corticomotor output during pain reflects reduced activity in M1.

A novel finding during pain was the non-specificity of the corticomotor response. Pooled analyses of data from two TMS studies show that muscles in the same body

segment, but not directly subjected to pain, exhibit reduced corticomotor output (Le Pera et al. 2001, Martin et al. 2008). Although there is currently insufficient data to determine whether this effect is limited to muscles at rest, it is possible that a similar response may occur in active muscles but remains undetected due to the facilitatory influence of volitional contraction on measures of MEPs (Di Lazzaro et al. 1998b). This non-specificity suggests that an indiscriminate motor strategy is employed during pain. Although the functional significance remains unclear, it is possible that this adaptation serves to decrease muscle coordination and splint the affected body part (Schabrun et al. 2015b). Such *en masse* movement strategies would likely prevent symptom aggravation and afford protection to an injured limb (Hodges et al. 2011).

A strong reduction in corticomotor output was also found for painful muscles in the post pain period at rest. The relative strength of this response may explain why a low-moderate reduction in corticomotor output to actively contracting muscles was also detected at this time point. A strong trend towards MEP suppression was also observed for non-painful muscles at rest. In line with current theories, it is possible that these reductions persist in the post-pain period as a defense against the threat of further pain and injury (Hodges et al. 2011).

### 2.5.3 Relationship between S1 and M1 activity and the symptoms of pain

There is currently insufficient evidence to determine whether the neurophysiological changes described in this review are related to subjective assessments of pain severity. Although all fMRI investigations were conducted within the 'during pain' time period, a relationship to pain severity is impeded by inconsistent findings. For

example, one study provided evidence of severity-dependent S1/M1 activations during pain (Loggia et al. 2012), whereas several others demonstrate strong cortical effects despite waning symptoms (Henderson et al. 2006, Nash et al. 2010b, Takahashi et al. 2011). As additional activations/deactivations were also observed in the ipsilateral hemisphere by some authors (Niddam et al. 2002, Henderson et al. 2006, Nash et al. 2010b, a, Owen et al. 2010, Loggia et al. 2012), there is also doubt regarding the specificity of this response. Furthermore, although the majority of EEG and TMS studies reported findings during pain (Romaniello et al. 2000, Le Pera et al. 2001, Rossi et al. 2003, Del Santo et al. 2007, Martin et al. 2008, Schabrun et al. 2013), few studies explicitly examined S1/M1 excitability in association with pain severity (Le Pera et al. 2001, Rossi et al. 2003). However, as meta-analyses of these studies show moderate-strong evidence of reduced corticomotor output and S1 excitability that persist after pain has resolved, a linear relationship between objective neurophysiological measures and subjective pain recordings appears unlikely.

### 2.5.4 Limitations and Recommendations

This review has a number of limitations that require consideration. First, findings from multiple methodologies with unique temporal and spatial resolutions were included to provide insight regarding the extent, time-course and direction of the effect of pain on S1/M1. However, as the relationship between hemodynamic and electrophysiological measures remains uncertain, comparison of findings across methodologies is limited. Future protocols that combine PET/fMRI (high spatial resolution) with TMS/EEG (high temporal resolution) would clarify the effect of acute muscle pain on S1/M1 and the relationship between these measures.

The small number of studies identified for each methodology, as well as differences in study design, pain modality and severity, also limit discussion. For example, pain severity was reported as a value corresponding to either 'peak' or 'average' and ranged in severity from 'mild' to 'severe'. As such, this review was unable to discern a clear effect of pain severity and S1/M1 change between or within outcome measures. There is also insufficient evidence to ascertain the duration of S1/M1 change following the resolution of muscle pain due to inconsistent time points in TMS/EEG studies and a lack of post-pain data from PET/fMRI. Standardized recording intervals, in addition to improved descriptions of whether the interval refers to the time elapsed since the cessation of pain or since the cessation of the pain intervention (pain remains), would help clarify the duration of these effects. Future protocols should also include objective measures of sensory and/or motor function in order to elucidate the functional significance of S1/M1 adaptations both during and post muscle pain.

# Chapter 3:

## Reduced short- and long-latency afferent inhibition following acute muscle pain: a potential role in the recovery of motor output

The findings of the Chapter 2 suggest that acute muscle pain exerts a net inhibitory effect on the primary motor (M1) and somatosensory cortices (S1). This chapter reports on the findings of an experimental study designed to investigate whether intracortical inhibitory mechanisms such as long interval intracortical inhibition (LICI) and short/long latency afferent inhibition (SAI/LAI) could plausibly contribute to such a response. The content of this chapter has been published in *Burns E, Chipchase LS, Schabrun SM (2016). Reduced Short- and Long-Latency Afferent Inhibition Following Acute Muscle Pain: A Potential Role in the Recovery of Motor Output. Pain Medicine. 17(7): 1343-52.* A copy of this publication is provided in Appendix B.

### *3.1 Abstract*

Corticomotor output is reduced in response to acute muscle pain, yet the mechanisms that underpin this effect remain unclear. Here the authors investigate the effect of acute muscle pain on short-latency afferent inhibition (SAI), long-latency afferent inhibition (LAI) and long-interval intra-cortical inhibition (LICI) to determine whether these cortical mechanisms could plausibly contribute to reduced motor output in pain. This observational, same subject, pre-post test design study was performed in a neuroplasticity research laboratory setting. Twenty-two healthy, right-handed human volunteers (nine males; mean age ± standard deviation, 22.6 ± 7.8 years) participated. Transcranial magnetic stimulation was used to assess corticomotor output, SAI, LAI and LICI before, during, immediately after and 15 minutes after hypertonic saline infusion into right first dorsal interosseous muscle. Pain intensity and quality were recorded using an 11-point numerical rating scale and the McGill Pain Questionnaire. Compared with baseline, corticomotor output was reduced at all time points ($p$ = 0.001). SAI was reduced immediately after ($p$ = 0.039), and LAI 15 minutes after ($p$ = 0.035), the resolution of pain. LICI was unchanged at any time point ($p$ = 0.36). These findings suggest SAI and LAI, mechanisms thought to reflect the integration of sensory information with motor output at the cortex, are reduced following acute muscle pain. Although the functional relevance is unclear, the authors hypothesize a reduction in these mechanisms may contribute to the restoration of normal motor output after an episode of acute muscle pain.

### *3.2 Introduction*

Pain affects sensorimotor function. When pain is present, the capacity of a muscle to generate force is diminished (Graven-Nielsen et al. 1997d, Graven-Nielsen et al. 2002b, Slater et al. 2003), muscle co-ordination is altered (Arendt-Nielsen et al. 1996, Henriksen et al. 2009, Henriksen et al. 2011, Jacobs et al. 2011), proprioception is distorted (Matre et al. 2002, Rossi et al. 2003, Weerakkody et al. 2008) and the ability to integrate sensory information with motor commands (sensorimotor integration) is impaired (Malmstrom et al. 2013). A number of authors have shown reduced corticomotor output during and after the resolution of muscle pain (Le Pera et al. 2001, Svensson et al. 2003c, Martin et al. 2008, Schabrun et al. 2012, Schabrun et al. 2013), and these changes are hypothesized to contribute to altered sensorimotor function. However, the mechanisms that underpin reduced corticomotor output in pain are unclear.

Changes occurring across multiple levels of the nervous system (peripheral, spinal, cortical) could reasonably contribute to altered motor output in pain. Previous studies have excluded changes at the muscle, as the peripheral M-wave (muscle compound action potential), contractile properties and conduction velocity of action potentials along the muscle fiber membrane are unchanged (Svensson et al. 1998, Graven-Nielsen et al. 2002b, Svensson et al. 2003c, Farina et al. 2004). At the level of the spinal cord, findings have been contradictory. One study demonstrated suppression of motoneurons in the late phase of pain (Le Pera et al. 2001), while another demonstrated facilitation (Martin et al. 2008). This discrepancy could be explained by the use of different measures (H-reflex vs. cervicomedullary-evoked

potentials) to record spinal excitability. Regardless of the direction of spinal effects, both studies reported depression of motor cortical excitability, suggesting cortical mechanisms may contribute to the reduced motor output observed in pain.

A recent study has provided the first evidence for altered activity in motor cortical inhibitory and facilitatory networks in response to pain (Schabrun et al. 2012). That study used transcranial magnetic stimulation (TMS) paired pulse paradigms to show a reduction in intracortical facilitatory (glutamate-mediated), and an increase in intracortical inhibitory (gamma-aminobutyric acid $[GABA]_A$ receptor mediated), networks during and immediately after the resolution of pain. The authors hypothesized that these changes act to restrict activity of painful muscles and limit the range and velocity of movement in pain, protecting the painful part from further pain and/or injury (Hodges et al. 2011). However, the impact of acute muscle pain on other intracortical networks such as those mediated by $GABA_B$ (long-interval intracortical inhibition; LICI), or circuits involved in the integration of sensory information with motor execution (sensorimotor integration, measured using short- [SAI] and long-latency [LAI] afferent inhibition protocols) has not been investigated. Thus, here we aimed to examine the effect of acute experimental muscle pain on intracortical inhibitory networks, specifically those mediated by $GABA_B$, and networks associated with sensorimotor integration.

## *3.3 Methods*

### 3.3.1 Participants

Twenty-two healthy, right-handed volunteers participated (mean ± standard deviation; nine males, age = 22.6 ± 7.8 years). As afferent inhibition and LICI are yet to be investigated in response to acute pain, sample size calculations were based on effect sizes from a previous study examining the effect of innocuous afferent input (water immersion) on SAI and LAI (Sato et al. 2013). Additional participants were recruited to account for the possibility of exclusions, as it has been shown that approximately 30% of individuals fail to respond to afferent inhibition protocols at baseline under uniform test conditions (Asmussen et al. 2013). Based on these assumptions, a sample size of 22 was sufficient to observe a statistically significant difference in response to pain (80 % power, alpha 0.05), should one exist. Handedness was determined using the Edinburgh Handedness inventory (laterality quotient, 88.8 ± 19.6) (Oldfield 1971). All volunteers completed a TMS safety screening questionnaire (Keel et al. 2001) and were excluded if they had a recent history of arm pain or injury, a personal or family history of epilepsy, major neurological, respiratory, orthopaedic or circulatory disorders, were pregnant, had metal in their head or jaw or were taking central nervous system acting medication (Keel et al. 2001). The study was approved by the institutional human medical research ethical committee and performed in accordance with the Declaration of Helsinki. All participants provided written, informed consent. All testing took place in a University laboratory.

### 3.3.2 Electromyography (EMG)

Surface EMG was recorded from FDI of the right hand. Dual silver-silver chloride disposable electrodes (spacing 2.0 cm; Noraxon Inc, USA) were positioned over the muscle in a belly-tendon arrangement and remained in place for the duration for the experiment. The ground electrode was positioned over the right olecranon. EMG signals were amplified x1000, band-pass filtered 20-1000 Hz (Neurolog System Pre–amplifier; Digitimer Ltd, UK), and sampled at 2000 Hz using a Micro3 1401 Data Acquisition System and Signal5 software (Cambridge Electronic Design, Cambridge, UK).

### 3.3.3 Corticomotor output

Corticomotor output was investigated using single pulse TMS delivered to the primary motor cortex (M1) using a Magstim $200^2$ stimulator (Magstim Co. Ltd., Dyfed, UK) connected to a figure–of -eight coil (70mm wing diameter). TMS was performed over the left hemisphere (contralateral to the side of pain) for all participants. The coil was oriented at a 45-degree angle to induce posterior-anterior current flow and positioned over the motor 'hotspot' of FDI (Groppa et al. 2012). The motor hotspot was determined via systematic application of TMS to the scalp until the site that produced the largest motor evoked potential (MEP) was identified. A Brainsight neuronavigation system (Rogue Research Inc, Quebec, Canada) was used to target this site and ensure accurate coil placement for the duration of the experiment. All procedures adhered to the TMS checklist for methodological quality and were performed with the muscle at rest (Chipchase et al. 2012). Fifteen MEPs (rate of 1

every 6s) were recorded using a constant stimulator intensity sufficient to evoke a MEP of approximately 1mV peak-to-peak amplitude in FDI at baseline.

### 3.3.4 Long-interval intra-cortical inhibition

A standard paired-pulse TMS protocol, using a supra-threshold conditioning stimulus delivered before a supra-threshold test stimulus (Claus et al. 1992), was used to evaluate LICI. Evidence suggests that spinal mechanisms may contribute to the inhibitory response when conditioning stimuli precedes the test stimulus by 80-100ms (McNeil et al. 2009, 2011). Thus, in order to reduce the likelihood of a spinal contribution in the present study, LICI was tested using an inter stimulus interval (ISI) of 160ms. The intensity for both the conditioning and test stimuli was set to evoke a peak-to-peak response of 1mV in FDI. As the amplitude of the test MEP is known to influence the magnitude of the LICI response (McNeil et al. 2011), it is possible that pain-induced fluctuations in test MEP amplitude may impact the accuracy of LICI estimation in this study. Thus, TMS intensity of the test stimulus was adjusted to maintain a consistent 1mV test response throughout the experimental protocol (pre, during and post pain). Twelve trials were recorded in random order at ISI 160ms (LICI) and for the test stimulus alone (rate of 1 every 6s; total of 24 trials). As test TMS intensity was adjusted where necessary, the peak-to-peak amplitude of the test MEP alone was not suitable to use as an additional measure of corticomotor output.

### 3.3.5 Short- and long-latency afferent inhibition

SAI and LAI were assessed by pairing a conditioning electrical stimulus with a suprathreshold TMS test stimulus at ISIs of 20ms and 200ms respectively (Tokimura

et al. 2000). Electrical stimuli were delivered via a Digitimer Constant Current stimulator (DS7A; Digitimer Ltd, UK) connected to surface electrodes placed 2 cm proximal to the wrist crease. Single electrical stimuli (100 μs duration, 400V) were applied to the ulnar nerve of the right arm at an intensity sufficient to elicit a visible muscle contraction (motor threshold) (Fischer et al. 2011). Intensity of the TMS test stimulus was adjusted to evoke a peak-to-peak response of 1mV in FDI. Where necessary, the amplitude of the test stimulus was adjusted to maintain a consistent response at all time points (Bertolasi et al. 1998). Twelve trials were recorded in random order for each ISI (20ms, 200ms) and for the test stimulus alone (rate of 1 every 6s; total of 36 trials). As test TMS intensity was adjusted where necessary, the peak-to-peak amplitude of the test MEP alone was not suitable to use as an additional measure of corticomotor output.

### 3.3.6 Compound muscle action potentials (M-waves)

To control for changes in excitability occurring at the muscle and neuromuscular junction, electrical stimuli (100μs duration, 400V, amplified x100) were applied to the right ulnar nerve using the set-up outlined for SAI/LAI above. Five trials were recorded at a stimulus intensity 50% above that required to elicit a maximal compound muscle action potential (M-wave) in the FDI muscle at rest.

### 3.3.7 Hypertonic saline infusion

Hypertonic saline was infused into right FDI following a standard procedure (Schabrun et al. 2012, Schabrun et al. 2013). A 25-gauge disposable cannula (Winged Infusion Set, Terumo, Japan) was placed into right FDI with the tip of the cannula at

a depth of approximately 0.5cm. A single bolus of 0.2ml of sterile saline (5 % NaCl) was infused over 20s using an infusion pump (Harvard Apparatus, SDR Scientific, USA), a 10ml plastic syringe (Terumo, Japan) and a low sorbing extension tube (140 cm length; Braun, Germany). Following the bolus, a steady infusion rate of 6 ml/hour was maintained for 10 minutes. Pain intensity and quality were evaluated using an 11-point numerical rating scale (NRS) anchored with '0' as no pain and '10' as worst pain imaginable and the McGill short form pain questionnaire (Melzack 1987).

### 3.3.8 Experimental protocol

As the LICI response has been shown to be influenced by concurrent recording of SAI and LAI measures (Sailer et al. 2002, Udupa et al. 2009), data collection was performed in two experiments: the effect of acute muscle pain on LICI was examined in Experiment 1 and the effect of acute muscle pain on SAI and LAI was assessed in Experiment 2. All 22 participants completed both experiments, separated by at least 36 hours, and experimental order was randomized. The experimental protocol was as follows: participants sat upright in a chair with their head and neck supported and their arms resting comfortably on a pillow. Data were recorded at four time points: (2) baseline, (2) during pain (measurement commenced once pain reached a moderate intensity of NRS 5/10), (3) post pain (once pain had returned to 0/10) and (4) follow up (15 minutes after pain had returned to 0/10). Measures at each time point were performed in the following order: 15 MEPs, either 24 LICI trials (Experiment 1) or 36 SAI/LAI trials (Experiment 2) and 5 M-waves. Following the completion of baseline assessments, a cannula was inserted into FDI and remained in situ for the duration of the 'during pain' time period. Immediately after the

infusion, the cannula was removed. Participants were asked to verbally rate their pain using the NRS after the completion of each measure within the 'during pain' time period and for every minute after the removal of the cannula, until NRS 0/10 was achieved. At the conclusion of each experiment, participants described the location, quality and intensity (subjective average) of muscle pain using the McGill short form pain questionnaire. The protocol for Experiments 1 and 2 are outlined in Figure 3.1.

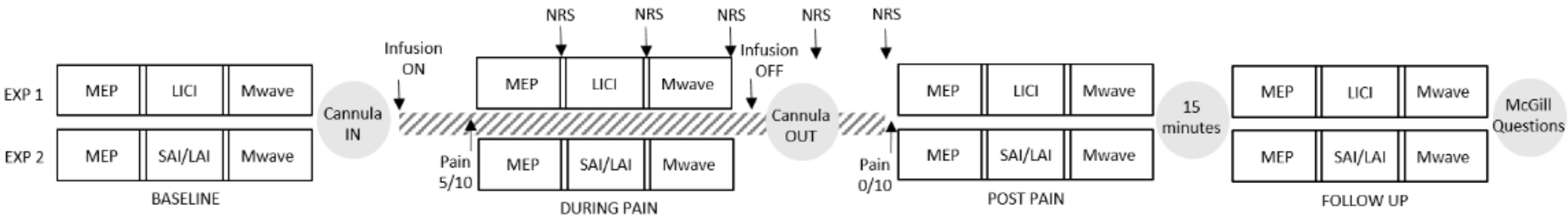


***Figure 3.1*** *Experimental protocol.*

### 3.3.9 Data and statistical analyses

MEP and Mwave data were measured as peak-to-peak amplitudes (mV) and averaged at each time point. To account for any activity-dependent changes in muscle fiber action potentials resulting from the induction of pain, statistical analyses for corticomotor output were performed with MEP amplitude expressed as a proportion of Mwave amplitude (MEP/Mwave amplitude ratio) (Groppa et al. 2012). To determine whether acute muscle pain induced a change in LICI (Experiment 1) or SAI and LAI (Experiment 2), peak-to-peak conditioned MEP amplitudes for these data were expressed as a percentage of the unconditioned (test) response. It has been shown that approximately 30% of individuals fail to respond to afferent inhibition protocols at baseline under uniform test conditions (Asmussen et al. 2013), thus, consistent with previous work (Asmussen et al. 2013), it was elected to exclude participants who did not display inhibition at baseline from further analyses.

All neurophysiological outcomes (MEP/Mwave amplitude ratio, LICI, SAI and LAI) were assessed for normality and compared between time-points (baseline, during pain, post pain, follow up) using one-way repeated measures analysis of variance (ANOVA) or where data was not normally distributed, a Friedman's repeated measures analysis on ranks. Post hoc analyses were performed using the Student Newman Keuls (SNK) test that corrected for multiple comparisons. Significance was set at 5%. All data in text is presented as mean ± standard deviation.

## *3.4 Results*

### 3.4.1 Pain characteristics

Infusion of hypertonic saline induced an average pain intensity of 5.2 ± 1.7cm for Experiment 1 and 5.0 ± 1.7cm for Experiment 2. The most frequent words used to describe pain were aching (82%), throbbing (73%), sharp (62%), and cramping (60%). The majority of participants reported symptoms localized to the dorsal surface of the hand. One participant reported numbness localized to the thumb and an additional two participants reported pain that extended beyond the wrist into the proximal forearm.

### 3.4.2 Corticomotor output is reduced during and after acute muscle pain

As the effect of acute muscle pain on corticomotor output (MEP/ Mwave amplitude ratio) was similar for Experiments 1 and 2 (ANOVA main effect of interaction, $F_{(3,21)}$ = 0.52, $p$ = 0.67), these data were pooled for analyses. Corticomotor output was reduced during pain (ANOVA main effect of time, $F_{(3,21)}$ = 5.77, $p$ = 0.001) relative to baseline (SNK $p$ = 0.003) and this reduction persisted immediately (SNK $p$ = 0.003) and 15 minutes following the resolution of pain (SNK $p$ = 0.047; Figure 3.2).

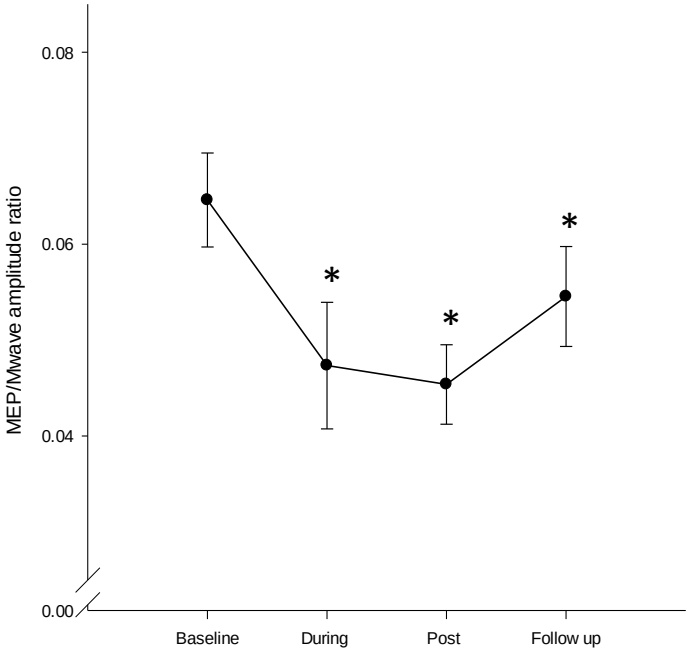


***Figure 3.2*** *Pooled group data from both experiments (n = 22 in each experiment) for corticomotor output before, during, immediately post, and 15 minutes following the resolution of experimental muscle pain. Data are expressed as MEP/Mwave amplitude ratio. Corticomotor output is reduced during, immediately post and 15 minutes following the resolution of muscle pain. *p < 0.05 when compared with baseline.*

### 3.4.3 Long-interval intra-cortical inhibition is unchanged by muscle pain

Two participants did not display inhibition at baseline (two females, aged 19 years) and these individuals were excluded from further analysis of LICI (Asmussen et al. 2013). Thus, data from 20 participants (nine males, age = 23.0 ± 8.4 years) were analyzed. It was necessary to adjust the intensity of TMS output at each time point to maintain test responses of approximately 1 mV peak-to-peak amplitude for all participants. The intensity at baseline was 57.3 ± 9.6%, 61.4 ± 9.5% during pain, 64.0 ± 10.2% post pain and 61.8 ± 12.2% at follow up. The induction of acute muscle pain

did not alter LICI at any time point (ANOVA main effect of time, $F_{(3,19)} = 1.09$, $p = 0.36$; Figure 3.3A). The amplitude of the test MEP remained stable over time (baseline: 1.10 ± 0.42mV, during: 0.96 ± 0.40mV, post: 1.02 ± 0.37mV, follow up: 0.99 ± 0.37mV; ANOVA main effect of time, $F_{(3,19)} = 0.73$, $p = 0.54$).

### 3.4.4 Short-latency afferent inhibition is reduced immediately after the resolution of acute muscle pain

Six participants did not display inhibition in response to the SAI protocol at baseline (two male, age = 26.0 ± 12.1 years) and were excluded (Asmussen et al. 2013)**.** Thus, data from sixteen participants were included in the analysis (seven male, age = 21.4 ± 4.9 years). It was necessary to adjust the intensity of TMS output at each time point to maintain test responses of approximately 1mV peak-to-peak amplitude for all participants. The intensity at baseline was 53.2 ± 10.0%, 57.6 ± 12.1% during pain, 60.9 ± 12.3% post pain and 59.9 ± 12.1% at follow up. Acute muscle pain reduced SAI (ANOVA main effect of time, $F_{(3,15)} = 3.03$, $p = 0.039$) relative to baseline immediately following the resolution of muscle pain (baseline vs. immediate, SNK $p = 0.044$; Figure 3.3B). This effect was not maintained at 15 minutes follow-up (baseline vs. follow-up, SNK $p = 0.16$). There was no change in SAI during pain (baseline vs. during, SNK $p = 0.55$). The amplitude of the test MEP remained constant over time (baseline: 1.00 ± 0.13mV, during: 0.93 ± 0.19mV, post: 0.95 ± 0.13mV, follow up: 0.95 ± 0.16mV; ANOVA main effect of time, $F_{(3,15)} = 1.79$, $p = 0.16$).

### 3.4.5 Long-latency afferent inhibition is reduced 15 minutes after the resolution of acute muscle pain

Six participants did not display inhibition in response to the LAI protocol at baseline (two males, age = 20.0 ± 1.7 years) and were excluded. Thus, data from sixteen participants were included in the analysis (seven male, age = 24.5 ± 10.4 years). It was necessary to adjust the intensity of TMS output at each time point to maintain test responses of approximately 1mV peak-to-peak amplitude for all participants. The intensity at baseline was 58.8 ± 10.1%, 57.3 ± 12.1% during pain, 60.6 ± 12.6% post pain and 59.7 ± 12.8% at follow up. As data was not normally distributed comparisons were performed using Friedman's repeated measures analysis on ranks. Acute muscle pain reduced LAI (Friedman: main effect of time, $\chi^2$ (3) = 8.63, $p$ = 0.035) 15 minutes after pain had resolved (follow-up vs. baseline SNK $p < 0.05$; Figure 3.3C). There was no change in LAI during (baseline vs. during, SNK $p > 0.05$) or immediately after the resolution of pain (baseline vs. immediate, SNK $p > 0.05$). The amplitude of the test MEP remained constant over time (baseline: 0.99 ± 0.26mV, during: 0.91 ± 0.29mV, post: 0.86 ± 0.28mV, follow up: 0.89 ± 0.32mV; ANOVA: main effect of time, $F_{(3,15)} = 1.40$, $p = 0.25$).

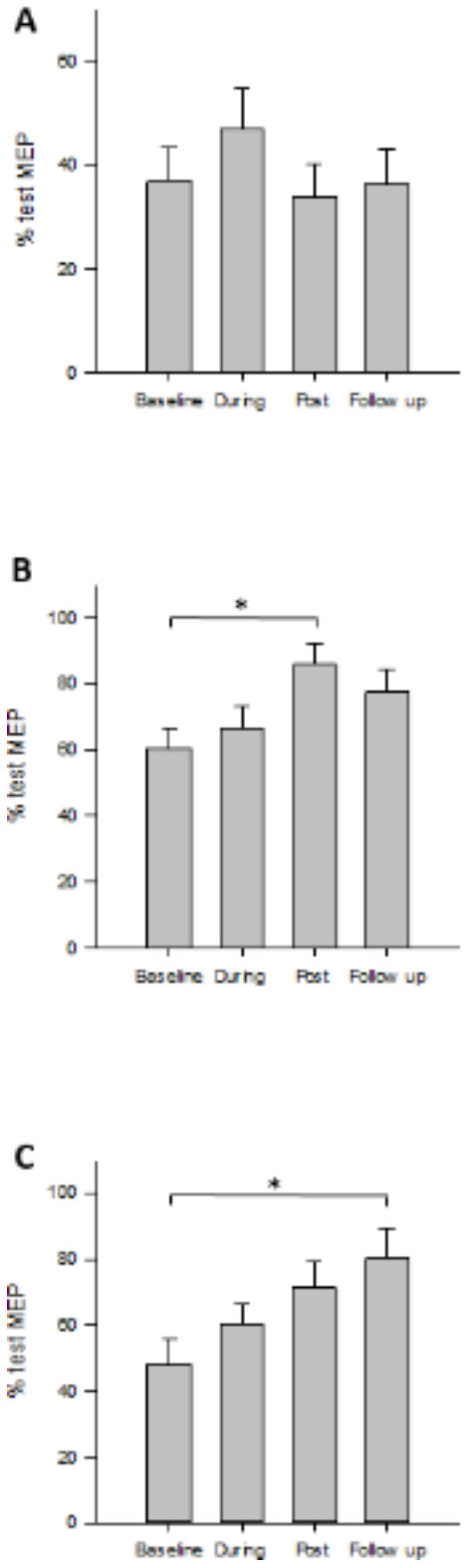


***Figure 3.3*** *Group data (mean ± standard error) for A) long-interval intracortical inhibition (LICI, n = 20), B) short-latency afferent inhibition (SAI, n = 16) and C) long-latency afferent inhibition (LAI, n = 16) at baseline, during, immediately post and 15 minutes following the resolution of experimental muscle pain. Individuals who did not show inhibition at baseline were excluded from analyses. LICI, SAI and LAI were determined by expressing the conditioned MEP as a percentage of the unconditioned test MEP (% of test MEP). LICI was not affected by acute muscle pain at any time point (A). Compared to baseline, SAI was reduced (less inhibition, higher proportion of the test MEP) immediately post pain (B) and LAI was reduced at follow up (C). * $p < 0.05$.*

### *3.5 Discussion*

To our knowledge, this study is the first to examine circuits involved in sensorimotor integration (SAI, LAI) and intracortical networks mediated by $GABA_B$ (LICI) in response to acute muscle pain. The novel finding is that experimental muscle pain reduces SAI and LAI (indicative of less inhibition) but only once pain has resolved, whereas intracortical networks mediated by $GABA_B$ appear unaffected by acute muscle pain. Our data also confirm previous reports of reduced corticomotor output during and following the resolution of pain. Taken together, our findings provide new insights into mechanisms that may contribute to recovery of motor output following acute muscle pain.

Short- and long-latency afferent inhibition are investigated by coupling peripheral nerve stimulation at the wrist with TMS at M1 (Tokimura et al. 2000). When paired at short (~20ms) and long (~200ms) inter-stimulus intervals, inhibition of the MEP is observed, an effect thought to reflect the interaction between sensory input and motor output at the level of the cortex (sensorimotor integration) (Chen et al. 1999, Tokimura et al. 2000). The latencies at which inhibition is induced suggest that SAI and LAI reflect the activation of distinct sensorimotor pathways and thus are likely to represent separate indices of sensorimotor integration. For instance, SAI is postulated to involve direct S1-M1 pathways (Tokimura et al. 2000, Sailer et al. 2002, Sailer et al. 2003), whereas LAI is thought to involve indirect basoganglia-thalamocortical pathways (Chen et al. 1999, Abbruzzese et al. 2001, Sailer et al. 2002, Sailer et al. 2003). This interpretation is further supported by evidence that SAI and LAI display unique interactions with other intracortical circuitry (Sailer et al. 2002,

Alle et al. 2009, Udupa et al. 2009), and are differentially affected by pathology (Abbruzzese et al. 2001, Sailer et al. 2003, Kessler et al. 2005) and drug formulations (Di Lazzaro et al. 2005b, Di Lazzaro et al. 2005c, Di Lazzaro et al. 2007a). In the present study, we show reduced SAI immediately following, and reduced LAI 15 minutes following, an episode of acute muscle pain. Hence, the different cortical pathways that underpin these mechanisms are likely to explain these differential time effects. Although it is beyond the scope of the present study to infer causation, it is possible is that the timing of these effects may relate to the relative temporal sensitivity of the cortical generators of SAI and LAI to salient cues (i.e. the cessation of nociceptive input) (Legrain et al. 2011).

Although the functional significance of reduced SAI and LAI in response to acute muscle pain is unclear, evidence suggests that appropriate sensorimotor integration constitutes an essential component of fluid, coordinated motor control (Riemann et al. 2002). Work performed in non-pain conditions indicate that SAI and LAI support the operation of 'surround inhibition', a mechanism that facilitates fine motor control via regulation of the inhibitory drive to specific muscles (Hallett 2003). Indeed, it has been shown during selective movement of the index finger that SAI/LAI to muscles necessary for movement is decreased, whilst inhibition to redundant muscles is increased (Voller et al. 2005, Voller et al. 2006). One explanation for our data is that reduced afferent inhibition (SAI and LAI) following resolution of muscle pain acts to facilitate motor recovery, promoting a return to normal motor output of the painful part as the threat of pain and injury subsides. Indeed, although motor output remains suppressed compared with baseline in the post pain time period, our data

demonstrate that motor output has undergone recovery of approximately 50% 15 minutes after pain has resolved (Figure 3.2). Further work is necessary to determine the time course of recovery of motor output and the relationship between motor output and afferent inhibition to confirm this hypothesis.

We contend that motor output in the post-pain period may reflect a balance between the need for recovery of motor output and protection from the threat of further pain and injury. This is supported by evidence of altered activity in the cortical circuits thought to support each of these functions. For instance, although SAI and LAI are reduced (less inhibition) in the post-pain period, possibly to facilitate motor recovery, other inhibitory mechanisms are increased. Short-interval intracortical inhibition (SICI), an index of M1 intracortical activity mediated via $GABA_A$ receptors on inhibitory interneurons (Ziemann et al. 1996b, Di Lazzaro et al. 2000a), is increased immediately following the resolution of muscle pain and this is thought to maintain a protective motor strategy in the post-pain period by continuing to limit motor output (Schabrun et al. 2012). As SICI is known to inhibit the expression of SAI (Stefan et al. 2002, Alle et al. 2009), and interacts in an additive manner with LAI when activated concurrently (Sailer et al. 2002), it is possible that SICI is the dominant mechanism during this early post-pain time-period, ensuring a protective strategy persists until the threat of pain is completely removed. Once all threat has dissipated, SICI may reduce, and mechanisms such as SAI and LAI may become dominant, facilitating recovery of motor output. However, SICI as only been investigated in the period immediately following the resolution of acute pain (Schabrun et al. 2012), and it is unknown how the temporal profile of this mechanism is altered in relation to SAI,

LAI and motor output over an extend time-frame. Future work should seek to examine the interaction of these mechanisms over a prolonged time-period once pain has resolved.

Our observation that LICI remains unchanged in response to pain is further evidence that the response to pain varies across different inhibitory networks. Unlike SICI, the effects of LICI are mediated via $GABA_B$ receptors located on M1 interneurons (Werhahn et al. 1999, McDonnell et al. 2006), therefore the results of the present study suggest that the effect of pain differs amongst GABA receptor subtypes. Although it is unclear why $GABA_A$, but not $GABA_B$ mediated inhibition is affected by pain, one possible explanation may be that LICI is more robust than SICI and less sensitive to transient changes in afferent input. Indeed, since reductions in LICI have been observed in chronic pain conditions such as fibromyalgia and rheumatoid arthritis (Salerno et al. 2000), it is likely that the effect of pain on LICI may depend upon symptom duration. Alternatively, as LAI has been demonstrated to interact with LICI in an intensity dependent manner (Sailer et al. 2002), it is possible that LICI remained unaffected during or immediately post pain due to the stability of LAI at these time points. However, since LAI was reduced 15 minutes post pain but LICI was unchanged, further work is needed to determine whether LICI/LAI interactions are relevant during and/or following a painful episode.

A vehicle control was not included in the current study for several reasons. First, use of such a control is common in both animal and human studies of experimental pain and rarely, if ever, shows an effect (Adachi et al. 2008, Nash et al. 2010b). Indeed,

previous studies of similar methodology have demonstrated no effect of intramuscular isotonic saline solution on the amplitude or latency of MEPs to muscles of the upper limb (Le Pera et al. 2001, Martin et al. 2008). Second, numerous studies demonstrate that measures of M1 organisation and function are stable and reliable over time (Uy et al. 2002, Malcolm et al. 2006, Ngomo et al. 2012). For example, corticomotor output has been shown to be stable during 30 minutes of controlled, quiet sitting (Svensson et al. 2003c) and is reliable over short (0-4 days) and long (0-14 days, 0-1 months) intersession intervals (Malcolm et al. 2006, Ngomo et al. 2012). Similarly, LICI and SAI have been shown to be stable over testing intervals of one and seven days, respectively (Farzan et al. 2010, Fischer et al. 2011). Taken together, these observations indicate that the measures used in this study are not sensitive to time effects and our findings are unlikely to be replicated in a no pain control condition.

This study has several limitations that should be acknowledged. First, we did not collect data from muscles other than FDI and thus, this study is not able to determine whether reductions in SAI and LAI are muscle specific. However, results from previous studies suggest a non-specific, but localised, effect of hand pain on corticomotor output. For example, muscle pain induced in FDI has been shown to reduce corticomotor output of distant hand muscles (abductor digiti minimi) (Le Pera et al. 2001, Schabrun et al. 2012), but not of proximal forearm muscles (flexor carpi ulnaris) (Svensson et al. 2003c). Further research is required to determine whether reductions in SAI and LAI are confined to the muscle in pain, or extend to other local hand and forearm muscles.

The ISIs selected for the assessment of SAI, LAI and LICI also require discussion. In early studies of SAI, the ISIs tested were based on calculations of the specific latency of the N20 component of the somatosensory evoked potential observed following median nerve stimulation for each participant (Tokimura et al. 2000). Following ulnar nerve stimulation, the latency of this component is known to occur within the range of 19.93 ± 1.1ms (Fischer et al. 2011) to 20.7 ± 0.7ms (Alle et al. 2009). As inter-individual variability for SAI has been shown to be high within the optimal test range (Wassermann 2002, Fischer et al. 2011) and SAI is decreased or absent if afferent stimulation fails to correspond with the latency of N20, it is possible that that the stimulus parameters used in this present study may have been suboptimal for some participants. As this limitation necessitated the exclusion of six participants from SAI and LAI analysis, future research should seek to use stimulus parameters specific to induce inhibition in each individual participant. Two participants who responded with MEP facilitation to the LICI protocol were also excluded as there is evidence that such facilitation is due to the interference of intracortical facilitatory mechanisms (Ni et al. 2007). Future work concerning LICI should seek to investigate the effect of pain at intervals < 160ms as recent evidence suggests that LICI evoked using early (~100ms) and late (~150ms) test intervals (Vallence et al. 2012, Vallence et al. 2014) may differentially reflect pre- and postsynaptic inhibitory processes (Chu et al. 2008).

Finally, the reliability of these findings may be improved by increasing the number of pulses used for cortical assessments. In the present study, 15 MEPs were recorded to provide an estimate of corticomotor output, and 12 MEPs for measures of afferent/intracortical inhibition. Although trials of 12-15 MEPs are common among

TMS studies of acute muscle pain (Schabrun et al. 2012, Schabrun et al. 2013, Schabrun et al. 2016), recent literature indicates that higher numbers of MEPs may be required for reliable assessment (Chang et al. 2016, Goldsworthy et al. 2016). While increasing the number of TMS pulses may improve accuracy, it is also time consuming. As time is a precious commodity in acute pain studies, extending the number of MEPs within each testing block should be done with caution. For example, it is likely that increasing the number of trials in the present study from 12/15 to the recommended 30 MEPs (Goldsworthy et al. 2016) would have affected the timing between measures and thus negatively impacted the integrity of each experiment. In the future, studies with similar experimental designs should use the least number of trials necessary to achieve reliable outcomes. The minimum number of pulses required for the reliable assessment of corticomotor output and intracortical inhibition are currently estimated to be 21 and 20, respectively (Chang et al. 2016).

### *3.6 Conclusions*

This study is the first to provide evidence of reduced SAI and LAI following acute muscle pain. We hypothesize that these responses may reflect the early activation of mechanisms that restore normal motor function after the resolution of pain. It is possible that a protective motor strategy (reduced corticomotor output) prevails early after the resolution of pain, despite reductions in SAI and LAI, due to the existence of competitive interactions between SAI/LAI and other inhibitory networks. Further studies are required to confirm this hypothesis. If confirmed, these data may have relevance for the design of clinical interventions that aim to restore motor output in musculoskeletal pain conditions.

# Chapter 4:

## Altered function of intracortical networks in chronic lateral epicondylalgia

There is consistent evidence of reduced, or indeed absent, intracortical inhibition in chronic musculoskeletal pain. These findings are hypothesized to underpin altered M1 function, M1 reorganisation and contribute to pain maintenance in some conditions. This chapter reports on the findings of an observational study designed to investigate whether intracortical networks associated with long interval intracortical inhibition (LICI), short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) function differently in persons with chronic elbow pain (lateral epicondylalgia) compared to pain-free individuals. The content of this chapter has been published in *Burns E, Chipchase LS, Schabrun SM (2016). Altered function of intracortical networks in chronic lateral epicondylalgia. European Journal of Pain. 20(7): 1166-75.* A copy of this publication is provided in Appendix C.

### *4.1 Abstract*

Lateral epicondylalgia (LE) is a musculotendinous condition characterized by persistent pain, sensorimotor dysfunction and motor cortex reorganisation. Although there is evidence linking cortical reorganisation with clinical symptoms in LE, the mechanisms underpinning these changes are unknown. Here we investigated activity in motor cortical (M1) intracortical inhibitory and facilitatory networks in individuals with chronic LE and healthy controls. Surface electromyography was recorded bilaterally from the extensor carpi radialis brevis (ECRB) muscle of 14 LE (four males, 41.5 ± 9.9 years) and 14 control participants (four males, 42.1 ± 11.1 years). Transcranial magnetic stimulation of M1 was used to evaluate resting and active motor threshold, corticomotor output, short- (SICI) and long-latency intracortical inhibition (LICI) and intracortical facilitation (ICF) of both hemispheres. In individuals with LE, SICI ($p$ = 0.005), ICF ($p$ = 0.026) and LICI ($p$ = 0.046) were less in the M1 contralateral to the affected ECRB muscle compared with healthy controls. Motor cortical threshold (rest: $p$ = 0.57, active: $p$ = 0.97) and corticomotor output ($p$ = 0.15) were similar between groups. No differences were observed between individuals with LE and healthy controls for the M1 contralateral to the unaffected ECRB muscle. These data provide evidence of less intracortical inhibition mediated by both $GABA_A$ and $GABA_B$ receptors, and less intracortical facilitation in the M1 contralateral to the affected ECRB in individuals with LE compared with healthy controls. Similar changes were not present in the M1 contralateral to the unaffected ECRB. These changes may provide the substrate for M1 reorganisation in chronic LE and could provide a target for future therapy.

### *4.2 Introduction*

Lateral epicondylalgia (LE), commonly termed 'tennis elbow', is a disabling condition affecting the musculotendinous structures at the lateral epicondyle, characterized by symptoms of persistent pain and sensorimotor dysfunction (Bisset et al. 2006, Skinner et al. 2007, Juul-Kristensen et al. 2008, Coombes et al. 2012). Thought to be triggered by repetitive, forceful use of the forearm extensor muscles (Fan et al. 2009), LE affects 1-3 per cent of the general population and 15% of workers, with high rates of symptom persistence and recurrence (Shiri et al. 2006). Recent evidence suggests that altered primary motor cortex (M1) organisation could contribute to symptoms of pain and motor dysfunction in this condition. For instance, increased excitability, greater overlap and a reduced number of discrete cortical peaks have been demonstrated in the representations of the elbow extensor muscles in chronic LE, and these changes are associated with pain severity (Schabrun et al. 2015b). As M1 representations are known to be maintained and adjusted by intracortical inhibitory (ICI) and facilitatory (ICF) networks (Liepert et al. 1998a), altered activity in these networks could underpin altered M1 organisation in LE.

Intracortical networks can be probed in the human M1 using paired pulse transcranial magnetic stimulation protocols. Intracortical inhibition (ICI) measured at short (SICI)- and long (LICI)- latencies is thought to reflect activity in $GABA_A$ and $GABA_B$ receptor systems respectively, while intracortical facilitation (ICF) is thought to reflect activity in the glutamatergic (NMDA receptor) system, with a relative spinal contribution (Ziemann et al. 1998, Werhahn et al. 1999, McDonnell et al. 2006). Studies have shown increased ICI, and reduced ICF, in response to acute muscle pain, and these

findings are hypothesized to underpin reduced M1 excitability and restriction of motor output observed in acute pain (Hodges et al. 2011, Schabrun et al. 2012). Conversely, in persistent neuropathic pain, complex regional pain syndrome and low back pain, ICI is reduced, a finding that may underpin increased excitability and altered organisation of M1 in these conditions (Schwenkreis et al. 2003, Eisenberg et al. 2005, Schwenkreis et al. 2010, Masse-Alarie et al. 2012). Similar alterations in intracortical networks could be present in LE, yet no study has investigated these mechanisms in this condition.

Understanding the mechanisms that contribute to chronic LE has the potential to provide new targets for therapy. Indeed, use of repetitive transcranial magnetic stimulation to restore ICI in chronic neuropathic pain is associated with a reduction in pain severity, suggesting that therapies designed to target intracortical networks may be effective in ameliorating persistent pain (Lefaucheur et al. 2006). Here we aimed to investigate the function of M1 intracortical networks mediated by $GABA_A$ (short-interval intracortical inhibition, SICI), $GABA_B$ (long-interval intracortical inhibition; LICI) and NMDA receptors (intracortical facilitation, ICF) in individuals with persistent LE and healthy controls. Consistent with findings in other persistent pain conditions, we hypothesized a reduction in intracortical inhibition in individuals with LE.

## *4.3 Method*

### 4.3.1 Participants

Fourteen individuals with LE (mean ± standard deviation; four males, aged 41.5 ± 9.9 years) and 14 age and gender matched healthy controls (four males, aged matched ± 5 years: 42.1 ± 11.1) participated. As M1 intracortical mechanism are yet to be investigated in LE, sample size calculations were based on effect sizes from a previous study examining cortical excitability (motor evoked potential amplitude) in LE (Schabrun et al. 2015b). Based on these data (difference of means between patients and controls: 83%, standard deviation: 0.625mV), it was calculated that a minimum sample size of nine participants in each group were needed to observe a statistically significant difference (80% power, alpha 0.05) should one exist (Kadam et al. 2010). Individuals with LE were included if they had experienced elbow pain over the lateral epicondyle for greater than 6 weeks that was provoked by palpation, gripping, resisted wrist and/or middle finger extension (Linaker et al. 1999). Exclusion criteria included: (1) use of oral or topical pain-relief medication in the preceding 48 hours (2) concomitant neck or arm pain that prevented participation in usual work or recreational activities (3) corticosteroid injections in the last 6 months and (5) evidence of sensory disturbances, history of fractures, elbow surgery, arthritic or inflammatory disorders or pain localized to the radiohumeral joint (Coombes et al. 2009, Coombes et al. 2013). In addition, participants completed a TMS safety screening questionnaire and were excluded from enrolment if they had a personal or family history of epilepsy, major neurological, respiratory, orthopaedic or circulatory disorders, if they were pregnant, had metal in their head or jaw or were taking central

nervous system acting medications (Keel et al. 2001). The study was approved by the institutional human medical research ethical committee and performed in accordance with the Declaration of Helsinki. All participants provided written, informed consent.

### 4.3.2 Clinical measures of LE

#### *4.3.2.1 Pain and disability*

The Patient Rated Tennis Elbow Evaluation (PRTEE) was used to assess pain and disability for the week preceding the experiment (Macdermid 2005). Scores for pain (sum of five items out of 50) and function (sum of ten items, divided by 2, out of 50) were combined to give a total score ranging from 0 (no pain and no functional impairment) to 100 (worst pain imaginable with significant functional impairment). Current pain intensity was recorded using an 11-point numerical rating scale (NRS) anchored with '0' as no pain and '10' as worst pain imaginable.

#### *4.3.2.2 Pressure pain thresholds (PPT)*

A handheld pressure algometer (Commander probe size 1cm$^2$, JTECH Medical) was applied perpendicular to the extensor carpi radialis brevis (ECRB) muscle at a steadily increasing rate of pressure. ECRB was located via palpation during resisted middle finger and wrist extension. The location of the muscle belly was marked on the skin with an oily-tipped pen to ensure accurate probe placement between trials. Participants were instructed to vocalize at the exact moment they perceived the sensation of pressure first turn to pain. Pressure pain threshold (N) was assessed for

each arm and defined as the average of five consecutive trials (rate of 1 trial every 10s).

#### *4.3.2.3 Pain-free grip strength and maximum grip strength*

Pain-free and maximum grip strength were assessed using a hand-held dynamometer (Baseline Digital Hand dynamometer, Chattanooga Group, UK). Participants assumed a seated position and were assisted in placing their arm in 90° shoulder flexion, full elbow extension and neutral forearm pronation (De Smet et al. 1998). For pain-free grip strength, participants were instructed to squeeze the dynamometer, but to cease immediately at the onset of pain. To assess maximal grip strength, participants were instructed to squeeze the dynamometer as hard as possible, regardless of pain. Grip strength (kg) of each arm was determined based on the average of three consecutive trials (rate 1 trial every 30s).

### 4.3.3 Neurophysiological measures

#### *4.3.3.1 Electromyographic recordings*

Surface electromyography (EMG) was recorded from ECRB using dual silver-silver chloride disposable electrodes (spacing 2.0cm). Electrode position was determined following palpation of ECRB during resisted wrist and middle finger extension. The ground electrode was positioned over the olecranon. EMG signals were amplified x1000 (NL844, Digitimer Ltd, Welwyn Garden City, UK), band-pass filtered: 20-1000 Hz and sampled at 2000 Hz using a Micro3 1401 Data Acquisition System and Signal5 software (Cambridge Electronic Design, Cambridge, UK).

*4.3.3.2 Motor threshold and corticomotor output*

Transcranial magnetic stimulation (TMS) was delivered to the primary motor cortex using a Magstim 200 stimulator (Magstim Co. Ltd., Dyfed, UK) connected to a circular coil (90mm diameter) and all procedures adhered to the TMS checklist for methodological quality (Chipchase et al. 2012). Early pilot trials showed that MEPs were more reliably elicited from ECRB when TMS was performed using a circular coil. Circular coils provide a strong, deep and broad induced electric current and are formally recommended for diagnostic TMS (Groppa et al. 2012). The coil was oriented to induce posterior-anterior current flow (left hemisphere: anticlockwise current direction; right hemisphere: clockwise current direction), and positioned over the optimal scalp position for ECRB (Groppa et al. 2012). This position was determined by systematic application of TMS to the scalp until the site that produced the largest motor evoked potential (MEP) was identified. A Brainsight neuronavigation system (Rogue Research Inc, Quebec, Canada) ensured accurate coil placement throughout each experiment. Resting and active motor threshold were determined at the optimal position via the maximum-likelihood protocol (Motor threshold Assessment Tool, version 2.0: http://www.clinicalresearcher.org/software.htm) (Awiszus 2003). Resting motor threshold (RMT) was defined as the minimum stimulus intensity that evoked a MEP greater than 50µV in the target muscle at rest (Rossini et al. 1994). Active motor threshold (AMT) was defined as the minimum stimulus intensity that evoked a MEP greater than 200µV during isometric contraction of ECRB (10% maximum voluntary contraction [MVC]) (Rossini et al. 1994). Thirty MEPs were recorded from resting ECRB at 120% RMT to assess corticomotor output (rate of 1

pulse every 6s). Trials containing muscle activity within 50ms preceding the TMS pulse were discarded (Groppa et al. 2012).

*4.3.3.3 Short-interval intra-cortical inhibition (SICI) and intra-cortical facilitation (ICF)*

A standard paired-pulse TMS protocol that consisted of a sub-threshold conditioning stimulus delivered 2 or 10ms before a supra-threshold test stimulus, was used to evaluate short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF), respectively (Kujirai et al. 1993). Magnetic stimulation was delivered at the optimal scalp site to evoke a response in ECRB with the muscle at rest. Conditioning intensity was based on a percentage of AMT to decrease the likelihood of SICI and ICF measurements being influenced by other intracortical mechanisms, such as short-interval intracortical facilitation (sICF) (Ortu et al. 2008, Peurala et al. 2008). However, as individual variability for SICI thresholds is known to be high (Orth et al. 2003), testing SICI at a single 'optimal' conditioning intensity is unlikely to reflect the true maximum inhibition for all individuals. Thus, to account for between-subject variability to paired-pulse TMS (Boroojerdi et al. 2000, Maeda et al. 2002, Wassermann 2002) and to investigate the stimulus-response profile of SICI and ICF, three conditioning intensities were used in this experiment. The intensities 70, 80, 90% of AMT were selected as these are known to elicit reliable SICI and ICF in healthy individuals (Ortu et al. 2008, Peurala et al. 2008). As MEPs from proximal upper limb muscles are typically smaller and less defined than those evoked from distal muscles (Chen et al. 1998, Groppa et al. 2012), test stimulus intensity was set to evoke a test MEP with a peak-to-peak amplitude of approximately 0.3 - 0.5mV in all subjects (Perez et al. 2008, Schwenkreis et al. 2011). SICI/ICF at 70%, 80% and 90% AMT

conditioning intensity were tested in separate blocks. Within each block, twelve trials were recorded in pseudorandom order at each inter-stimulus interval (ISI; 2ms, 10ms) and for the test stimulus alone (rate of 1 every 6s; total of 36 trials).

#### *4.3.3.4 Long-interval intra-cortical inhibition (LICI)*

Long-interval intracortical inhibition (LICI) was evaluated using a supra-threshold conditioning stimulus delivered before a supra-threshold test stimulus (Claus et al. 1992). Evidence suggests that spinal mechanisms may contribute to the inhibitory response when conditioning stimuli precedes the test stimulus by 80-100ms (McNeil et al. 2009, 2011). Thus, in order to reduce the likelihood of a spinal contribution in the present study, LICI was tested using an inter stimulus interval (ISI) of 160ms. Stimulations were performed at the optimal scalp site to evoke a response in ECRB at rest. The test and conditioning intensities were equal and set to evoke a peak-to-peak response of approximately 0.3 - 0.5mV in ECRB. Twelve trials were recorded in pseudorandom order at ISI 160ms and for the test stimulus alone (rate of 1 every 6s; total of 24 trials).

### 4.3.4 Experimental protocol

All experimental procedures were conducted in a single test session. Data collection was performed in the following order: (1) clinical outcome measures (LE participants only), (2) resting and active motor threshold, (3) corticomotor output (MEPs), (4) SICI and ICF (three conditioning intensities: 70, 80, 90% of AMT) and (5) LICI. TMS measures were repeated over both hemispheres for consenting participants. Rest intervals of at least 2 minutes were provided between each measure.

### 4.3.5 Data and statistical analysis

#### *4.3.5.1 Clinical measures of LE*

Measures of PPT, maximum grip strength and pain-free grip strength were compared between sides (affected vs. unaffected arm) for individuals with LE. Pearson correlation coefficients were used to evaluate relationships between (1) pain (NRS) and disability (PRTEE), sensorimotor function (PPT, max grip strength, pain-free grip strength) and (2) SICI, ICF and LICI of the corresponding hemisphere.

#### *4.3.5.2 Neurophysiological measures*

MEP data were measured as peak-to-peak amplitudes and averaged for each trial. Intracortical inhibition (SICI, LICI) and facilitation (ICF) were calculated by expressing the mean peak-to-peak conditioned MEP amplitudes as a percentage of the unconditioned (test) response.

All neurophysiological outcomes (RMT, AMT, MEP, SICI, ICF and LICI) were assessed for normality via the Shapiro-Wilk test, transformed if necessary, and compared between groups (LE vs. control) for each hemisphere using separate one-way ANOVAs. To account for any potential influence of handedness, data corresponding to the affected/non-affected limb of LE participants was appropriately matched to the dominant/non-dominant limb of their specific age and gender matched control. As the amplitude of the unconditioned (test) MEP can affect the magnitude of SICI, ICF and LICI (Chen et al. 1998, Udupa et al. 2010), test MEP amplitude was also compared between groups with one-way ANOVAs. One-way repeated measures

ANOVA were used to compare data contralateral and ipsilateral to the affected limb within the LE group. Post hoc analyses were corrected for multiple comparisons using the Holm-Sidak method. Statistical significance was set at $P < 0.05$. Data in text are expressed as mean ± standard deviation unless stated otherwise.

## *4.4 Results*

### 4.4.1 Clinical measures of LE

Patient characteristics are summarized in Table 4.1. Average current pain intensity was 3.5 ± 2.8 on the NRS and the average combined score for the pain and function subscale of the PRTEE was 38.4 ± 19.0. Individuals with LE demonstrated reduced strength for the affected arm compared to the unaffected arm in the pain-free (affected: 18.6 ± 18.2kg; unaffected: 32.1 ± 12.8kg; main effect, $F_{(1,13)} = 12.09$, $p = 0.004$) and maximal grip strength tasks, however results for the latter failed to reach statistical significance (affected: 30.3 ± 15.0 kg; unaffected: 34.7 ± 11.2kg; main effect, $F_{(1,13)} = 3.75$, $p = 0.075$). Pressure pain thresholds were less for ECRB of the affected arm compared to the unaffected arm (affected: 17.8 ± 8.7kg; unaffected: 21.4 ± 7.4kg; main effect, $F_{(1,13)} = 7.42$, $p = 0.017$), suggesting increased sensitivity to mechanical stimuli in the affected limb.

***Table 4.1*** *Demographic and clinical characteristics for individuals with lateral epicondylalgia (n = 14).*

| **SUBJECT** | **GENDER** | **AGE** *(years)* | **DURATION** *(months)* | **DOMINANT ARM** | **AFFECTED ARM** | **PRTEE** *(/100)* | **NRS** *(/10)* |
|---|---|---|---|---|---|---|---|
| **1** | F | 52 | 288 | R | R | 35 | 2 |
| **2** | F | 38 | 3 | R | R | 79 | 7 |
| **3** | M | 44 | 12 | R | R | 14 | 0.5 |
| **4** | F | 45 | 7 | R | R | 45 | 6 |
| **5** | F | 48 | 7 | R | R | 31.5 | 2 |
| **6** | F | 43 | 3 | R | R | 17 | 0 |
| **7** | F | 50 | 24 | L | R | 24 | 0.5 |
| **8** | M | 46 | 60 | R | R | 22 | 2 |
| **9** | F | 45 | 3 | R | R | 67 | 7.5 |
| **10** | M | 20 | 3 | R | R | 32.5 | 1 |
| **11** | M | 37 | 4 | R | R | 34.5 | 6 |
| **12** | F | 21 | 24 | R | L | 56 | 7 |
| **13** | F | 50 | 60 | R | L | 30 | 3 |
| **14** | F | 42 | 24 | R | R | 50.5 | 5 |

PRTEE, patient rated tennis elbow evaluation (pain and disability); NRS, numerical rating scale.

4.4.2 Neurophysiological measures

*4.4.2.1 TMS of the hemisphere contralateral to the 'affected' arm in LE and the matched hemisphere in healthy controls*

There was no difference in resting motor threshold (LE: 48.6 ± 8.5; Control: 50.7 ± 10.4; main effect, $F_{(1,26)}$ = 0.33, $p$ = 0.57), active motor threshold (LE: 45.6 ± 9.3; Control: 45.4 ± 8.9; main effect, $F_{(1,26)}$ = 0.002, $p$ = 0.97) or corticomotor output (LE MEP amplitude: 0.30 ± 0.14mV; Control: 0.53 ± 0.46mV; main effect, $F_{(1,26)}$ = 2.19, $p$ = 0.15) between individuals with LE and healthy controls.

The magnitude of SICI and ICF observed for ECRB of healthy controls was consistent with previous investigations of similar methodology (circular coil, 2ms, 10ms ISI) (Shimizu et al. 1999). Short-interval intracortical inhibition (SICI) was less in individuals with LE compared to healthy controls at a conditioning intensity of 90% AMT (main effect, $F_{(1,26)}$ = 9.58, $p$ = 0.005), but was similar between groups at conditioning intensities of 70% and 80% AMT (70%: main effect, $F_{(1,26)}$ = 1.46, $p$ = 0.24; 80%: main effect, $F_{(1,26)}$ = 0.47, $p$ = 0.50; Figure 4.1A). Similarly, long-interval intracortical inhibition (LICI) was less in individuals with LE when compared with controls (main effect, $F_{(1,26)}$ = 4.40, $p$ = 0.046; Figure 4.2A). Intracortical facilitation was less in LE participants compared with healthy controls at a conditioning intensity of 80% AMT (main effect, $F_{(1,26)}$ = 5.58, $p$ = 0.026; Figure 4.1C), but not at 70% (main effect, $F_{(1,26)}$ = 0.004, $p$ = 0.95) or 90% AMT (main effect, $F_{(1,26)}$ = 0.64, $p$ = 0.43). Test MEP amplitudes were comparable between groups for LICI (main effect, $F_{(1,26)}$ = 1.10, $p$ = 0.30) and for each SICI and ICF conditioning intensity (70% AMT: main effect, $F_{(1,26)}$

= 1.10, $p$ = 0.30; 80% AMT: main effect, $F_{(1,26)}$ = 0.003, $p$ = 0.96; 90% AMT: main effect, $F_{(1,26)}$ = 0.31, $p$ = 0.58). Taken together, these findings suggest that inhibition and facilitation are reduced under certain test conditions, in individuals with LE. The magnitude of SICI 90% AMT, ICF80% AMT and LICI did not correlate with pain (SICI: r = 0.035, $p$ = 0.91; ICF: r = -0.13, $p$ = 0.65; LICI: r = 0.013, $p$ = 0.97), PRTEE disability (SICI: r = -0.11, $p$ = 0.71; ICF: r = -0.20, $p$ = 0.50; LICI: r = -0.01, $p$ = 0.97), PPT (SICI: r = -0.35, $p$ = 0.22; ICF: r = -0.40, $p$ = 0.16; LICI: r = -0.34, $p$ = 0.23), max grip strength (SICI: r = -0.39, $p$ = 0.17; ICF: r = -0.31, $p$ = 0.29; LICI: r = -0.37, $p$ = 0.19) or pain-free grip strength (SICI: r = -0.34, $p$ = 0.23; ICF: r = -0.22, $p$ = 0.46; LICI: r = -0.27, $p$ = 0.35)in the affected arm .

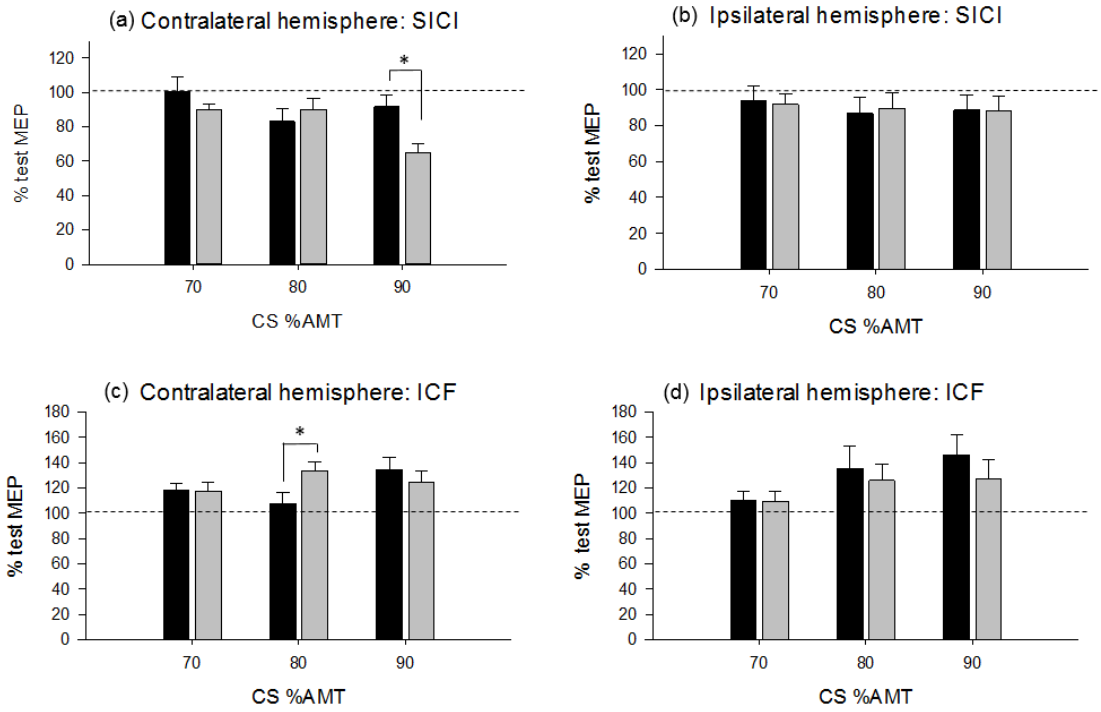


***Figure 4.1*** *Group data (mean ± standard error) for short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) over the hemisphere contralateral (LE, n = 14; control, n = 14) and ipsilateral (LE, n = 10; control, n = 10) to the affected arm of LE participants (black bars) and the matched arm of healthy controls (grey bars). Trials were performed using conditioning stimulus (CS) intensities 70%, 80% and 90% of active motor threshold (AMT). SICI and ICF were determined by expressing the conditioned MEP as a percentage of the unconditioned test MEP (percentage of test MEP). A) LE participants displayed less SICI (less inhibition, higher percentage of the test MEP) for the contralateral hemisphere compared to healthy controls at CS 90% AMT. B) There was no difference in SICI between groups for the ipsilateral hemisphere. C) LE participants displayed less ICF (less facilitation, lower percentage of the test MEP) for the contralateral hemisphere compared to healthy controls at CS 80% AMT. D) There was no difference in ICF between groups for the ipsilateral hemisphere. * p < 0.05.*

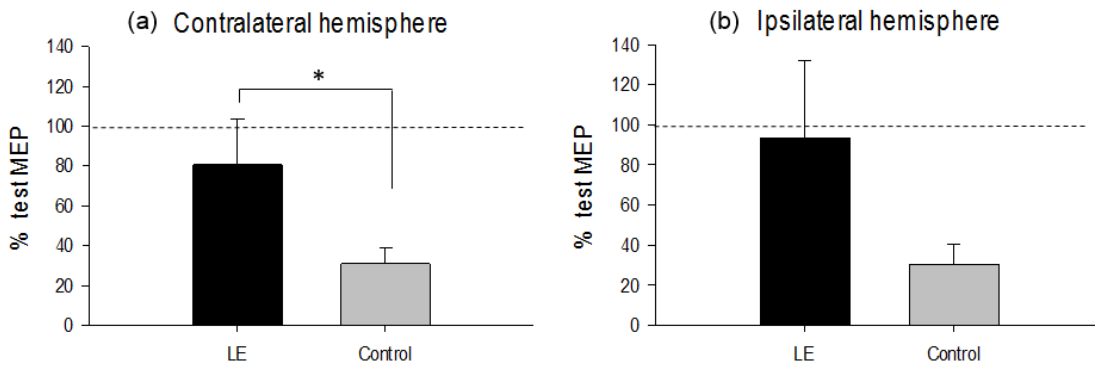


***Figure 4.2*** *Group data (mean ± standard error) for long-interval intracortical inhibition (LICI) over the hemisphere contralateral (LE, n = 14; control, n = 14) and ipsilateral (LE, n = 10; control, n = 10) to the affected arm of LE participants (black bars) and the matched arm of healthy controls (grey bars). LICI was determined by expressing the conditioned MEP as a percentage of the unconditioned test MEP (percentage of test MEP). LE participants displayed less LICI (less inhibition, higher percentage of the test MEP) for the contralateral hemisphere compared to healthy controls (A), however no difference was detected between groups for the ipsilateral hemisphere (B). * p < 0.05.*

*4.4.2.2 TMS of the hemisphere ipsilateral to the 'affected' arm in LE and the matched hemisphere in the healthy controls*

Ten LE (three males, aged 40.2 ± 11.8 years) and 10 control participants (three males, aged 42.0 ± 12.3 years) consented to TMS of the ipsilateral hemisphere. Of the participants who chose to withdraw from this part of the study, all cited poor tolerability to TMS as the reason for their decision. Similar to data obtained from the contralateral hemisphere, there was no difference in resting motor threshold (LE: 47.8 ± 10.7; Control: 49.5 ± 13.7; main effect, $F_{(1,18)} = 0.10$, $p = 0.76$), active motor threshold (LE: 45.6 ± 10.0; Control: 46.0 ± 9.9; main effect, $F_{(1,18)} = 0.008$, $p = 0.93$) or

corticomotor output (LE: 0.28 ± 0.14mV; Control: 0.33 ± 0.22mV; main effect, $F_{(1,18)}$ = 0.39, $p$ = 0.54) between groups.

The magnitude of SICI was similar between those with and without LE under all test conditions (70%: main effect, $F_{(1,18)}$ = 0.01, $p$ = 0.91; 80%: main effect, $F_{(1,18)}$ = 0.004, $p$ = 0.95; 90%: main effect, $F_{(1,18)}$ = 0.02, $p$ = 0.89; Figure 4.1B), and this was the same for measures of LICI (main effect, $F_{(1,18)}$ = 2.36, $p$ = 0.14; Figure 4.2B) and ICF (70% AMT: main effect, $F_{(1,18)}$ = 0.0003, $p$ = 0.99; 80%: main effect, $F_{(1,18)}$ = 0.14, $p$ = 0.71; 90%: main effect, $F_{(1,18)}$ = 0.65, $p$ = 0.43; Figure 4.1D). The amplitude of the test MEP was comparable between groups for LICI (main effect, $F_{(1,18)}$ = 2.64, $p$ = 0.15) and for each SICI and ICF conditioning intensity (70% AMT: main effect, $F_{(1,18)}$ = 0.84, $p$ = 0.37; 80% AMT: main effect, $F_{(1,18)}$ = 3.61, $p$ = 0.07; 90% AMT: main effect, $F_{(1,18)}$ = 1.84, $p$ = 0.19).

*4.4.2.3 Comparison of TMS of the hemisphere contralateral and ipsilateral to the 'affected' arm in LE*

There was no difference in resting motor threshold ($F_{(1,9)}$ = 0.08, $p$ = 0.78), active motor threshold ($F_{(1,9)}$ = 0.57, $p$ = 0.47) or corticomotor output ($F_{(1,9)}$ = 0.48, $p$ = 0.50) between the motor representations of ECRB of the affected or unaffected arm for individuals with LE consenting to TMS of both hemispheres (n = 10). Similarly, there was no difference in SICI (70%: $F_{(1,9)}$ = 1.87, $p$ = 0.21, 80%: $F_{(1,9)}$ = 0.00003, $p$ = 0.99, 90%: $F_{(1,9)}$ = 0.07, $p$ = 0.80) or ICF (70%: $F_{(1,9)}$ = 0.78, $p$ = 0.40, 80%: $F_{(1,9)}$ = 2.55, $p$ = 0.15, 90%: $F_{(1,9)}$ = 2.34, $p$ = 0.16) at any conditioning intensity. LICI was also comparable between hemispheres ($F_{(1,9)}$ = 0.04, $p$ = 0.86) as were test MEP

amplitudes for the paired-pulse protocols (SICI/ICF 70%: $F_{(1,9)} = 0.009$, $p = 0.93$, 80%: $F_{(1,9)} = 3.24$, $p = 0.11$, 90%: $F_{(1,9)} = 0.19$, $p = 0.68$; LICI: $F_{(1,9)} = 0.06$, $p = 0.81$).

### *4.5 Discussion*

This study is the first to investigate M1 intracortical networks in individuals with chronic LE. We demonstrate less intracortical inhibition mediated by both $GABA_A$ and $GABA_B$ receptors, and less intracortical facilitation, in the M1 contralateral to the affected ECRB in individuals with LE compared with healthy controls. Similar changes were not present in the M1 contralateral to the unaffected ECRB. These changes may provide the substrate for altered M1 organisation in chronic LE and could provide a target for future therapy.

Individuals with LE displayed on average, 27% less inhibition in networks mediated by $GABA_A$ (SICI) and 50 % less inhibition in networks mediated by $GABA_B$ (LICI) for the motor representation of the affected ECRB muscle compared with healthy controls. These data are suggestive of cortical disinhibition (shift towards greater excitability) in LE. However, individuals with LE also displayed 26% less intracortical facilitation (ICF) than healthy controls, suggesting that ICI and ICF are differentially affected by the presence of chronic elbow pain. This finding is not surprising given that ICI and ICF are mediated by different receptor systems and are thought to act independently (Ziemann et al. 1996a, Ziemann et al. 1998, Werhahn et al. 1999, Ilic et al. 2002, McDonnell et al. 2006, Di Lazzaro et al. 2007a). Indeed, despite similar conditioning requirements, ICI and ICF are differentially affected by current direction and intensity

(Ziemann et al. 1996c), and evoke different patterns of cerebral blood flow (Strafella et al. 2001).

Previous studies investigating intracortical networks typically report no difference or, like the present findings, reduced inhibition/facilitation in persons with chronic musculoskeletal pain. For example, some studies report less SICI in fibromyalgia (Mhalla et al. 2010, Schwenkreis et al. 2011), low back pain (Masse-Alarie et al. 2012) and complex regional pain syndrome (Schwenkreis et al. 2003, Eisenberg et al. 2005) compared with healthy controls, others report normal levels of inhibition (Salerno et al. 2000). Similarly, less ICF has been demonstrated in fibromyalgia (Salerno et al. 2000, Mhalla et al. 2010) compared with controls, whereas studies of people with osteoarthritis and complex regional pain syndrome report no difference (Schwenkreis et al. 2003, Eisenberg et al. 2005, Schwenkreis et al. 2010). Only one study has examined the effect of musculoskeletal pain on LICI. That study reported less inhibition in individuals with fibromyalgia than controls (Salerno et al. 2000). As each of these mechanisms are controlled by independent cortical networks (Ziemann et al. 1996c), it is plausible for both intracortical inhibition *and* facilitation to be reduced in individuals with LE. However, since the circuits underpinning these mechanisms demonstrate complex interactions (Sanger et al. 2001), the relative contribution of each mechanism towards corticomotor output in LE remains uncertain.

Since M1 disinhibition has been demonstrated in conditions characterised by pain of neuropathic (neuralgia), but not nociceptive (osteoarthritis) origin (Schwenkreis et al.

2010), it has been suggested that discrepancies between studies (no change vs. decreased inhibition/facilitation) might reflect differences in the pathological process of different chronic conditions. Alternatively, it is possible that symptom duration is a significant determinant of the direction and extent of M1 cortical change. Indeed, although there is currently little data detailing the progression of cortical change during the transition from the acute to the chronic pain state, there is evidence to suggest that the direction of these effects differ for acute experimental pain (e.g. SICI increased), compared to clinical chronic pain (e.g. SICI decreased or unchanged) (Schabrun et al. 2012). In addition to differences in patient characteristics, discrepancies between studies in identical pain conditions may also relate to methodological factors. For example, in fibromyalgia, ICI is reduced when TMS pulses are separated by 2ms (Mhalla et al. 2010, Schwenkreis et al. 2011), but not at intervals of 4ms (Salerno et al. 2000) and in the present study, between-group differences were only detected using a conditioning intensity of 90% active motor threshold for SICI and 80% active motor threshold for ICF. These data indicate that studies investigating M1 intracortical networks should use a range of conditioning stimulus intensities and inter-stimulus intervals.

The conditioning stimulus intensity required to observe differences between people with LE and healthy controls provides further information on the integrity of intracortical networks in this condition. Data from healthy individuals indicate that SICI is strongest at a conditioning intensity of 90% active motor threshold and ICF is strongest at a conditioning intensity of 80% active motor threshold (Ortu et al. 2008). However, individuals with LE displayed the strongest SICI and ICF at 80% and 90%

active motor threshold respectively. As the threshold for evoking ICI/ICF is hypothesized to reflect the threshold for stimulating axons belonging to GABAergic and glutamatergic interneurons respectively (Ziemann et al. 1998, Ilic et al. 2002, Orth et al. 2003), these findings suggest that the electrophysiological properties of circuits involving these populations may be altered in LE (Di Lazzaro et al. 1998c, Hanajima et al. 1998, Di Lazzaro et al. 2006b). Changes to the structure of these circuits, such as the proximity of each population to the stimulating coil and the orientation of their axons with respect to the induced current, may also contribute to altered ICI/ICF thresholds in this condition (Orth et al. 2003).

The results of the present study provide insight into mechanisms that may contribute to the development of altered M1 organisation and motor dysfunction in LE. Rapid reorganisation of M1 is thought to depend on changes in synaptic efficacy that rely on GABAergic disinhibition and NMDA receptor dependent long-term potentiation-like mechanisms (Liepert et al. 1998a). Altered function of intracortical networks is therefore a plausible mechanism to explain increased map volume, greater MEP amplitude and less separation in the cortical representations of the ED and ECRB muscles in LE (Schabrun et al. 2015b). In addition, a key function of intracortical networks is to facilitate contraction of muscles required for a motor task while preventing unwanted movements, muscle overflow and co-contraction of surrounding muscles (Liepert et al. 1998b). Motor dysfunction characterized by altered muscle synergies between ECRB and other extensor/flexor muscles of the wrist (Alizadehkhaiyat et al. 2007), adoption of a flexed wrist posture (Bisset et al. 2006), diminished ability to generate force (De Smet et al. 1997, Slater et al. 2005)

and reduced fine motor performance (Skinner et al. 2007) have been reported in LE. Finally, LE is common in people who perform manual tasks with repeated, rapid movements of the wrist and forearm (Fan et al. 2009, Descatha et al. 2013) and repetitive movement training has been shown to reduce expression of SICI and ICF (Nordstrom et al. 2002, Cirillo et al. 2011). One possibility is that repetitive movements drive intracortical changes (reduced ICI and reduced ICF as observed in the present study) leading to altered M1 organisation, motor dysfunction and pain persistence in LE. However, as this study was exploratory in nature and not designed to determine causality, further work using longitudinal study designs are needed to confirm this hypothesis. Studies investigating larger cohorts of participants and studies including individuals in the acute (< 6 weeks) and chronic (> 3 months) stage of disease are required to determine the time-course of altered cortical organisation and function in LE.

We did not observe a relationship between ICI/ICF and measures of pain or disability. However, previous studies of M1 organisation in LE have shown a relationship between the degree of cortical reorganisation (specifically the degree of overlap between the representations of ED and ECRB) and pain severity in the last 6 months (Schabrun et al. 2015b). It is conceivable that any relationship between ICI/ICF and outcomes of pain and disability is non-linear, and depends on the relative contribution of changes in ICI and ICF to M1 organisation. This hypothesis would explain why features of altered M1 organisation are associated with pain severity in LE while intracortical mechanisms are not. Furthermore, although SICI has been shown to correlate with pain measures in myofascial pain syndrome (Volz et al. 2013)

and complex regional pain syndrome (Schwenkreis et al. 2003), similar findings have not been reported for other conditions such as low back pain or fibromyalgia (Mhalla et al. 2010, Schwenkreis et al. 2011, Masse-Alarie et al. 2012). Despite these findings, it remains possible that therapies that normalise activity in intracortical networks could have a role in the treatment of LE and other chronic pain conditions by preventing maladaptive reorganisation of M1. Future studies should seek to investigate whether therapies capable of targeting intracortical networks, such as repetitive TMS, are of benefit in persistent LE and whether normalization of these networks is associated with improved M1 organisation.

Finally, there are several limitations of the present study that should be acknowledged. First, it is unclear whether the present findings are typical of the general LE population as data were collected from a small sample of convenience. Studies involving a larger number of participants are required to confirm our results and to further examine potential relationships between neurophysiological outcomes and clinical characteristics. Larger trials would also be better able to control for confounding variables such as 'handedness'- a factor not accounted for in the in the present study due to the small proportion of LE participants (n = 3) experiencing symptoms in the non-dominant arm. Second, since we did not assess muscle representations other than ECRB, it is not clear whether the observed changes are restricted to the 'painful' muscle. Indeed, since the M1 representation of a muscle adjacent to ECRB (extensor digitorum) has been found to be similarly altered in LE in a previous study (Schabrun et al. 2015b), it may be anticipated that altered inhibitory and/or facilitatory network activity may extend to other local muscles. Further

research is required to determine whether M1 cortical change is confined to muscles located within the immediate vicinity or pain in LE or whether these effects also extend to muscles distal and/or proximal to ECRB. Third and final, the reliability of these findings may be improved by increasing the number of pulses used for intracortical assessments. In the present study, intracortical inhibition/facilitation was estimated from the average of twelve pulses at each ISI. As previous investigations of these mechanisms in persons with chronic musculoskeletal pain have been based on four to ten pulses (Salerno et al. 2000, Mhalla et al. 2010, Schwenkreis et al. 2011, Masse-Alarie et al. 2012, Volz et al. 2013, Caumo et al. 2016, Masse-Alarie et al. 2016), it was assumed that twelve would be adequate to provide a reliable measure of SICI, ICF and LICI in LE. However, more recent evidence suggests that a minimum of 20 pulses are required to achieve a reliable estimate of SICI, while 23 are required for ICF (Chang et al. 2016). Thus, extending the number of pulses used to assess these mechanisms is an important step towards increasing the reliability and quality of findings in the future.

### *4.6 Conclusion*

This study is the first to provide evidence of reduced intracortical activity mediated by $GABA_A$ (short-interval intracortical inhibition, SICI), $GABA_B$ (long-interval intracortical inhibition; LICI) and NMDA (intracortical facilitation, ICF) receptors in individuals with chronic LE. We hypothesize that these mechanisms may drive altered M1 organisation and aspects of motor dysfunction in this condition. However, longitudinal trials on larger subject numbers are required to confirm this relationship.

If confirmed, therapies that restore intracortical function may have the potential to normalise cortical abnormalities and improve outcomes in LE.

# Chapter 5:

## Temporal and spatial characteristics of post silent period electromyographic bursting in low back muscles: comparison between persons with and without low back pain

The findings of the previous chapter, in conjunction with previous research in this field, suggest that cortical disinhibition may be common amongst chronic musculoskeletal pain conditions. This chapter reports on the findings of an observational study designed to investigate the temporal and spatial characteristics of a novel, direct measure of corticomotor disinhibition (EMG bursting) in persons with chronic low back pain and pain-free individuals. The content of this chapter has been published in *Burns E, Chipchase LS, Schabrun SM (2017). Temporal and spatial characteristics of post silent period electromyographic bursting in low back muscles: comparisons between persons with and without low back pain. International Journal of Neuroscience. 1-23.* A copy of this publication is provided in Appendix D.

### *5.1 Abstract*

Recently, a novel measure of cortical disinhibition was identified using transcranial magnetic stimulation (TMS). This measure, described as post silent period electromyographic (EMG) bursting, may inform on the corticomotor control of movement in health and disease, however it has not been investigated for muscles outside the hand or in musculoskeletal conditions. Thus, the aim of this study was to investigate the temporal and spatial characteristics of 'EMG bursting' in individuals with and without low back pain (LBP). TMS was used to map the motor cortical representation of paraspinal muscles in eleven individuals with LBP and eleven pain-free controls. The latency, duration and magnitude of bursting, number of active burst sites, map volume and coordinates of the burst 'hotspot' were compared between groups. In pain-free controls, the latency, duration and magnitude of bursts were similar to the hand however bursts occurred earlier and were of smaller magnitude in LBP. Bursting was widespread throughout the cortical representation in both groups, however there was a trend towards smaller mean EMG burst and map volume in LBP. Here we confirm the presence of EMG bursting in back muscles and provide a description of the spatial profile of this mechanism. Our observations in LBP suggest that cortical disinhibition may be altered in this condition.

### *5.2 Introduction*

Transcranial magnetic stimulation (TMS) can be used to assess features of the corticomotor control of movement in health and disease. In particular, paired pulse paradigms have been used to investigate inhibitory activity mediated by gamma-Aminobutyric acid (GABA) in healthy individuals and in a range of pathological conditions. In low back pain (LBP) for example, there is evidence of reduced inhibition in networks mediated by $GABA_A$ receptors, suggesting changes in these networks might be involved in this condition. Recently, a novel single pulse measure of intracortical activity has been identified. This response, described as post silent period 'EMG bursting', has been hypothesized to reflect the activation of $GABA_B$ receptors on inhibitory interneurons and represent a measure of corticomotor disinhibition (Chin et al. 2012). However, despite recurrent observations (Ferbert et al. 1992, Wilson et al. 1995, Kimiskidis et al. 2005, King et al. 2006), few studies have specifically examined this measure in healthy individuals and there have been no studies in musculoskeletal pain conditions.

EMG burst responses following the cortical silent period have been demonstrated following magnetic stimulation of the cortical territory devoted to the hand (Wilson et al. 1995, Chin et al. 2012). In a recent investigation, TMS delivered over the motor hotspot during volitional contraction consistently evoked transient (~60ms) but distinct bursts of muscle activity up to three times the amplitude of background EMG. A GABAergic origin for this response was proposed since bursts were largest following

longer cortical silent periods ($GABA_B$ mediated) and occurred at latencies corresponding with a known period of reduced corticomotor inhibition (also $GABA_B$ mediated) (Cash et al. 2010). Hence, measurement of EMG bursting may provide further insight into cortical processes involving GABA mediated networks in pathological conditions such as LBP. However, the temporal characteristics and the cortical distribution of EMG bursts have yet to be investigated for muscles outside the hand. It is also unknown whether EMG bursting differs between healthy individuals and those with musculoskeletal pathology. Thus, the aims of the present study were to: (1) confirm the presence of post silent period EMG bursting in low back muscles and (2) examine the spatial and temporal profile of EMG bursting using TMS mapping, in persons with and without LBP. We hypothesize that examination of burst characteristics in individuals with LBP will reveal further evidence of reduced cortical inhibition in this condition.

## *5.3 Methods*

### 5.3.1 Participants

Eleven right-handed individuals with a history of recurring episodes of non-specific LBP (six males, aged 29 ± 7 years) and eleven age and gender matched pain-free controls (six males, 27 ± 5 years) participated. Sample size calculations were based on effect sizes from a previous study examining GABAergic cortical inhibition in LBP(Masse-Alarie et al. 2012). Based on these data (difference of means between patients and controls: 56%, standard deviation: ± 40.05), it was calculated that a minimum sample size of eight participants in each group were needed to observe a statistically significant difference

(80% power, alpha 0.05) should one exist (Kadam et al. 2010). Individuals with LBP were recruited during an active episode of low back pain (with or without buttock pain). To be eligible for inclusion, average pain intensity was required to be greater than 3 on an 11-point numerical rating scale (NRS) anchored with "no pain" at zero and "worst pain imaginable" at 10, and of sufficient intensity to interfere with at least three important activities of daily living (assessed via a Patient-Specific Functional Scale) (Stratford et al. 1995). All participants completed a TMS safety screening questionnaire and were excluded from enrolment if they had a personal or family history of epilepsy, major neurological, respiratory, orthopaedic or circulatory disorders, if they were pregnant, had metal in their head or jaw or were taking central nervous system acting medications (Rossi et al. 2011). Additional exclusion criteria for LBP participants included previous spinal surgery, the use of analgesic or anti-inflammatory medication in the last month or the receipt of treatment from a health professional in the last month. The study was approved by the institutional human medical research ethical committee and performed in accordance with the Declaration of Helsinki. All participants provided written, informed consent.

### 5.3.2 Electromyography (EMG)

Surface electromyography (EMG) was recorded bilaterally from the paraspinal muscles 3cm lateral to the spinous process of L3 and 1cm lateral to the spinous process of L5 via dual silver-silver chloride disposable electrodes (spacing 2.0cm, Noraxon USA Inc, Az, USA). These sites record EMG from deep and superficial back muscles including

multifidus and erector spinae (Lariviere et al. 2003). Ground electrodes were positioned over the anterior superior iliac spine of the same side. EMG signals were amplified x1000 (NL844, Digitimer Ltd, Welwyn Garden City, UK), band-pass filtered: 20-1000 Hz and sampled at 2000 Hz using a Micro1401 data acquisition System and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

### 5.3.3 Transcranial magnetic stimulation (TMS)

TMS mapping of the cortical representation of the lumbar paraspinal muscles was performed according to procedures outlined previously (O'Connell et al. 2007, Schabrun et al. 2014, Schabrun et al. 2015a). In brief, TMS was delivered to the primary motor cortex contralateral to the side of worst pain (or the matched side for pain-free controls) using a Magstim 200 stimulator (Magstim Co. Ltd., Dyfed, UK) connected to a figure-of-eight coil (70 mm wing diameter), oriented with the handle facing posteriorly with respect to the midline. The location of the vertex (Cz) was determined using the 10/20 International EEG Electrode Placement system and registered using a Brainsight neuronavigation system (Rogue Research Inc, Quebec, Canada). Starting at the vertex, five magnetic stimuli were delivered at 1cm intervals on a 6 x 7cm grid with the aid of the neuronavigation instrument. Stimuli were applied at 100% of stimulator output with an inter-stimulus interval of 6 s while participants performed a low-level voluntary contraction (20% maximum) of the paraspinal muscles. Target amplitude was determined based on the largest root mean square (RMS) EMG achieved during three 3 second maximal trunk extension efforts performed against manual resistance in sitting

(Schabrun et al. 2015a). During testing, participants maintained the appropriate level of muscle contraction by sitting forward with the back straight (Tsao et al. 2011a, Schabrun et al. 2015a). Visual feedback was provided on a computer monitor to ensure symmetrical pre-activation. To ensure that the prolonged sitting and high TMS stimulator output required during the mapping procedure did not exacerbate LBP symptoms, pain severity was monitored verbally throughout, and evaluated on completion of TMS mapping using an 11-point NRS. All procedures adhered to the TMS checklist for methodological quality (Chipchase et al. 2012).

### 5.3.4 Data analyses

Analysis of TMS map data was performed using MATLAB 7 (The Mathsworks, USA). EMG was full-wave rectified and trials (five) at each scalp site were averaged. Five parameters were extracted from these data: (1) MEP amplitude, (2) cortical silent period, (3) percentage burst ratio (PBR), (4) burst duration, and (5) burst silent period. To account for pre-activation, MEP amplitude (uV) was calculated by subtracting the RMS EMG recorded 55 to 5ms prior to stimulation (background EMG) from the RMS EMG between MEP onset and offset (Strutton et al. 2005, Tsao et al. 2008, Tsao et al. 2010, Tsao et al. 2011b). The duration of the cortical silent period (ms) was determined as the time between MEP offset and the resumption of EMG equivalent to or greater than that present pre-stimulus. If a clear burst in EMG activity was identified following the cortical silent period, the RMS of the burst was calculated by manually cursoring burst onset and offset. Where a burst was not apparent in a trial, but could be identified in other trials

within the set of five, the mean of those bursts were used to calculate RMS EMG for that map site (Chin et al. 2012). If no bursts were identified within the set of five trials, RMS EMG for that map site was calculated from background EMG 0 - 50ms after the cortical silent period. The PBR (%) was calculated by expressing mean burst RMS EMG as a percentage of mean background EMG (Chin et al. 2012). The duration of the burst silent period (ms) was determined to be the period between burst offset and the resumption of RMS EMG equivalent to or greater than that observed pre-stimulus. To examine the spatial profile of EMG bursting in M1, PBR were superimposed over the respective scalp sites to generate a 'PBR map' for each participant. The number of active burst sites, mean PBR of the map (%), PBR map volume and the anterior-posterior and medial-lateral location (cm) of the largest MEP (the 'motor hotspot') and PBR ('burst hotspot') were identified for each map. For a map site to be considered 'active', at least one trial out of five was required to display evidence of EMG bursting. Mean PBR of the map (%) and PBR map volume were calculated as the average or sum of PBR recorded at each active site, respectively.

### 5.3.5 Statistical analyses

Data for MEP amplitude, cortical silent period, PBR, map volume, burst duration, burst silent period were assessed for normality via the Shapiro-Wilk test and compared between groups (Control vs. LBP) using separate one-way analyses of variance (ANOVA). To ensure that pre-activation during TMS testing did not aggravate pain in the LBP group, pain intensity before and after TMS were compared using one-way repeated measures

ANOVA. Separate two-way ANOVA were used to compare map hotspots (MEP vs. PBR) between groups (Control vs. LBP) in the anterior-posterior and medial-lateral direction. Post hoc analyses were corrected for multiple comparisons using the Holm-Sidak method. Eta-squared was calculated as a measure of the effect size for each outcome. Pearson correlation analyses were performed to examine the relationship between (1) cortical silent period duration and PBR in both groups and (2) pain severity and neurophysiological outcomes in LBP. Statistical significance was set at $P < 0.05$. Data in text are expressed as mean ± standard deviation unless stated otherwise.

### *5.4 Results*

As there was no difference between responses at L3 and L5 within the LBP or pain-free groups ($p > 0.42$), data generated from both recording sites were pooled and compared between groups. Normative data and data from individuals with LBP are presented in Table 5.1.

***Table 5.1*** *Group data (mean ± SD) for controls and individuals with LBP. *p < 0.05.*

| | **CONTROL** *(n = 11)* | **LBP** *(n = 11)* |
|---|---|---|
| **Active burst sites (n/42)** | 38.6 ± 3.5 | 36.5 ± 7.6 |
| **Map volume (%)** | 5406 ± 734 | 4794 ± 1327 |
| **Mean PBR of map (%)** | 140 ± 13 | 131 ± 18 |
| **Max PBR of map (%)** | 229 ± 59 | 209 ± 77 |
| **MEP at motor hotspot (mV)** | 0.019 ± 0.013 | 0.010 ± 0.009* |
| **Cortical silent period at motor hotspot (ms)** | 81.4 ± 49.3 | 53.7 ± 21.3* |
| **PBR at motor hotspot (%)** | 150 ± 62 | 117 ± 46* |
| **Burst onset at motor hotspot (ms)** | 123 ± 54 | 88 ± 23* |
| **Burst duration at motor hotspot (ms)** | 57.0 ± 33.6 | 61.2 ± 25.4 |
| **Burst silent period duration at motor hotspot (ms)** | 54.3 ± 34.2 | 54.7 ± 33.1 |

PBR, percentage burst ratio; MEP, motor evoked potential.

## 5.4.1 EMG bursting is present in the low back muscles of pain-free individuals

### *5.4.1.1 Burst characteristics of the map in pain-free individuals*

The present data indicate that EMG bursting can be elicited by stimulating multiple scalp sites overlying the cortical territory of the low back muscles. Example traces of EMG bursting are presented in Figure 5.1 and average PBR maps are presented in Figure 5.2.

In pain-free controls, approximately 92% of map sites showed evidence of EMG bursting. In this group, the largest burst was more than double the amplitude of background RMS EMG (PBR ~229%) and was located 1.3 ± 1.8cm anterior and 1.7 ± 1.7cm lateral to Cz. Mean PBR of the map was 140 ± 13% and map volume was 5406 ± 734%.

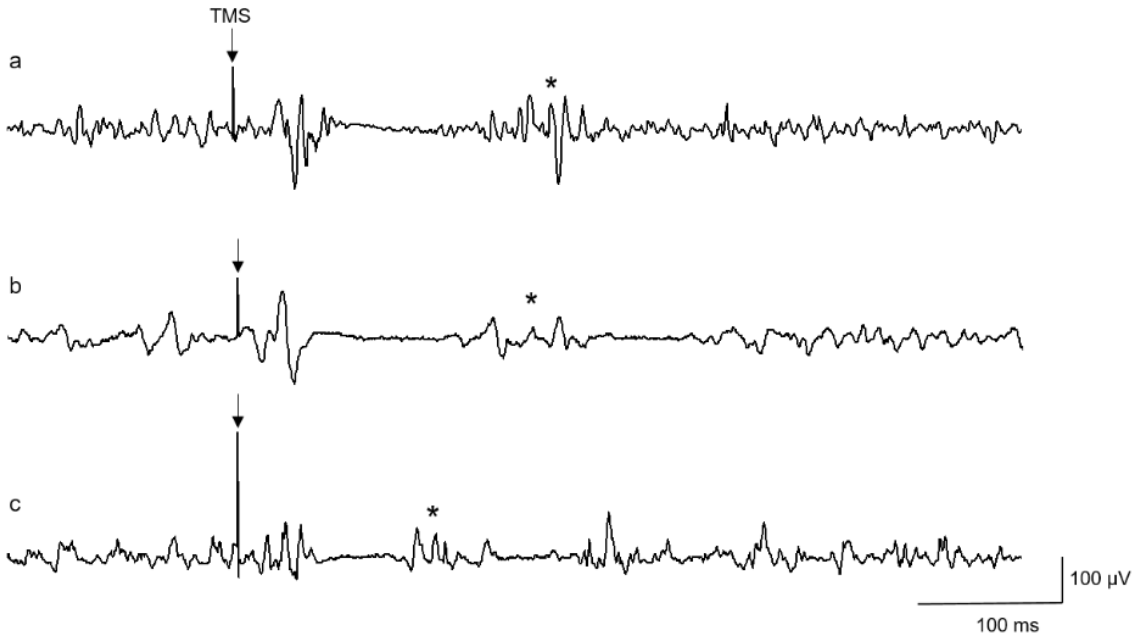


***Figure 5.1*** *Representative waveforms demonstrating EMG bursting in the lumbar paraspinal muscles of two pain-free subjects (a, b) and one LBP subject (c) during sustained 20% MVC. Note that two types of responses were typically observed: a) the cortical silent period is terminated by a burst of EMG activity, or b/c) the cortical silent period is interrupted by a burst of EMG activity. MEP amplitudes were typically smaller and bursts occurred earlier in persons with LBP compared to controls. *Indicates position of burst*

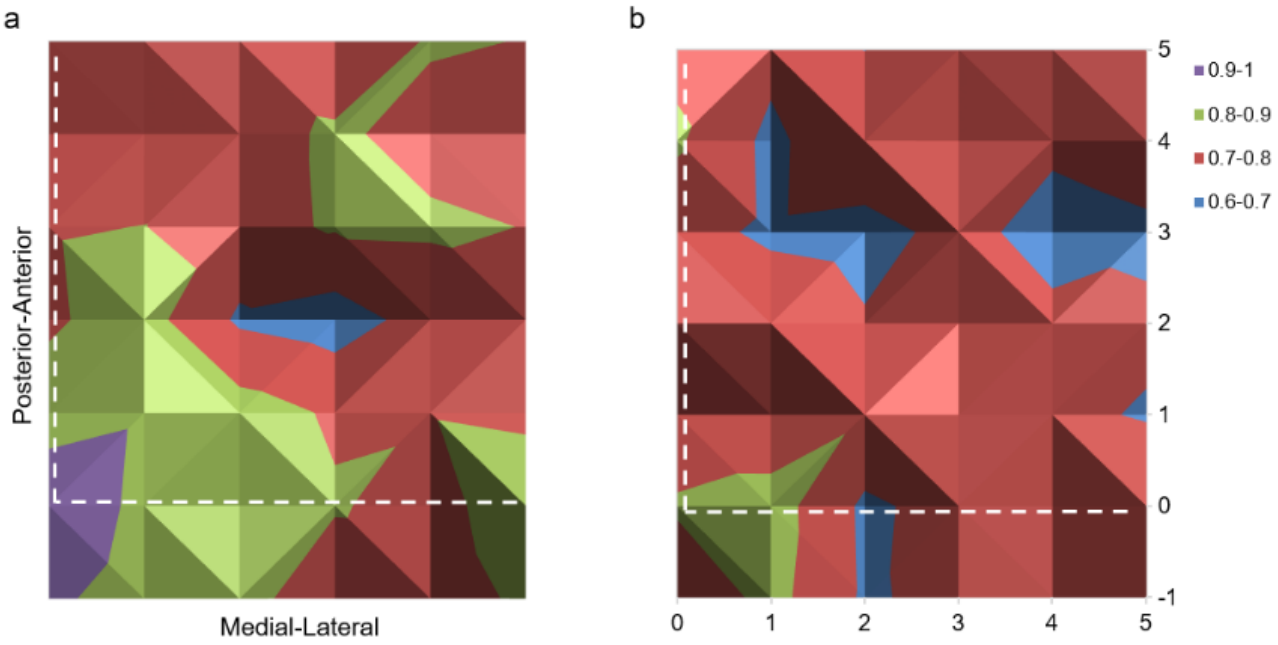


***Figure 5.2*** *Averaged and normalised PBR maps obtained from the lumbar paraspinal muscles of a) pain-free individuals and b) individuals with LBP. The horizontal dashed line represents the inter-aural line and the vertical dashed line represents the line from the nasion to the inion. Cz is located at coordinate 0, 0. Note the difference in magnitude and distribution of EMG bursting between groups.*

*5.4.1.2 Burst characteristics at the motor hotspot in pain-free individuals*

At the motor hotspot, normative data indicated that burst amplitude was approximately 1.5 times larger than that of background RMS EMG. On average, burst activity commenced 123 ± 54ms following magnetic stimulation and lasted 57 ± 33ms. This was immediately followed by a period of EMG silence in 10 out of 11 control participants (Figure 5.1B). In these participants, the duration of the burst silent period was 54 ± 34ms.

A positive correlation between the PBR and the duration of the cortical silent period was detected at the motor hotspot (r = 0.54, $p$ = 0.0096).

### 5.4.2 Burst characteristics differ for individuals with low back pain compared to pain-free controls

Patient characteristics are summarized in Table 5.2. Average pain intensity on the day of testing was 4.0 ± 2.0 on the NRS and the time elapsed since first pain episode was 56 ± 40 months. The procedure of TMS mapping did not alter pain intensity ($F_{(1,10)}$ = 2.41, $p$ = 0.15, $\eta^2$ = 0.028). At the motor hotspot, EMG bursts occurred earlier ($F_{(1,42)}$ = 7.62, $p$ = 0.009, $\eta^2$ = 0.15) and were of smaller magnitude in individuals with LBP compared to controls ($F_{(1,42)}$ = 4.09, $p$ = 0.050 , $\eta^2$ = 0.091). There was, however, no detectable correlation between pain severity and PBR (r = 0.27, $p$ = 0.22) or pain severity and burst onset (r = 0.26, $p$ = 0.24). Other characteristics including the duration of the burst ($F_{(1,42)}$ = 0.23, $p$ = 0.64, $\eta^2$ = 0.005) and burst silent period did not differ ($F_{(1,30)}$ = 0.001, $p$ = 0.97, $\eta^2$ < 0.001) between groups. The number of active burst sites were also similar for individuals with and without LBP ($F_{(1,42)}$ = 1.43, $p$ = 0.24, $\eta^2$ = 0.033; Figure 5.2A,B), as was the maximum PBR of the map ($F_{(1,42)}$ = 1.03, $P$ = 0.32, $\eta^2$ = 0.024). A trend towards smaller mean PBR and map volume was detected for LBP but failed to reach statistical significance (mean: $F_{(1,42)}$ = 3.48, $p$ = 0.069, $\eta^2$ = 0.076; volume: $F_{(1,42)}$ = 3.58, $p$ = 0.065, $\eta^2$ = 0.079). The PBR hotspot was located 1.2 ± 1.7cm anterior and 1.8 ± 1.7cm lateral to Cz. Two-way ANOVA revealed that the burst hotspot was located significantly closer to the midline compared to the MEP hotspot in both groups (Figure 5.3; post hoc: $p$ =0.007)

however did not identify an interaction between groups (Medial-Lateral: $F_{(1,84)} = 0.38$, $p = 0.54$, $\eta^2 = 0.004$).

***Table 5.2*** *Demographic and clinical characteristics for individuals with LBP (n = 11).*

| SUBJECT | GENDER | AGE *(years)* | SIDE OF PAIN | CURRENT PAIN *(NRS/10)* | PAIN DURATION *(months)* |
|---|---|---|---|---|---|
| **1** | F | 27 | L | 1 | 84 |
| **2** | M | 36 | R | 7.5 | 60 |
| **3** | M | 39 | R | 4 | 100 |
| **4** | F | 19 | R | 2 | 60 |
| **5** | M | 27 | R | 6 | 18 |
| **6** | M | 41 | L | 3 | 60 |
| **7** | F | 31 | R | 5 | 6 |
| **8** | M | 31 | R | 6 | 108 |
| **9** | M | 24 | L | 5 | 6 |
| **10** | F | 27 | L | 2 | 100 |
| **11** | F | 19 | R | 3 | 17 |

NRS, numerical rating scale.

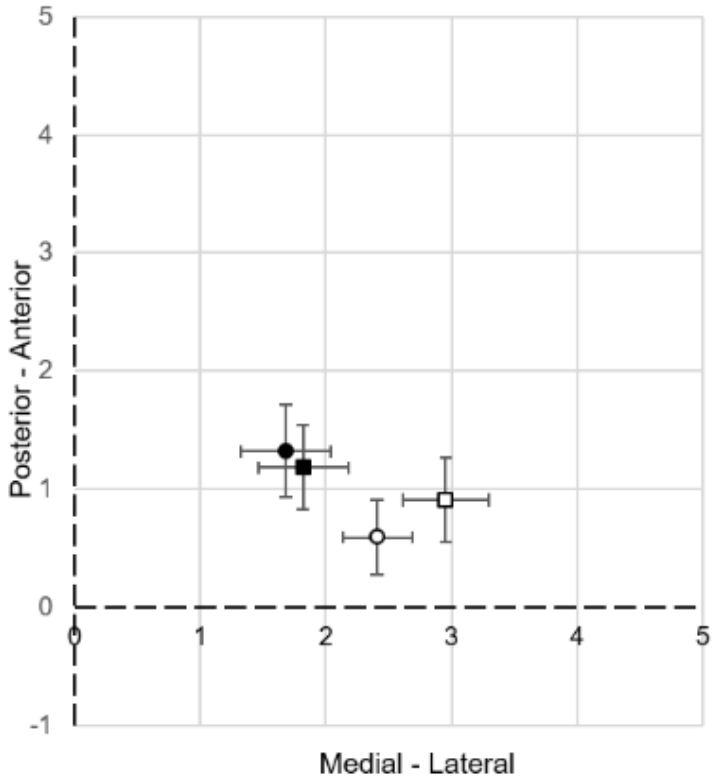


***Figure 5.3*** *Topographical location (mean ± standard error) of MEP hotspot (open markers) and PBR hotspot (filled markers) for LBP (squares) and pain-free controls (circles). The horizontal dashed line represents the inter-aural line and the vertical dashed line represents the line from the nasion to the inion. Cz is located at coordinate 0, 0. Each grid square represents 1 x 1cm. Note that the PBR hotspot is positioned medially to the MEP hotspot in both groups.*

### 5.4.3 MEP amplitude and the duration of the cortical silent period are less in LBP compared to pain-free controls

At the motor hotspot, MEP amplitudes were smaller ($F_{(1,42)}$ = 5.90, $p$ = 0.019, $\eta^2$ = 0.12) and the cortical silent period was shorter ($F_{(1,42)}$ = 5.87, $p$ = 0.02, $\eta^2$ = 0.12) for individuals with LBP compared to controls. In contrast to pain-free controls, there was no correlation

between PBR and cortical silent period duration at this site for individuals with LBP (r = 0.21, *p* = 0.34). There was also no significant correlation between pain severity and MEP amplitude (r = -0.20, *p* = 0.37) or pain severity and cortical silent period duration (r = 0.074, *p* = 0.75) in this group.

## *5.5 Discussion*

The results of the present study confirm the presence of post silent period EMG bursting in muscles of the low back. We also provide a description of the spatial distribution of bursting in the primary motor cortex (M1), and the first account of differences between healthy individuals and those with musculoskeletal pain.

### 5.5.1 Spatial and temporal characteristics of EMG bursting in the back

This study utilised TMS mapping techniques to examine the spatial profile of post silent period EMG bursting in M1. Our findings show that, like MEPs, EMG bursts may be elicited from a number of scalp sites overlying the cortical representation of the lumbar paraspinal muscles in individuals with and without LBP. While the magnitude of bursting varied across sites, we found the largest responses to be distributed within the posterior half of the map. Here, we observed a clear peak in PBR approximately 2cm medial to the location of the motor hotspot (Figure 5. 3). In pain-free individuals, PBR at the burst hotspot was 53% greater than that recorded at the motor hotspot. Interestingly, this difference was more pronounced in LBP (~77%). Differences in PBR at the burst and motor hotspots could be evidence that burst and MEP responses are generated by

independent cortical networks and may explain previous accounts of bursting in the absence of an evoked potential (Ferbert et al. 1992, Wilson et al. 1995). These findings also suggest that the motor hotspot may not be the optimal location to assess EMG bursting, particularly in patient populations.

In pain-free individuals, EMG bursts at the motor hotspot demonstrated a similar latency, duration and magnitude to those reported in the hand under similar test conditions (Chin et al. 2012). Similarly, a positive correlation between the cortical silent period and PBR was detected in the present study. Taken together, these findings suggest that burst characteristics may be similar across distal and axial muscles. If confirmed, responses recorded from the hand could be used to provide a global estimate of EMG bursting in M1. This method would be particularly advantageous in patient studies where the cortical representation of the affected muscle is positioned deep in the cortex and is difficult to target with TMS (e.g. low back or leg muscles) or where volitional contraction aggravates pain. However, as we only investigated EMG bursting at the site of pain in LBP, further work is needed to determine whether burst characteristics are identical for painful and pain-free muscles in this and other musculoskeletal pain conditions.

By confirming the latency and duration of the burst and its affiliation with the cortical silent period, our data further supports the hypothesis that post silent period EMG bursting represents a measure of the depth and magnitude of disinhibition in M1 (Chin et al. 2012). As was the case in the hand, the latency (~120ms) and duration (~50ms) of

bursting in the back muscles is consistent with that of a recently described period of late cortical disinhibition (Cash et al. 2010). In that study, a period of raised corticomotor excitability was noted following the evocation of a form of $GABA_B$-mediated inhibition known as long-interval intracortical inhibition (LICI). This effect was attributed to the action of pre-synaptic $GABA_B$ receptors which function to negatively regulate GABA release and facilitate excitatory post-synaptic potentials (Mott et al. 1991, Otis et al. 1993). This, taken together with evidence linking EMG bursts with the cortical silent period (also $GABA_B$-mediated), suggests that this mechanism may reflect the depth and magnitude of disinhibition in M1. However, as LICI and late cortical disinhibition have yet to be investigated in muscles of the trunk, further work is necessary to determine whether these mechanisms are present in the low back and whether this hypothesis also holds for our results.

A novel outcome of the present study was identification of the 'burst silent period'. While previous studies show evidence of EMG bursts terminating the cortical silent period or trains of bursts interspersed with low-level EMG in a proportion of test subjects, EMG bursts in the present study were followed by a clear period of EMG silence in 20 of 22 participants (Ferbert et al. 1992, Chin et al. 2012). In pain-free individuals, this episode appeared 200ms post stimulus and persisted for ~50ms. Since cortical silent periods longer than 85ms have yet to be demonstrated in muscles of the low back (Ferbert et al. 1992, Strutton et al. 2005), we suggest that this response represents a separate entity, rather than a continuation of the cortical silent period. It is possible that this 'burst silent

period' may signify a return of post-synaptic GABAergic activity following the conclusion of the EMG burst, however further work is necessary to establish causation and identify the mechanisms involved.

### 5.5.2 EMG bursting in Low Back Pain

At the location of the motor hotspot, bursts occurred earlier and were smaller in magnitude in individuals with LBP compared to pain-free controls. In keeping with current hypotheses, we interpret smaller bursts in LBP as evidence of reduced cortical disinhibition in this condition. Previous estimates of disinhibition in LBP have been based on chance observations in paired pulse studies investigating inhibitory networks in M1. In contrast to the present study, those studies cite lower levels of short interval intracortical inhibition (SICI) in abdominal and paravertebral muscles in LBP as evidence of increased disinhibition in this condition (Masse-Alarie et al. 2012, Masse-Alarie et al. 2016). Although it is common to interpret a reduction in SICI as a form of disinhibition, pharmacological evidence indicates that this mechanism reflects inhibitory processes mediated by post-synaptic $GABA_A$ receptors (Ziemann et al. 1996a, Di Lazzaro et al. 2000a), which, unlike the pre-synaptic $GABA_B$ receptors proposed to underlie EMG bursting (Chin et al. 2012), do not have the innate capacity to negatively regulate GABAergic transmission. Furthermore, a number of competitive interactions have been documented between SICI and other inhibitory and excitatory mechanisms in M1 (Sanger et al. 2001, Stefan et al. 2002, Ortu et al. 2008, Alle et al. 2009). Therefore, we suggest that the present findings may provide a more accurate account of the depth and

duration of M1 disinhibition in LBP. However, as we found no evidence of a relationship between burst characteristics and pain, the clinical significance of these findings remains unclear.

Despite no statistical difference between the location of hotspots between groups, there was a trend towards smaller mean PBR and smaller map volume in individuals with LBP. These findings are further evidence that the topography and mean excitability of the motor representation of low back muscles is altered compared to controls (Tsao et al. 2011b, Schabrun et al. 2014, Schabrun et al. 2015a). As these changes are hypothesized to underpin altered trunk muscle coordination and postural control in LBP, it is possible that an abnormal burst mechanism may similarly contribute to motor dysfunction in this condition (Tsao et al. 2011b).

In addition to altered burst characteristics, we also report reduced MEP amplitudes and shorter cortical silent periods at the motor hotspot in LBP. While these findings complement previous observations of decreased corticomotor excitability (Strutton et al. 2005, Tsao et al. 2011c, Schabrun et al. 2015a) and dysfunctional GABAergic disinhibition in LBP (Masse-Alarie et al. 2012, Masse-Alarie et al. 2016), they are not entirely compatible with our conclusion of reduced cortical disinhibition. Discrepant responses to TMS are not uncommon in observational studies and serve to highlight the intricate nature of M1, however, methodological factors also have the potential to affect study outcomes. For example, in the present study TMS mapping was performed with maximum stimulator output (100% intensity) in all participants. While in line with

previous studies using surface EMG over muscles of the low back during volitional contraction (Ferbert et al. 1992, O'Connell et al. 2007, Schabrun et al. 2014, Schabrun et al. 2015a), this methodology may give rise to erroneous results if differences in corticomotor excitability exist between groups. Indeed, since the magnitude of the MEP, the cortical silent period and EMG burst are reportedly intensity-dependent (Chin et al. 2012, Kojima et al. 2013), it is possible that our observation of smaller MEPs, smaller bursts, shorter silent periods and earlier burst onset in LBP may be a consequence of raised motor threshold. However, as motor threshold was not an outcome of the present study and there is a lack of consensus regarding this aspect of cortical excitability for low back muscles in LBP (Strutton et al. 2005, Masse-Alarie et al. 2016), the impact of this factor on our results remains unclear. To prevent such ambiguity in the future, we recommend that future studies requiring maximum stimulator output to generate M1 maps include a measure of active motor threshold to validate their findings, especially if abnormal motor threshold is suspected.

### 5.5.3 Conclusion

This study confirms the presence of post silent period EMG bursting in low back muscles. We found that the latency, duration and magnitude of bursting in healthy persons was similar to that reported previously in the hand however our novel observation of a 'burst silent period' suggests that this feature may be specific to the back. TMS mapping revealed a graded distribution of EMG bursting throughout M1, culminating in one definitive 'hotspot'. Our observation of spatial and temporal differences between

individuals with and without LBP may be evidence of altered cortical disinhibition in this condition, however further work is necessary to confirm this hypothesis.

# Chapter 6: General discussion

The primary aim of this thesis was to determine the effect of acute (lasting minutes) and chronic (lasting months) muscle pain on intracortical inhibition within the primary motor cortex (M1). In this chapter, findings from the four studies will be synthesized and discussed *'in toto'* to provide insight into the role of cortical inhibition in acute and chronic musculoskeletal pain. Limitations, clinical implications and future directions for research will be discussed.

### *6.1 Contribution of the thesis to the body of evidence*

This thesis provides novel and original data on the role of cortical inhibitory mechanisms in acute and chronic musculoskeletal pain. Although musculoskeletal pain is a common health complaint affecting up to 47% of the general population (Cimmino et al. 2011), the mechanisms associated with the development, maintenance and resolution of pain are poorly understood. Current evidence suggests that the organisation and function of the primary motor cortex (M1) is altered when pain is present and these changes contribute to symptoms of pain and movement dysfunction. Altered cortical inhibition is one mechanism that could underpin changes in M1 organisation and function in the acute and persistent stages of pain. However, only a small number of studies have investigated cortical inhibitory mechanisms in pain. Understanding these mechanisms is essential to improve our understanding of a challenging condition and to reveal new targets for future treatment.

A number of different forms of cortical inhibition exist within M1 that can be quantified using specific single pulse, paired pulse and mixed stimulation TMS protocols. Inhibitory activity mediated by post-synaptic $GABA_A$ and $GABA_B$ receptors located on M1 interneurons can be examined via delivery of paired pulses at specific short (short interval intracortical inhibition; SICI) and long (long interval intracortical inhibition; LICI) inter-stimulus intervals, while pre-synaptic $GABA_B$ mediated disinhibition can be observed in electromyographic recordings following single pulse stimulation (post silent period electromyographic bursting) (Claus et al. 1992, Kujirai et al. 1993, Ziemann et al.

1998, Werhahn et al. 1999, McDonnell et al. 2006, Chin et al. 2012). The interaction between sensory input and motor output at the level of the cortex (sensorimotor integration) can also be assessed by pairing peripheral nerve stimulation at the wrist with cortical stimuli (Chen et al. 1999, Tokimura et al. 2000). Each of these mechanisms has the capacity to influence the overall level of inhibition within M1, however the relative contribution of each is dependent on the presiding state of cortical excitability and complex interactions between specific neural populations. Indeed, results from triple pulse protocols indicate that $GABA_B$, $GABA_A$ and cholinergic forms of inhibition (SAI/LAI) are activated in a hierarchical manner. For example, SICI is suppressed in the presence of LICI, yet dominates SAI and is completely independent of LAI (Sanger et al. 2001, Sailer et al. 2002, Alle et al. 2009). Thus, investigation of each form of intracortical inhibition is essential to inform our understanding of these mechanisms in pain. This thesis explored each of these inhibitory mechanisms in the M1 of people experiencing acute and chronic pain. In brief, the findings of each study were:

Study 1: Provides the first systematic evaluation of the primary sensory (S1) and primary motor (M1) cortex response to acute experimental muscle pain in healthy volunteers. Synthesis of data from a range of methodologies showed moderate to strong evidence of reduced S1 and M1 excitability during, and following the resolution of acute experimental muscle pain.

Study 2: Provides the first description of the effect of acute experimental muscle pain on SAI, LAI and LICI in healthy volunteers. Results demonstrated a

reduction in sensorimotor integration (SAI, LAI), but not $GABA_B$ mediated inhibition (LICI), following the resolution of acute muscle pain.

Study 3: Provides the first evidence of reduced intracortical inhibition mediated via $GABA_{A/B}$ receptors (SICI and LICI) and reduced intracortical facilitation (ICF) in the M1 contralateral to the affected extensor carpi radialis brevis muscle in persons with chronic lateral epicondylalgia (elbow pain) compared to healthy volunteers.

Study 4: Provides the first description of the temporal and spatial profile of electromyographic bursting in low back muscles of persons with chronic low back pain. Results suggest that cortical disinhibition ($GABA_B$ mediated) is reduced in persons with chronic low back pain compared to healthy volunteers.

Although each study provided an original contribution to the body of evidence, findings from the four studies can be synthesized and discussed *'in toto'* to provide greater insights into the role of cortical inhibition in acute and chronic musculoskeletal pain conditions.

### *6.2 Cortical inhibition in acute musculoskeletal pain*

Acute musculoskeletal pain is experienced by almost everyone at some stage in his or her life. The onset of acute musculoskeletal pain is accompanied by disturbances in sensory and motor function yet, the mechanisms that underpin these changes are poorly understood. The first two studies in this thesis provide a comprehensive understanding of cortical inhibition in the presence of acute musculoskeletal pain. The first study, a systematic review and meta-analysis, aimed to establish the effect of acute muscle pain on the primary motor and somatosensory cortices, whereas the second study, extended our understanding of several forms of cortical inhibition (SAI, LAI, LICI) that had not previously been examined in acute musculoskeletal pain.

Study 1 provides the first systematic evidence of reduced corticomotor output both during and following the resolution of acute experimental muscle pain in healthy volunteers. This effect was strongest for painful muscles tested at rest in the period following the resolution of pain, however a moderate reduction in corticomotor output was also detected during pain. Due to the small number of studies and differences in study design, the duration of corticomotor depression following the resolution of pain remains unclear, however data from individual studies suggests that this effect may persist for up to 20 minutes following pain (Le Pera et al. 2001). Study 2 confirmed this profile of corticomotor depression for the period up to and including 15 minutes post pain (Figure 6.1). As a similar effect and time course were also detected for S1 (S1 excitability was strongly reduced during pain and moderately reduced post pain), it may

be concluded that acute muscle pain affects S1 excitability and corticomotor output similarly.

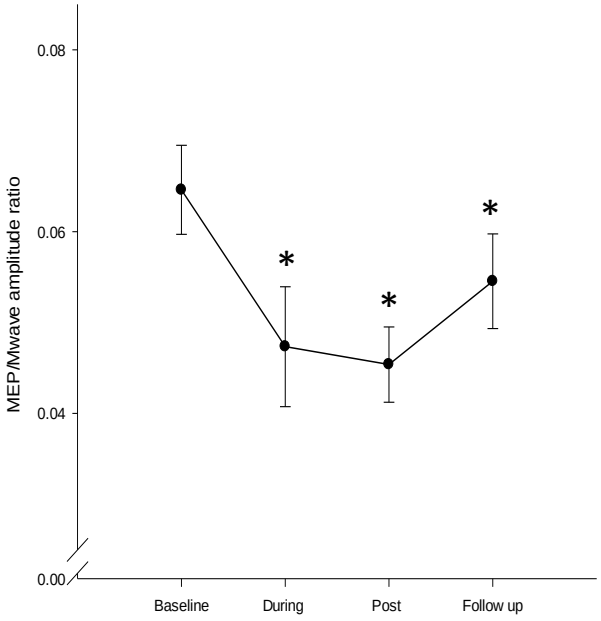


***Figure 6.1*** *<u>Temporal profile of the corticomotor response to acute muscle pain.</u> Data points correspond to before (Baseline), during, immediately post (Post), and 15 minutes following (Follow up) the resolution of pain. Corticomotor output is expressed as MEP/Mwave amplitude ratio. *p < 0.05. Figure appropriated from (Burns et al. 2016c).*

The results of the first two studies demonstrate that reductions in corticomotor output are accompanied by altered intracortical inhibition. As inhibitory mechanisms play an important role in controlling the direction and magnitude of plastic change of excitatory corticospinal projections (Murakami et al. 2012), it is possible that altered intracortical inhibition may underpin the corticomotor response to pain. For example, increased

activity in inhibitory networks (and/or decreased activity in excitatory networks) could produce a reduction in M1 excitability that in turn, would manifest as a reduction in corticomotor output when tested using TMS. Since the systematic review identified evidence of increased SICI (enhanced inhibition) in the immediate post pain period and reduced ICF (reduced excitation) during and post pain, it is possible that these mechanisms underpin reductions in corticomotor output at these time points. However, study 2 provided evidence of the opposite effect (less intracortical inhibition) in the post pain period when measures of afferent inhibition were made (decreased SAI, LAI). Taken together, these data suggest that reductions in corticomotor output could be exclusively due to reduced ICF during pain (since SICI, SAI, LAI, LICI are unchanged), but due to a combination of reduced ICF and increased inhibition (due to the relative dominance of SICI over SAI) in the immediate post pain period. Following this, the corticomotor response, although still depressed, begins to recover, perhaps due to the rising influence of LAI (decreased inhibition) and potential normalisation of SICI/ICF. The temporal profile of these mechanisms in relation to pain and corticomotor output are provided in Figure 6.2. Together, these data provide novel insight into the temporal profile of intracortical inhibitory and facilitatory mechanisms in acute muscle pain.

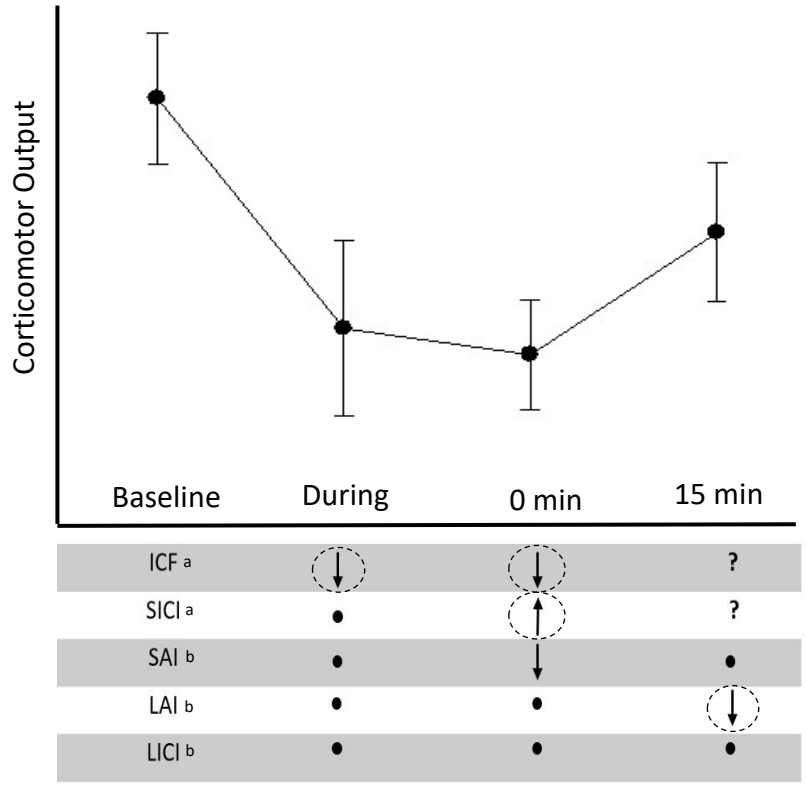


***Figure 6.2*** *Intracortical activity before, during, immediately post (0 min), and 15 minutes following the resolution of acute experimental muscle pain. Dominant mechanisms at each time point are marked by dashed circles. [a] (Schabrun et al. 2012); [b] (Burns et al. 2016b); ICF, intracortical facilitation; SICI, short interval intracortical inhibition; SAI, short latency afferent inhibition; LAI, long latency afferent inhibition; LICI, long interval intracortical inhibition; ?, unknown; •, no change.*

While study 1 and 2 provide detail on the temporal characteristics of intracortical change during acute pain, causation remains uncertain. The systematic review found insufficient evidence to determine whether reductions in S1 and M1 activity are directly related to acute pain, while study 2 demonstrated intracortical change only after the resolution of

pain. Thus, a linear relationship between intracortical measures and pain appears unlikely. While further work is needed to identify the internal and/or external factors triggering the SICI and ICF response to pain, the findings of study 1 provide insight into the origins of reduced SAI/LAI. For example, since the afferent volley that conditions SAI and LAI traverses a number of sensory areas before facilitating the inhibitory response at M1 (Chen et al. 1999, Abbruzzese et al. 2001, Sailer et al. 2002, Sailer et al. 2003), it is possible that reduced SAI/LAI may be a direct consequence of reduced S1 excitability during and post pain. Indeed, changes in sensory processing would likely affect propagation of the afferent signal along sensorimotor pathways underpinning SAI and LAI and culminate in reduced expression of these forms of inhibition at M1. The alternate timing of reductions (SAI: immediately post, LAI: 15 minutes post) may reflect the differential effect of acute muscle pain on sensorimotor integration involving direct (S1-M1: SAI) and indirect (basoganglia-thalamocortical: LAI) pathways.

The M1 response to pain likely reflects a balance between the timely recovery of motor output and protection from the threat of further pain and injury. This hypothesis is supported by current theory which suggests decreased output to muscles at the site of pain may be an adaptation that serves to decrease muscle coordination, prevent symptom aggravation and protect an injured limb (Hodges et al. 2011). The present results suggest that this protective strategy may be sustained in the early post pain period by an increase in SICI. Although the temporal profile of SICI after this time is unknown, it is possible that SICI may normalise as the threat of pain and injury subsides,

allowing mechanisms such as SAI and LAI to become dominant, facilitating a return to normal corticomotor output to the painful body part.

### *6.3 Cortical inhibition in chronic musculoskeletal pain*

In contrast to observations in acute pain, the effect of chronic musculoskeletal pain on M1 excitability and corticomotor output is unclear. For example, motor threshold (a measure of the resting membrane potential of corticospinal neurons and a key determinant of corticomotor output) is increased for painful muscles in low back pain (Strutton et al. 2005), fibromyalgia (Salerno et al. 2000, Mhalla et al. 2010) and the shoulder (Bradnam et al. 2016), but unchanged in osteoarthritis and lateral epicondylalgia (Schwenkreis et al. 2010, Schabrun et al. 2015b). Map volume (a measure of the aggregate excitability of cells projecting to a given muscle) is reduced for painful muscles in low back pain and patellofemoral pain (Tsao et al. 2011b, Te et al. 2017), but increased for painful forearm muscles in lateral epicondylalgia (Schabrun et al. 2015b). While these findings are generally suggestive of reduced M1 excitability in chronic pain (increased motor threshold and decreased map volume), reports of unchanged or increased excitability imply that these effects may be disease-specific.

Contrary to observations of cortical inhibitory pathways in acute musculoskeletal pain, there is evidence of less intracortical inhibition in chronic musculoskeletal pain conditions when compared with pain free individuals. For example, SICI is reduced in low back pain (Masse-Alarie et al. 2012, Masse-Alarie et al. 2016), SICI and LICI are reduced

in persons with diffuse pain due to fibromyalgia (Salerno et al. 2000, Mhalla et al. 2010, Schwenkreis et al. 2011, Caumo et al. 2016) and SAI is reduced in chronic shoulder pain (Bradnam et al. 2016). This thesis expands on these findings by examining inhibitory mechanisms previously unexplored in lateral epicondylalgia (SICI, LICI) and chronic low back pain (post silent period electromyographic bursting).

The results of study 3 and 4 demonstrate a reduction in GABAergic inhibition in persons with low back pain (LBP) and lateral epicondylalgia (LE). This effect was present for forms of inhibition mediated by both $GABA_A$ (SICI) and $GABA_B$ receptor subtypes (LICI, EMG bursting). Reductions in SICI and LICI in LE suggest a shift towards cortical disinhibition (greater excitability) whereas the reduced magnitude of EMG bursting in LBP is indicative of less cortical disinhibition. Taken together, these findings appear contradictory, however since SICI and LICI (at ~150ms latency) are hypothesized to reflect activity primarily at the postsynaptic terminal of M1 interneurons (Chu et al. 2008) and EMG bursting is thought to reflect the action of presynaptic $GABA_B$ receptors (Chin et al. 2012), it is physiologically possible that these observations coexist. Indeed, although there has yet to be an investigation of EMG bursting in LE or any other musculoskeletal condition, previous studies indicate that SICI is also reduced in LBP and fibromyalgia (Mhalla et al. 2010, Schwenkreis et al. 2011, Masse-Alarie et al. 2012, Caumo et al. 2016, Masse-Alarie et al. 2016). Thus, it is possible that simultaneous reductions in these forms of cortical inhibition may be present across a range of musculoskeletal conditions, regardless of the anatomical site of pain.

An additional finding of study 3 was the complete absence of inhibition in some individuals with chronic musculoskeletal pain. Although not explicitly stated within the manuscript text (due to publishing restrictions), a significant number of individuals with LE (7/14) were found to display MEPs greater than 100% of baseline amplitude at one or more inhibitory inter stimulus interval (i.e. a facilitatory response). These findings are consistent with those of a recent investigation in which SICI was found to be absent in six of eight participants with chronic LBP (Masse-Alarie et al. 2016). Like many biological characteristics, responses to paired pulse TMS conform to a normal distribution (Wassermann 2002) and demonstrate high interindividual variation (Boroojerdi et al. 2000). Thus, it is possible that responses falling outside of the expected range of values merely sit on the tail of this distribution. Indeed, normative values for SICI in the first dorsal interosseous muscle have been shown to range from 0% (total inhibition of the test MEP) to 330% (test MEPs 3.3 times larger than baseline) for individuals aged 50 years or younger (median SICI ~ 20%) (Cueva et al. 2016). Interindividual variability is also common for other forms of inhibition, as demonstrated by the small number of subjects that did not display SAI, LAI or LICI at baseline in study 2 (Burns et al. 2016c). While correlational studies cite physiological differences between individuals such as skull thickness or intrinsic neuronal properties as the main source of variation within the normal population (Wassermann 2002), it is unclear why such a significant proportion of individuals with chronic musculoskeletal pain fail to respond to paired pulse inhibitory protocols. Although a pathological origin is possible, current literature has yet to identify evidence of a relationship between measures of cortical inhibition and pain severity,

level of disability or duration of musculoskeletal pain (Schwenkreis et al. 2011, Kittelson et al. 2014, Burns et al. 2016a, Masse-Alarie et al. 2016). A relationship has, however, been found between less inhibition and increased levels of catastrophizing and depression in persons with fibromyalgia and myofascial pain syndrome (Mhalla et al. 2010, Volz et al. 2013). As individuals with chronic pain often present with comorbid mood/anxiety disorders (Askari et al. 2017), it is possible that behavioural and psychological traits could also underpin the tendency towards absent inhibition in LE and LBP.

Taken together, the results of study 3 and 4 provide insight into mechanisms that may contribute to the development and maintenance of chronic musculoskeletal pain. Inhibitory mechanisms play an important role in the maintenance and adjustment of M1 motor representations (Liepert et al. 1998a). Thus, although neither study provided direct evidence linking neurophysiological outcomes with pain, disability or sensorimotor impairments in LE or LBP, it is plausible that these mechanisms underpin previous observations of altered map volume, M1 reorganisation and associated motor dysfunction in these conditions (Tsao et al. 2008, Tsao et al. 2011b, Schabrun et al. 2014, Schabrun et al. 2015a, Schabrun et al. 2015b). Our observation of reduced $GABA_B$ mediated inhibition in chronic (Burns et al. 2016a, 2017), but not acute musculoskeletal pain (Burns et al. 2016c), also suggests that these forms of inhibition may be involved in the transition to chronicity. While it remains unclear why LICI is unaffected by acute pain, one explanation could be that changes in this mechanism depend upon symptom

duration (Parker et al. 2017). In the event of delayed healing and/or ongoing nociceptive input, it is possible that $GABA_B$ mediated inhibition reduces, which, due to competitive interactions with other inhibitory circuits (LICI inhibits SICI, SICI inhibits SAI), ultimately influences a shift towards decreased cortical inhibition. This reversal of inhibitory activity in chronic musculoskeletal pain may facilitate M1 remodeling and support motor adaptations designed to unload painful joints and muscles, redistribute muscle activity and minimize discomfort (Hodges et al. 2011).

### *6.4 Clinical Implications*

This thesis provides a comprehensive examination of cortical inhibition in acute and chronic musculoskeletal pain that can assist our understanding of this condition and inform the development of future treatments. Rehabilitation of movement is a mainstay of interventions for musculoskeletal pain but there is considerable debate over the type, timing and quantity of movement needed to improve symptoms and effect sizes of most movement based treatments are at best, modest (Menke 2014). One explanation for these small effects is that current treatments are not optimized to target the mechanisms of pain. This thesis provides evidence of increased intracortical inhibition in acute pain and decreased intracortical inhibition in chronic pain. While both effects likely afford protection to the injured body part (either by limiting or modifying movement), this distinction highlights the importance of tailoring treatment to the current stage of pain and underlying mechanisms.

Over the course of the past decade, there has been a dramatic increase in research regarding the use of peripheral and central neuromodulatory interventions as standalone and/or adjunct therapies for the management of persistent pain conditions (Nitsche et al. 2011, O'Connell et al. 2011). Interventions such as repetitive transcranial magnetic stimulation (rTMS), transcranial direct current stimulation (tDCS) and peripheral nerve stimulation (PNS), have the capacity to induce shifts in cortical excitability and therefore the potential to benefit persons in both the acute and chronic stages of pain. For example, 1mA cathodal-tDCS applied over M1 (presumed to shift neuronal membrane potentials towards hyperpolarization and decreased cortical excitability) has been demonstrated to increase SICI (Batsikadze et al. 2013) while anodal-tDCS (shift towards depolarization, increased cortical excitability) decreases SICI (Antal et al. 2010). Thus, it is possible that such interventions could be efficacious in restoring defective inhibitory processes in chronic and acute pain, respectively. Multimodal interventions may also have a place in future treatment programs since preliminary evidence in low back pain indicates that PNS in conjunction with motor training restores SICI and reduces pain (Masse-Alarie et al. 2013), while concurrent application of PNS and tDCS reduces pain, improves sensorimotor function and normalises motor cortical organisation (Schabrun et al. 2014). However, the translation of neuromodulatory interventions into clinical practice is currently hampered by the tendency to adopt a 'one-size fits all' strategy that fails to consider the presiding state of cortical excitability and high inter-individual variability. The successful transition of these therapies from experimental to clinical use depends on identification of specific cortical

mechanisms present in different musculoskeletal pain conditions and an understanding of the variability present between individuals. Thus, further research building on the work presented in this thesis is essential to drive the development and application of efficacious mechanism based therapies in the future.

### *6.5 Limitations*

Limitations are acknowledged and discussed within each individual study and thus, the limitations presented here are those relevant to the framework of this thesis as a whole.

One criticism that may be leveled against this thesis is that it is comprised of standalone research projects examining different mechanisms, muscle groups and populations. At first glance, this composition may appear haphazard, especially compared to theses constructed from a series of interdependent experiments. However, each study was subject to careful consideration and planning in order to ensure that this work, as whole, would provide a broad contribution to our understanding of cortical inhibition in acute and chronic musculoskeletal pain. For example, the muscles studied in each project were purposely selected based on their proximity to pain rather than anatomical position in order to better enable comparisons with previous and future studies. Ordinarily, comparisons between TMS studies with different target muscles is difficult due to the distal-proximal and upper-lower limb attenuation of MEP amplitudes (Groppa et al. 2012), however, this is less of an issue for measures of cortical inhibition which are expressed as relative (%), rather than absolute (mV) values. Furthermore, the magnitude

of SAI, SICI and ICF have all been found to be similar for muscles of the hand and forearm (Bikmullina et al. 2009). Thus, it is likely that the findings of study 2 and 3, for example, may be confidently compared with previous and future research in the upper limb. However, as cortical inhibition was not investigated for muscles other than those immediately affected by pain, it is unknown whether the results of the present studies are muscle specific. Future work is needed to clarify the extent of these observations and to determine whether, as suggested by the systematic review, a non-specific, but localised effect also occurs for non-painful muscles within the immediate vicinity of pain (Burns et al. 2016b).

It is also unclear whether the findings presented in this thesis are typical of the populations investigated (pain-free, LE, LBP) as data were collected from relatively small samples of convenience. Although small sample sizes are not uncommon in this field (Burns et al. 2016b) or for the neurosciences in general (Button et al. 2013), this is a noteworthy limitation as studies with small sample sizes often demonstrate low statistical power. Median statistical power in the neurosciences is estimated to sit around 21% (Button et al. 2013). This means that for every 100 genuine significant observations, these studies would, at best, be capable of identifying 21 significant findings (Sterne et al. 2001). Thus, it is possible that the small sample sizes in this thesis precluded the detection of significant findings between time points (study 2) and patient/control groups (study 3 and 4). However, since sample size calculations were performed prior to conducting each research project, the likelihood of these studies

being underpowered is low. Yet the possibility remains, since the studies used as reference for these statistics also involved small samples and, thus, were themselves potentially underpowered (Masse-Alarie et al. 2012, Sato et al. 2013, Schabrun et al. 2015b). This is important to note as it is also possible for low powered studies to produce inflated estimates of effects as the relative impact of sampling variation and random error is much greater in studies with small samples (Ioannidis 2008). Therefore, it is imperative that the findings described in Chapters 3 to 5, as well as those included in the systematic review (Chapter 2), are examined in replication studies with larger sample sizes and adequate statistical power (i.e. ≥ 80%) (Cohen 1992).

Larger sample sizes would also improve the rigor of the main experimental studies by allowing for increased control of extraneous variables. For example, participant age and sex were accounted for in the experimental design of studies 3 and 4, however, due to the heterogeneity of clinical presentations and small sample size it was difficult, if not impossible, to control for other patient characteristics. In the future, studies should be large enough to confidently employ statistical methods of control, such as covariate analysis, to determine whether variables such as symptom duration, pain severity or level of disability have significant bearing on study outcomes.

Another limitation of this work and the field in general is the use of experimental pain models to study acute pain, rather than clinical populations. There are several advantages to this approach, including the ability to carefully regulate the dose, intensity and duration of pain as well as the ability to perform standardized assessments. It is also

considerably easier to recruit healthy participants for these studies compared to acute patient populations, due to the typically short duration of clinical pain. However, the appropriateness of extrapolating findings from 'artificial' pain studies to clinical populations is somewhat questionable since responses to acute experimental pain can be variable (Graven-Nielsen et al. 1997b) and often contrary to what is observed in clinical conditions (Schabrun et al. 2012). Thus, although experimentally induced pain remains well practiced, and is a valuable method for studying the healthy nociceptive system, it is important that this methodology continues to be used as a precursor for studying clinical pain, rather than a substitute for population based studies.

There are a few other limitations relevant to the studies conducted within this thesis. For example, it is important to recognise that data collected within a single test session cannot be used to establish causation in patient studies. Hence, while this thesis identifies a number of mechanisms that could plausibly contribute to the transition from acute to chronic pain, longitudinal trials are required to confirm these hypotheses. It is also difficult to comment on the functional significance of altered intracortical activity in musculoskeletal pain states based on this thesis alone since there was no discernable relationship between neurophysiological outcomes and sensorimotor function in study 3 and a relative lack of functional measures in studies 2 and 4. Thus, replication studies examining intracortical activity in LE and LBP should consider including additional functional measures such as upper limb reaction times/speed of movement tests or postural tasks to determine whether intracortical changes could be linked to impaired

motor control in these conditions (Bisset et al. 2006, Masse-Alarie et al. 2012). Offline analysis of concurrent EMG recordings may also be useful in determining whether intracortical mechanisms directly contribute to transient changes in muscle behaviour during and following the resolution of acute experimental pain (Del Santo et al. 2007, Martin et al. 2008).

A final limitation to consider is that the investigator was not blinded to the time point of testing in study 2 or to the group in study 3 and 4. To improve internal validity and reduce the risk of bias, future studies should consider employing blinding practices during data analysis.

### *6.6 Directions for future research*

As mentioned in the preceding section, longitudinal trials are required to clarify the direction and extent of cortical change in acute and chronic musculoskeletal pain conditions. Longitudinal studies involving larger cohorts of healthy participants as well as individuals in acute (< 6 weeks) and chronic (> 3 months) stages of disease will improve our understanding of the intracortical response to musculoskeletal pain, the relationship between mechanisms and symptoms, and has the potential to drive the development of neuromodulatory therapies in the future. Longer follow up times in experimental studies would also improve our understanding of the temporal dispersion of cortical effects in the acute phase of pain. For example, the work described in Chapter 3 (study 2) could be improved in the future by extending post pain recording intervals by 30 minutes or until

baseline cortical function is restored. Future studies would also benefit from addressing the limitations and recommendations outlined in the systematic review (Burns et al. 2016b). For example, implementation of standardized recording intervals and objective measures of sensory/motor function would enable comparisons between studies and inform the functional significance of findings in the acute and chronic stages of pain. Future studies of cortical inhibition should also note the methodology adopted in this thesis. A novel finding of study 3 was that significant differences between health and disease were only detected under certain test conditions (Burns et al. 2016a). Thus, future studies examining forms of inhibition present over a range of latencies and/or stimulation intensities (i.e. SICI, LICI, SAI, LAI) should include a variety of test conditions to provide a comprehensive and complete description of the effect of pain on these mechanisms. Inclusion of a base measure of cortical excitability such as motor threshold or MEP amplitude would also be ideal to account for the high interindividual variability associated with cortical measures. Optimization of stimulation parameters to each individual participant is also likely to be particularly important if neurophysiological measures are used to prescribe courses of neuromodulatory treatment in the future.

### *6.7 Conclusion*

This thesis provides evidence of altered cortical inhibition in acute and chronic musculoskeletal pain. Immediate, transient changes in inhibition in the acute stage of pain likely reflect the initiation of a protective motor strategy designed to restrict movement, whereas lasting changes in chronic conditions probably support the

development and maintenance of motor adaptations that facilitate task performance yet minimize pain. Further work is needed to confirm these hypotheses and the effect of pain on the intracortical mechanisms investigated in this thesis. Confirmation of these findings in larger trials and other musculoskeletal conditions may lead to the development of novel mechanism based therapies for those living with musculoskeletal pain and, ideally, interventions that prevent the transition from acute to chronic musculoskeletal pain states.

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# Appendix A

EJP
European Journal of Pain

REVIEW ARTICLE

## Primary sensory and motor cortex function in response to acute muscle pain: A systematic review and meta-analysis

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## Abstract

Acute muscle pain has both motor and sensory consequences, yet the effect of muscle pain on the primary sensory (S1) and motor (M1) cortices has yet to be systematically evaluated. Here we aimed to determine the strength of the evidence for (1) altered activation of S1/M1 during and after pain, (2) the temporal profile of any change in activation and (3) the relationship between S1/M1 activity and the symptoms of pain. In September 2015, five electronic databases were systematically searched for neuroimaging and electrophysiological studies investigating the effect of acute experimental muscle pain on S1/M1 in healthy volunteers. Demographic data, methodological characteristics and primary outcomes for each study were extracted for critical appraisal. Meta-analyses were performed where appropriate. Twenty-five studies satisfied the inclusion criteria. There was consistent evidence from fMRI for increased S1 activation in the contralateral hemisphere during pain, but insufficient evidence to determine the effect at M1. Meta-analyses of TMS and EEG data revealed moderate to strong evidence of reduced S1 and corticomotor excitability during and following the resolution of muscle pain. A comprehensive understanding of the temporal profile of altered activity in S1/M1, and the relationship to symptoms of pain, is hampered by differences in methodological design, pain modality and pain severity between studies. Overall, the findings of this review indicate reduced S1 and corticomotor activity during and after resolution of acute muscle pain, mechanisms that could plausibly underpin altered sensorimotor function in pain.

**What does this review add?:** We provide the first systematic evaluation of the primary sensory (S1) and motor (M1) cortex response to acute experimental muscle pain in healthy volunteers. We present evidence from a range of methodologies to provide a comprehensive understanding of the effect of pain on S1/M1. Through meta-analyses we evaluate the strength of evidence concerning the direction and temporal profile of the S1/M1 response to acute muscle pain.

## 1. Introduction

It is well accepted that acute muscle pain alters sensory and motor function. Yet, the mechanisms that underpin these changes are poorly understood. Current theories on sensorimotor adaptation in pain hypothesize that the primary sensory (S1) and motor (M1) cortex contribute to altered sensorimotor function. For instance, reduced S1 activity is

hypothesized to underpin reduced kinaesthesia and position sense (Rossi et al., 2003), whereas reduced M1 activity is hypothesized to underpin restriction of motor output and afford protection from further pain and injury (Hodges and Tucker, 2011).

Numerous studies using a range of methodological tools, including positron emission tomography (PET), functional magnetic resonance imaging (fMRI), transcranial magnetic stimulation (TMS) and electroencephalography (EEG), have investigated how and when S1 and M1 activity are altered in response to acute muscle pain (Svensson et al., 1997; Niddam et al., 2002; Schabrun et al., 2013). These studies use similar *in vivo* experimental pain models to induce short-lasting muscle pain that is of a clinical quality (deep, constant, dull or a sharp ache) that allow collection of pre-pain baseline data that cannot be obtained in clinical pain populations (Graven-Nielsen et al., 1997). However, despite similarities in pain models and brain regions under investigation, there has been no systemic evaluation of S1 or M1 data in acute muscle pain. Integration of data obtained from studies using different methodologies is essential to drive a comprehensive understanding of the nature and time-course of altered S1 and M1 activity in response to acute muscle pain and to elucidate the relationship between S1/M1 and the symptoms of pain.

Here, we synthesized and critically evaluated data corresponding to activity in S1 and M1 cortical regions in order to: (1) examine S1/M1 activation in response to acute muscle pain, (2) quantify the direction and temporal profile of change and (3) determine the evidence for a relationship between altered S1/ M1 activity and symptoms of pain.

## 2. Literature search methods

### 2.1 Search strategy

Relevant studies were identified from five electronic databases (Scopus, Medline, Embase, Pubmed and Web of Science) using MeSH terms and free-text terms including *motor cortex, somatosensory cortex, muscle pain, acute pain and experimental pain*. The most recent search was performed in September 2015. Studies were first screened for relevance by title and abstract before analysis of full text. Inclusion was dependent upon the following criteria: (1) English language, (2) original, primary research, (3) healthy adult human subjects, (4) acute experimental pain was induced in a muscle, (5) acute muscle pain was induced in the absence of another stimulation or intervention and (6) outcome measures included full brain image analysis of regional cerebral blood flow (rCBF; measured using PET or fMRI) or blood-oxygen-level-dependent contrast imaging (BOLD; measured using fMRI), corticomotor excitability (motor evoked potentials; measured using TMS) and/or sensory cortex excitability (somatosensory evoked potentials; measured using EEG). To ensure the reliability of the process for inclusion/exclusion of studies, 10 abstracts were selected at random and independently reviewed by three assessors.

### 2.2 Data extraction and assessment of methodological quality

A standard form was used to extract subject demographics, methodological parameters (techniques, outcome measures) and pain characteristics (modality, location, intensity) for each study. Additional technical information specific to EEG (electrode orientation, conditioning location and intensity) and TMS (target muscle, muscles state, TMS intensity, coil type and position) methodologies was also recorded. Primary outcome measures included MEP amplitude/area to single and/or paired-pulse TMS, the amplitude/area of SEP components/complexes corresponding to S1 activation/processing and the direction of change (increase or decrease) in rCBF or BOLD contrast.

Methodological quality was appraised using a modified version of the Downs and Black's checklist (Downs and Black, 1998; Supporting Information Appendix S1). A maximum score of 17 points were awarded based on reporting within the text and external and internal validity. Studies involving TMS were further appraised using the TMS methodological checklist (Chipchase et al., 2012). The maximum score for reported and controlled items was 26 or 30 points, depending on methodology (single or paired-pulse TMS). The summed score for reported items ($r$) as a percentage of the maximum score [$r/(26 \text{ or } 30) \times 100$] to provide an indication of adherence to the checklist. The summed score for controlled items ($c$) was expressed as a percentage of reported items [$(c/r) \times 100$] to determine the extent to which reported items were controlled.

### 2.3 Meta-analyses

Meta-analyses were performed on MEP and SEP data but not for rCBF or BOLD contrast due to the heterogeneity of the analysis approaches used

between studies and inconsistent reporting of non-significant findings. Mean ± standard deviation for MEP and SEP amplitude/area were extracted at time points 'baseline', 'during pain' and 'post pain', where available. When data were not reported within the text, an email was sent to the corresponding author to request missing values. If authors were no longer contactable, did not respond, or declined requests for data, means ± standard deviation/standard error were estimated from illustrations or calculated from available $t$ values, $p$ values or $F$ statistics. Standardized mean differences (SMD) and 95% confidence intervals were calculated using a random-effects model in RevMan 5.2 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen) and heterogeneity determined using calculations of $I^2$. Effect estimates ≤0.2 were considered small, 0.5 moderate and ≥0.8 large.

## 3. Results

### 3.1 Search results

The search strategy retrieved 257 studies, minus duplicates (Fig. 1). Screening of title and abstract and evaluation of full text identified 20 suitable studies. Examination of reference lists revealed an additional five studies, thus, a total of 25 studies were included in the systematic review.

### 3.2 Study characteristics

Of the 25 included studies, four examined rCBF with PET (participants, $n = 56$), two examined rCBF with fMRI ($n = 32$), eight examined BOLD responses with fMRI ($n = 147$), eight examined corticomotor excitability (MEPs) with TMS ($n = 80$) and three examined sensory cortex excitability (SEPs) with

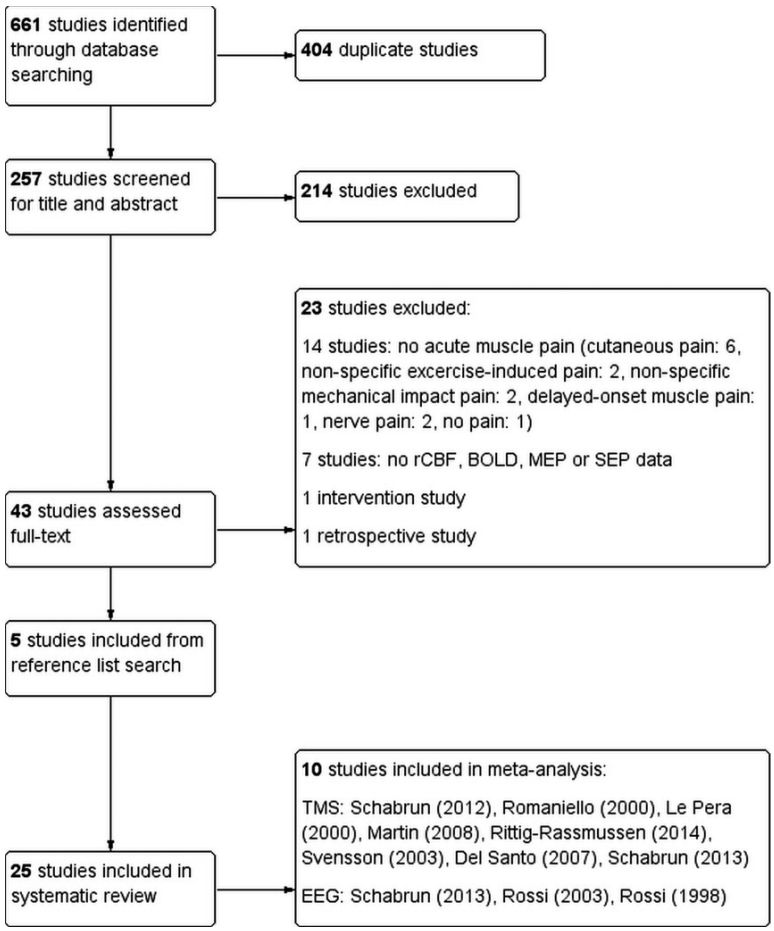


**Figure 1** Search strategy flow diagram.

EEG ($n = 25$). One study reported on both corticomotor and sensory cortex excitability (Schabrun et al., 2013). Hypertonic saline was the most common method of inducing experimental muscle pain ($n = 17$) and most studies ($n = 13$) induced pain into muscles of the upper extremity (hand, forearm or arm). Other sites included muscles of the leg (studies, $n = 7$), jaw ($n = 4$), neck ($n = 1$) and low back ($n = 1$). Demographic information and pain characteristics of included studies are presented in Table 1.

### 3.3 Methodological quality

Average scores for methodological quality were $12.1 \pm 1.5$ (out of 17) for MEP studies, $11.3 \pm 2.1$ for SEP studies, $13.8 \pm 0.5$ for rCBF studies and $12.9 \pm 0.9$ for BOLD studies. Items consistently unmet by reviewed studies related to internal and external validity. For example, male and female participants were unequally represented in the majority of study samples (Macefield et al., 2007) and sample size calculations were rarely performed a priori (Rittig-Rasmussen et al., 2014). No study blinded the investigator during data analysis and all recruited a sample of convenience.

The mean score for reported items of the TMS checklist was $78 \pm 8\%$. Compliance was high for methodological items, however, items relating to participant characteristics, were less well reported. This may be because authors did not consider it necessary to collect or report detailed data on health characteristics for participants considered 'healthy'. All studies reported on subject age and gender, position and contact of EMG electrodes, amount of relaxation/contraction of muscles, prior motor activity of the tested muscle, coil location and stability, type of stimulator, stimulation intensity, subject attention, number of MEP recordings and the method for determining MEP size in analysis. The mean score for controlled items was $88 \pm 7\%$. Items reported but poorly controlled included subject gender (bias towards male participants), prior activity of the muscle being tested, level of relaxation of muscles not being directly tested, and coil stability. Individual study scores are presented in Table 1.

### 3.4 The effect of experimental muscle pain at the primary sensory cortex (S1)

#### 3.4.1 Regional cerebral blood flow (rCBF)

Four studies investigated rCBF using PET and two studies investigated rCBF using fMRI and arterial spin labelling (ASL; Supporting Information Table S1). Full brain image analysis using PET (studies, $n = 4$) revealed no effect of muscle pain on rCBF at S1. There was also no effect on ASL-derived rCBF ($n = 2$) when pain was of moderate intensity (Owen et al., 2012), however a bilateral reduction at S1 was observed in response to severe pain (Owen et al., 2010). These conflicting findings may be explained by pain severity as reduced rCBF at S1 was found to negatively correlate with subjective pain ratings for the pain (hypertonic saline) and control groups (isotonic saline) in the latter study (contralateral S1: $R^2 = 0.75$, $p = 0.000$; ipsilateral S1: $R^2 = 0.69$, $p = 0.000$; Owen et al., 2010).

#### 3.4.2 Blood-oxygen-level-dependent (BOLD) contrast imaging

Seven of the nine studies that investigated BOLD responses using fMRI reported increased S1 activation in response to muscle pain (Supporting Information Table S1). Three studies reported increased BOLD contrast bilaterally at S1 (Niddam et al., 2002; Nash et al., 2010a,b), while three studies reported increases exclusive to the hemisphere contralateral to the side of hypertonic saline- or electrically induced muscle pain (Henderson et al., 2006; Macefield et al., 2007; Takahashi et al., 2011). In contrast, results for mechanically induced pain were conflicting. For example, Loggia et al. (2012) reported an increased BOLD response at S1 in the contralateral hemisphere and a decreased response in the ipsilateral hemisphere following painful pressure stimulation, whereas Uematsu et al. (2011) and Maeda et al. (2011) reported no change for either hemisphere. These discrepancies could be explained by differences in the spatial resolution of the pressure stimulation between these studies (pressure cuff vs. pressure probe).

#### 3.4.3 Somatosensory evoked potentials (SEPs)

Three studies used EEG to investigate the effect of acute muscle pain on S1 excitability following electrical stimulation of the ulnar (Rossi et al., 2003; Schabrun et al., 2013) or peroneal nerve (Rossi et al., 1998; Supporting Information Table S2). Ulnar SEPs were recorded using surface electrodes placed on the scalp 2 cm posterior to C3 using the 10–20 International EEG system, while peroneal SEPs were recorded subcutaneously at a location 2 cm posterior to Cz on the midline. All studies reported on components thought to reflect S1 activation ($P_{14}$–$N_{20}$, $P_{40}$–$N_{50}$) and pro-

**Table 1** Characteristics of included studies ($n$ = 25). Pain is described as mild, moderate, severe based on reported pain score (mild: <3.0, moderate: 3.0–5.4, severe: >5.4).

| | Demographics | | | Methodology | | Pain characteristics | | | Methodological quality | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Study (year) | *n* | Male, female | Age (years, mean ± SD) | Technique | Outcome measures | Model | Location | Intensity | Downs and Black Checklist (/17) | TMS Checklist Reported (%) | TMS Checklist Controlled (%) |
| Kupers (2004) | 10 | 6, 4 | 21–25 (range) | PET | rCBF, $^{15}$O-labelled water | Hypertonic saline | Masseter | Severe (p) | 14 | – | – |
| Svensson (1997) | 11 | 11, 0 | 30.4 ± 3.5 | PET | rCBF, $^{15}$O labelled water | Electrical stimulation | Brachioradialis | Mild (p) | 14 | – | – |
| Thunberg (2005) | 19 | 19, 0 | 26.7 ± 7.6 | PET | rCBF, $^{15}$O labelled water | Hypertonic saline | Erector spinae at L3 | Moderate (p) | 14 | – | – |
| Korotkov (2002) | 16 | 16, 0 | 24.3 ± 7.7 | PET | rCBF, $^{15}$O labelled water | Hypertonic saline | Triceps brachii | Moderate (a) | 13 | – | – |
| Owen (2010) | 13 | 13, 0 | 29.5 ± 5 | fMRI | CBF via ASL at 3 T | Hypertonic saline | Brachioradialis | Severe (a) | 14 | – | – |
| Owen (2012) | 19 | 19, 0 | 26 ± 5 | fMRI | CBF via ASL at 3 T | Hypertonic saline | Brachioradialis | Moderate (a) | 13 | – | – |
| Nash (2010a) | 25 | NR | NR | fMRI | BOLD at 3 T | Hypertonic saline | Masseter | Moderate (p) | 12 | – | – |
| Uematsu (2011) | 17 | 10, 7 | 23–33 (range) | fMRI | BOLD at 1.5 T | Pressure (skin anaesthetized), 10 mm probe | Gastrocnemius | Moderate (a) | 12 | – | – |
| Henderson (2006) | 15 | NR | NR | fMRI | BOLD at 3 T | Hypertonic saline | Tibialis anterior | Moderate (p) | 11 | – | – |
| Loggia (2012) | 16 | 11, 5 | 28.8 ± 9.7 | fMRI | BOLD at 3 T | Pressure, 13.5 cm cuff | Gastrocnemius | 7 increments: mild (p) - severe (p) | 14 | – | – |
| Macefield (2007) | 22 | 11, 11 | 22–49 (range) | fMRI | BOLD at 3 T | Hypertonic saline | Tibialis anterior, flexor carpi radialis | Severe (p) | 13 | – | – |
| Nash (2010b) | 17 | NR | NR | fMRI | BOLD at 3 T | Hypertonic saline | Masseter | Moderate (p) | 13 | – | – |
| Maeda (2011) | 12 | 7, 5 | 24–56 (range) | fMRI | BOLD at 1.5 T | Pressure, 10 mm probe | Gastrocnemius | Moderate (a), severe (a) | 14 | – | – |
| Niddam (2002) | 10 | 10, 0 | 26.8 ± NR | Event-related fMRI | BOLD at 3 T | Electrical stimulation | Abductor pollicis brevis | NR | 13 | – | – |
| Takahashi (2011) | 13 | 13, 0 | 20–36 (range) | Event-related fMRI | BOLD at 3 T | Electrical stimulation | Tibialis anterior | Mild (p), moderate (p), severe (p) | 13 | – | – |
| Schabrun (2012) | 11 | 7, 4 | 23.3 ± 6.5 | TMS | MEP amp, SICI, ICF | Hypertonic saline | First dorsal interosseous | Severe (a) | 13 | 87 | 91 |
| Svensson (2003) | 10 | 8, 2 | 34.3 ± 4.0 | TMS | MEP amp | Hypertonic saline | First dorsal interosseous | Moderate (p) | 13 | 81 | 81 |
| Del Santo (2007) | 8 | 5, 3 | 33.9 ± 11.5 | TMS | MEP area | Ascorbic acid | Abductor digiti minimi, biceps brachii | Severe (p) | 13 | 66 | 95 |
| Martin (2008) | 6 | NR | NR | TMS | MEP amp | Hypertonic saline | Biceps brachii | Moderate (p) | 11 | 66 | 83 |

**Table 1** (*Continued*)

| | Demographics | | | Methodology | | Pain characteristics | | | Methodological quality | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Study (year) | *n* | Male, female | Age (years, mean ± SD) | Technique | Outcome measures | Model | Location | Intensity | Downs and Black Checklist (/17) | TMS Checklist Reported (%) | TMS Checklist Controlled (%) |
| Rittig-Rasmussen (2014) | 12 | NR | 23 ± 2 | TMS | MEP amp | Hypertonic saline | Neck muscular tissue 2 cm lateral to C3 | Moderate (a) | 13 | 74 | 90 |
| Romaniello (2000) | 10 | NR | NR | TMS | MEP amp | Hypertonic saline | Masseter | Moderate (a) | 9 | 81 | 96 |
| Le Pera (2001) | 10; 11 | 7, 3; 8, 3 | 23.5 ± 2.7; 26.1 ± 4.8 | TMS | MEP amp | Hypertonic saline | Abductor digiti minimi, first dorsal interosseous; flexor carpi radialis | Severe (p) & moderate (p); moderate (p) | 12 | 85 | 91 |
| Schabrun (2013) | 12 | 5, 7 | 28 ± 9 | TMS, EEG | MEP amp, SEP area | Hypertonic saline | First dorsal interosseous | Moderate (a) | 13 | 81 | 77 |
| Rossi (2003) | 6 | NR | NR | EEG | SEP area | Levo-ascorbic acid | First dorsal interosseous | Severe (p) | 12 | – | – |
| Rossi (1998) | 7 | 5, 2 | 22–40 (range) | EEG | SEP area | Levo-ascorbic acid | Extensor digitor brevis | Severe (p) | 9 | – | – |

TMS, transcranial magnetic stimulation; EEG, electroencephalography; PET, positron emission tomography; fMRI, functional magnetic resonance imaging; rCBF, regional cerebral blood flow; ASL, arterial spin labelling; BOLD, blood-oxygen-dependent level contrast imaging; MEP, motor evoked potential; SEP, somatosensory evoked potential; (a), average pain score; (p), peak pain score; NR, not reported.

cessing ($N_{20}$–$P_{25}$–$N_{33}$, $P_{60}$–$N_{75}$). As pooled analyses revealed that the area of components corresponding to S1 activation was unchanged both during (Supporting Information Fig. S1) and following (Supporting Information Fig. S2) the resolution of muscle pain [−0.36 (−0.86, 0.14), −0.23 (−0.72, 0.26)], this suggests that input to S1 remains stable over time. In contrast, pooled effect estimates during pain show a strong reduction in the area of components corresponding to S1 processing [1.99 (0.64, 3.34)]. A moderate reduction [0.65 (0.15, 1.16)] is also present post pain. Such findings suggest that acute muscle pain may exert a lasing inhibitory effect on S1 excitability.

### 3.5 The effect of experimental muscle pain on the primary motor cortex (M1) and corticomotor pathway

#### 3.5.1 Regional cerebral blood flow (rCBF)

Full brain image analysis (PET $n = 4$, fMRI $n = 2$) revealed no change in rCBF at M1 in any study (Supporting Information Table S1).

#### 3.5.2 Blood-oxygen-level-dependent (BOLD) contrast imaging

At the location of M1, findings for BOLD contrast were mixed (Supporting Information Table S1). For example, four studies reported increased BOLD responses in the hemisphere contralateral to the side of pain (Henderson et al., 2006; Nash et al., 2010b; Takahashi et al., 2011; Loggia et al., 2012), while five studies observed no change (Niddam et al., 2002; Macefield et al., 2007; Nash et al., 2010a; Maeda et al., 2011; Uematsu et al., 2011). These discrepancies do not appear to be related to the use of different pain models between studies but may be related to pain severity since a positive relationship between increased BOLD and pain was observed in one study (Loggia et al., 2012). However, as the results from two additional studies indicate that increased BOLD outlasts peak pain and persists during waning pain (Henderson et al., 2006; Takahashi et al., 2011), the nature of this relationship remains uncertain. Interpretation of these findings is further complicated by observations from Nash et al., (2010b), who reported that initial increases in BOLD contrast were followed by lasting decreases in signal intensity. With the exception of one study (Henderson et al., 2006), there is no evidence of an effect of muscle pain on ipsilateral BOLD responses.

#### 3.5.3 Transcranial magnetic stimulation: assessment of corticomotor excitability

Eight studies investigated corticomotor excitability using TMS (Supporting Information Table S3). Separate meta-analyses were performed for data collected with the muscle at rest and during active contraction and sub group analysis was performed for 'target' or 'non-target' muscles. 'Target' refers to the muscle where experimental pain was induced, and was reported in all eight studies. The term 'non-target' refers to muscles remote, synergistic or antagonistic to the 'target muscle' and was reported in three studies (Le Pera et al., 2001; Svensson et al., 2003; Martin et al., 2008).

The size of the MEP to single-pulse TMS was reported by five studies 'during pain' (Supporting Information Fig. S3). Pooled effect estimates revealed a moderate reduction in corticomotor excitability (MEP amplitude/area) from 'baseline' for target and non-target muscles at rest [0.52 (−0.01, 1.06), 0.72 (0.01, 1.42)], but not for target muscles during active contraction [−0.13 (−0.61, 0.35)]. 'Post-pain' data collected either '0' (immediately post), '20' or '30' minutes following the cessation of pain or painful stimulation was available from all eight studies (Supporting Information Fig. S4). Compared to 'baseline' there was a strong reduction in MEP amplitude/area for target muscles at rest [0.97 (0.59, 1.35)] and a similar trend for non-target muscles at rest [0.37 (−0.02, 0.76)]. A moderate reduction in MEP amplitude/area was also detected for actively contracting target muscles [0.44 (0.05, 0.83)].

#### 3.5.4 Transcranial magnetic stimulation: intracortical inhibitory and facilitatory networks

The MEP response to paired-pulse TMS was examined by one study (Schabrun and Hodges, 2012). Short-interval intra-cortical inhibition (at 2 and 3 ms inter-stimulus intervals) was increased immediately following (2 ms: $p = 0.012$, 3 ms: $p = 0.007$), but not during muscle pain (2 ms: $p = 0.24$; 3 ms: $p = 0.61$). In contrast, intra-cortical facilitation (13 ms inter-stimulus interval) was reduced compared to baseline both during ($p = 0.009$) and immediately post ($p = 0.001$) muscle pain.

## 4. Discussion

The aim of this review was to synthesize and critically evaluate evidence for altered S1 and M1 activity in response to acute muscle pain. The findings

provide evidence of reduced activity in the contralateral S1 both during and following pain and moderate-strong evidence of reduced corticomotor output to the painful muscle. Currently, there is insufficient evidence to draw conclusions regarding effects at ipsilateral S1/M1.

### 4.1 The effect of acute muscle pain on S1

Eight of nine studies using fMRI provide evidence of altered activation (increased BOLD contrast) at S1 in the hemisphere contralateral to the side of induced pain, during pain. As BOLD contrast is a measure of cerebral metabolism based on the level deoxyhaemoglobin content, and oxygen demands are assumed to be higher at areas of increased cortical function (Howseman and Bowtell, 1999), such observations imply increased synaptic activation. However, as fMRI detects hemodynamic and not electrophysiological changes, it is unclear whether increased synaptic activation reflects increased or decreased S1 excitability. The inclusion of studies that record SEPs in response to pain provides insight into the direction of the effects observed with fMRI. The area of the $N_{20}$–$P_{25}$–$N_{33}$ and $P_{60}$–$N_{75}$ potential is thought to reflect early cortical processing of low-threshold somatosensory afferent information related to kinaesthesia and position sense (Rossi et al., 1998, 2003) and is commonly used to infer changes in S1 excitability. Pooled analysis of the three EEG studies provides strong evidence that acute muscle pain activates synaptic processes that reduce S1 excitability. Thus, taken together, data from fMRI and SEP studies suggest that S1 is altered during acute muscle pain and that this change is in the direction of reduced S1 excitability. However, as the relationship between SEP amplitude and BOLD contrast has not been characterized at stimulation intensities at or above pain threshold, evidence to support a correlation between these methodologies under pain conditions is limited.

Although the functional relevance of altered S1 activity during pain was not directly investigated in this review, previous studies have suggested that reduced S1 excitability may reflect a defensive adaptation designed to orient cortical attention towards stimuli that threaten the body's integrity (Legrain et al., 2011). Such a mechanism may reduce processing of non-painful afferent information and thus contribute to reduced sensorimotor performance when pain is present (Rossi et al., 1998, 2003). However, further work is needed to determine the relationship between reduced S1 excitability during pain and altered sensorimotor function.

The present review provides moderate evidence of reduced S1 excitability following the resolution of pain. However, this evidence is drawn exclusively from SEP data collected immediately post pain (Rossi et al., 2003; Schabrun et al., 2013) and further research is required to determine the duration and reliability of these findings. The effect of pain on the ipsilateral S1 also remains unclear as synthesis of fMRI data was largely inconclusive and SEPs were not investigated in this hemisphere. The results of all five PET studies show no S1 activation during pain. Since previous studies have shown that fMRI and PET activations typically correlate under identical stimulus conditions (Dettmers et al., 1996; Sadato et al., 1998), these findings were unexpected. One explanation may be that the lower temporal resolution of PET precluded the identification of rapid and/or transient pain-related neural activity. fMRI images are typically sampled between 0.3 and 3 s, whereas PET image acquisition is performed over a longer time period (50–120 s). If pain-related changes in S1 occurred prior to 50 s they may not have been detected using PET methodologies. Alternatively, as analyses are typically performed on data averaged over the entire scanning period, it is possible that changes were not detected due to a dynamic, fluctuating pattern of cortical activation during acute muscle pain.

### 4.2 The effect of acute muscle pain on M1

The effect of acute muscle pain on BOLD contrast at M1 in the hemisphere contralateral to the side of induced pain varied between studies with some reporting increased activation ($n = 4$), some decreased activation ($n = 1$) and some no change ($n = 5$) during pain. With the exception of one study (Henderson et al., 2006), there was no evidence for an effect of acute muscle pain on BOLD contrast in the ipsilateral M1. In contrast, meta-analyses of MEP data show moderate evidence of reduced corticomotor output to painful muscles when pain is present. Inconsistent findings between fMRI and TMS could also be explained by differences in temporal resolution between methodologies or alternatively, by changes occurring at spinal and/or peripheral, rather than cortical, level. As the MEP is a summative measure of cortico-cortical, cortico-motoneuronal and spinal motoneuron synaptic excitability, changes occurring at cortical, spinal and/or peripheral level may have contributed to the reduction in corticomotor output observed during pain. Few TMS studies controlled for changes in peripheral ($n = 3$) or spinal

excitability ($n = 3$) and only one study examined mechanisms thought to directly reflect activity in M1. That study reported enhanced activity of M1 intra-cortical circuits mediated by the inhibitory neurotransmitter GABA, and decreased activity of facilitatory circuits acting through NMDA receptors on glutamatergic interneurons (Schabrun and Hodges, 2012). Therefore, further research is required to determine whether reduced corticomotor output during pain reflects reduced activity in M1.

A novel finding during pain was the non-specificity of the corticomotor response. Pooled analyses of data from two TMS studies show that muscles in the same body segment, but not directly subjected to pain, exhibit reduced corticomotor output (Le Pera et al., 2001; Martin et al., 2008). Although there is currently insufficient data to determine whether this effect is limited to muscles at rest, it is possible that a similar response may occur in active muscles but remains undetected due to the facilitatory influence of volitional contraction on measures of MEPs (Di Lazzaro et al., 1998). This non-specificity suggests that an indiscriminate motor strategy is employed during pain. Although the functional significance remains unclear, it is possible that this adaptation serves to decrease muscle coordination and splint the affected body part (Schabrun et al., 2014). Such *en masse* movement strategies would likely prevent symptom aggravation and afford protection to an injured limb (Hodges and Tucker, 2011).

A strong reduction in corticomotor output was also found for painful muscles in the post-pain period at rest. The relative strength of this response may explain why a low-moderate reduction in corticomotor output to actively contracting muscles was also detected at this time point. A strong trend towards MEP suppression was also observed for non-painful muscles at rest. In line with current theories, it is possible that these reductions persist in the post-pain period as a defence against the threat of further pain and injury (Hodges and Tucker, 2011).

### 4.3 Relationship between S1 and M1 activity and the symptoms of pain

There is currently insufficient evidence to determine whether the neurophysiological changes described in this review are related to subjective assessments of pain severity. Although all fMRI investigations were conducted within the 'during pain' time period, a relationship to pain severity is impeded by inconsistent findings. For example, one study provided evidence of severity-dependent S1/M1 activations during pain (Loggia et al., 2012), whereas several others demonstrate strong cortical effects despite waning symptoms (Henderson et al., 2006; Nash et al., 2010b; Takahashi et al., 2011). As additional activations/deactivations were also observed in the ipsilateral hemisphere by some authors (Niddam et al., 2002; Henderson et al., 2006; Nash et al., 2010a,b; Owen et al., 2010; Loggia et al., 2012), there is also doubt regarding the specificity of this response. Furthermore, although the majority of EEG and TMS studies reported findings during pain (Romaniello et al., 2000; Le Pera et al., 2001; Rossi et al., 2003; Del Santo et al., 2007; Martin et al., 2008; Schabrun et al., 2013), few studies explicitly examined S1/M1 excitability in association with pain severity (Le Pera et al., 2001; Rossi et al., 2003). However, as meta-analyses of these studies show moderate-strong evidence of reduced corticomotor output and S1 excitability that persist after pain has resolved, a linear relationship between objective neurophysiological measures and subjective pain recordings appears unlikely.

### 4.4 Limitations and recommendations

This review has a number of limitations that require consideration. First, findings from multiple methodologies with unique temporal and spatial resolutions were included to provide insight regarding the extent, time-course and direction of the effect of pain on S1/M1. However, as the relationship between hemodynamic and electrophysiological measures remains uncertain, comparison of findings across methodologies is limited. Future protocols that combine PET/fMRI (high spatial resolution) with TMS/EEG (high temporal resolution) would clarify the effect of acute muscle pain on S1/M1 and the relationship between these measures.

The small number of studies identified for each methodology, as well as differences in study design, pain modality and severity, also limit discussion. For example, pain severity was reported as a value corresponding to either 'peak' or 'average' and ranged in severity from 'mild' to 'severe'. As such, this review was unable to discern a clear effect of pain severity and S1/M1 change between or within outcome measures. There is also insufficient evidence to ascertain the duration of S1/M1 change following the resolution of muscle pain due to inconsistent time points in TMS/EEG studies and a lack of post-pain data from PET/fMRI. Standardized recording intervals, in addition to improved descriptions of whether the interval refers to the time elapsed since the cessation

of pain or since the cessation of the pain intervention (pain remains), would help clarify the duration of these effects. Future protocols should also include objective measures of sensory and/or motor function in order to elucidate the functional significance of S1/M1 adaptations both during and post muscle pain.

## Author contributions

E.B., L.C. and S.S. designed the study. E.B. collected and analysed the data, interpreted the results and drafted the manuscript. E.B., L.C. and S.S discussed the results, commented on the manuscript and approved the final version.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1.** The effect of acute muscle pain on S1 excitability: a forest plot of baseline versus during pain data.

**Figure S2.** The effect of acute muscle pain on S1 excitability: a forest plot of baseline versus post-pain data.

**Figure S3.** The effect of acute muscle pain on corticomotor excitability: a forest plot of baseline versus during pain data for resting and actively contracting muscles.

**Figure S4.** The effect of acute muscle pain on corticomotor excitability: a forest plot of baseline versus post-pain data for resting and actively contracting muscles.

**Table S1.** Summary of S1/M1 activations (as determined via rCBF and BOLD contrast), during pain.

**Table S2**. Summary of study characteristics for studies investigating somatosensory evoked potentials (SEPs).

**Table S3.** Summary of study characteristics for studies investigating corticomotor excitability (motor evoked potentials: MEPs).

**Appendix S1.** Modified Downs and Black (1998) checklist for assessment of methodological quality of observational trials.

# Appendix A.1

Modified Downs and Black (1998) checklist for assessment of methodological quality of observational trials

***Reporting***

1 *Is the hypothesis/aim/objective of the study clearly described?*

| | |
|---|---|
| yes | 1 |
| no | 0 |

2 *Are the main outcomes to be measured clearly described in the Introduction or Methods section?*
If the main outcomes are first metioned in the Results section, the question should be answered no.

| | |
|---|---|
| yes | 1 |
| no | 0 |

3 *Are the characteristics of the patients included in the study clearly described?*
In cohort studies and trials, inclusion and/or exclusion criteria should be given. In case-control studies, a case-definition and the source for controls should be given.

| | |
|---|---|
| yes | 1 |
| no | 0 |

4 Are the distributions of principal confounders in each group of subjects to be compared clearly described?

A list of principal confounders is provided. Possible confounding variables include age, gender, duration of pain and severity of pain.

| | |
|---|---|
| yes | 2 |
| partially | 1 |
| no | 0 |

5 *Are the main findings of the study clearly described?*

6 *Does the study provide estimates of the random variability in the data for the main outcomes?*
In non normally distributed data the inter-quartile range of results should be reported. In normally distributed data the standard error, standard deviation or confidence intervals should be reported. If the distribution of the data is not described, it must be assumed that the estimates used were appropriate and the question should be answered yes.

| | |
|---|---|
| yes | 1 |
| no | 0 |

7 *Have the characteristics of patients lost to follow-up been described?*

This should be answered yes where there were no losses to follow-up or where losses to follow-up were so small that findings would be unaffected by their inclusion. This should be answered no where a study does not report the number of patients lost to follow-up.

| | |
|---|---|
| yes | 1 |
| no | 0 |
| unable to determine/ not applicable | 0 |

8 Have actual probability values been reported ( e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?

| | |
|---|---|
| yes | 1 |
| no | 0 |

Simple outcome data (including denominators and numerators) should be reported for all major findings so that the reader can check the major analyses and conclusions. (This question does not cover statistical tests which are considered below).

| | |
|---|---|
| yes | 1 |
| no | 0 |

**External validity**

All the following criteria attempt to address the representatives of the findings of the study and whether they may be generalised to the population from which the study subjects were derived.

9 *Were the subjects asked to participate in the study representative of the entire population from which they were recruited?*

The study must identify the source population for patients were selected. Patients would be representative if they comprised the entire source population, an unselected sample of consecutive patients, or a random sample. Random sampling is only feasible where a list of all members of the relevant population exists. Where a study does not report the proportion of the source population from which the patients are derived, the question should be answered as unable to determine.

| | |
|---|---|
| yes | 1 |
| no | 0 |
| unable to determine | 0 |

10 *Were those subjects who were prepared to participate representative of the entire population from which they were recruited?*

The proportion of those asked who agreed should be stated. Validation that the sample was representative would include demonstrating that the distribution of the main confounding factors was the same in the study sample and the source population.

| | |
|---|---|
| yes | 1 |
| no | 0 |

**Internal validity - bias**

11 *Was an attempt made to blind those measuring the main outcomes?*

| | |
|---|---|
| yes | |
| no | 0 |
| unable to determine | 0 |

12 *If any of the results of the study were based on "data dredging", was this made clear?*
Any analyses that had not been planned at the outset of the study should be clearly indicated. If no retrospective unplanned subgroup analyses were reported, then answer yes.

| | |
|---|---|
| yes | 1 |
| no | 0 |
| unable to determine | 0 |

13 *Were the statistical tests used to assess the main outcomes appropriate?*
The statistical techniques used must be appropriate to the data. For example non-parametric methods should be used for small sample sizes. Where little statistical analysis has been undertaken but where there is no evidence of bias, the question should be answered yes. If the distribution of the data (normal or not) is not described it must be assumed that the estimates used were appropriate and the question should be answered yes.

| | |
|---|---|
| yes | 1 |
| no | 0 |
| unable to determine | 0 |

14 *Were the main outcome measures used accurate (valid and reliable)?*
For studies where the outcome measures are clearly described, the question should be answered yes. For studies which refer to other work or that demonstrates the outcome measures are accurate, the question should be answered as yes.

| | |
|---|---|
| yes | 1 |
| no | 0 |
| unable to determine | 0 |

**Internal validity - confounding (selection bias)**

15 Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?
This question should be answered no for trials if: the distribution of known confounders in the different treatment groups was not described; the distribution of known confounders differed between treatment groups but was not taken into account in the analyses; or the confounding variables were not mentioned. For studies where covariate analysis (including confounding) was performed or non-significant difference between confounding variables was found, the question should be answered as yes.

| | |
|---|---|
| yes | 1 |
| no | 0 |
| unable to determine | 0 |

16 *Were losses of patients to follow-up taken into account?*

If the numbers of patients lost to follow-up are not reported, the question should be answered as unable to determine. If the proportion lost to follow-up was too small to affect the main findings, the question should be answered yes.

| | |
|---|---|
| yes | 1 |
| no | 0 |
| unable to determine/ not applicable | 0 |

**Power**

17 *Was sample size calculation done a priori?*

| | |
|---|---|
| yes | 1 |
| no | 0 |

## Appendix A.2

**Table S1.** Summary of S1/M1 activations (as determined via rCBF and BOLD-contrast), during pain.

| | | | **S1** | | **M1** | |
|---|---|---|---|---|---|---|
| *Study (year)* | *Technique* | *Outcome* | *contralateral* | *ipsilateral* | *contralateral* | *ipsilateral* |
| ***Kupers (2004)*** | PET | rCBF | ● | ● | ● | ● |
| ***Svensson (1997)*** | PET | rCBF | ● | ● | ● | ● |
| ***Thunberg (2005)*** | PET | rCBF | ● | ● | ● | ● |
| ***Korotkov (2002)*** | PET | rCBF | ● | ● | ● | ● |
| ***Owen (2010)*** | fMRI | CBF (ASL) | ↓[a] | ↓[a] | ● | ● |
| ***Owen (2012)*** | fMRI | CBF (ASL) | ● | ● | ● | ● |
| ***Nash (2010a)*** | fMRI | BOLD | ↑ | ↑ | ● | ● |
| ***Uematsu (2011)*** | fMRI | BOLD | ● | ● | ● | ● |
| ***Henderson (2006)*** | fMRI | BOLD | ↑ | ● | ↑[b] | ↑[b] |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Loggia (2012)*** | fMRI | BOLD | ↑[a] | ↓ | ↑[a] | ● |
| ***Macefield (2007)*** | fMRI | BOLD | ↑ | ● | ● | ● |
| ***Nash (2010b)*** | fMRI | BOLD | ↑ | ↑ | ↑ then ↓[b] | ● |
| ***Niddam (2002)*** | fMRI | BOLD | ↑ | ↑ | ● | ● |
| ***Takahashi (2011)*** | fMRI | BOLD | ↑ | ● | ↑a | ● |
| ***Maeda (2011)*** | fMRI | BOLD | ● | ● | ● | ● |

Abbreviations; ↓: decreased, ↑: increased, ●: no change reported, [a] linear relationship to pain, [b] effect outlasts peak pain, persist during waning pain. Contralateral and ipsilateral refer to the hemisphere in relation to the side of induced muscle pain.

# Appendix A.3

**Table S2.** Summary of study characteristics for studies investigating somatosensory evoked potentials (SEPs).

| *Study (year)* | *Pain location* | *Electrode orientation* | *Conditioning location* | *Conditioning intensity* | *SEP components* | *baseline vs. during pain* | *baseline vs. post pain* |
|---|---|---|---|---|---|---|---|
| ***Schabrun (2013)*** | first dorsal interosseous | C3' | ulnar nerve | 300% sensory perceptual threshold | P14-N20, N20-P25-N33 | yes | yes (0 mins) |
| ***Rossi (2003)*** | first dorsal interosseous | C3' | ulnar nerve | 95% motor threshold | P14-N20, N20-P25-N33 | yes (1-3, 3-6, 6-9, 9-12, 12-15 mins) | yes (0, 20 mins) |
| ***Rossi (1998)*** | extensor digitor brevis | Cz' | peroneal nerve | NR (< motor threshold) | P40-N50, P60-N75 | yes (0-5, 5-10, 10-15, 15-20 minutes) | yes (0, 30 mins) |

## Appendix A.4

**Table S3**. Summary of study characteristics for studies investigating corticomotor excitability (motor evoked potentials: MEPs)

| *Study (year)* | *Pain location* | *Muscle(s) targeted with TMS* | *Muscle State* | *TMS intensity* | *Coil Type* | *Coil position* | *baseline vs. during pain* | *baseline vs. post pain* |
|---|---|---|---|---|---|---|---|---|
| ***Le Pera (2001)*** | abductor digiti minimi<br>first dorsal interosseous<br>flexor carpi radialis | abductor digiti minimi<br>abductor digiti minimi<br>flexor carpi radialis | rest | RMT+5% stimulator output | figure 8 | hotspot | yes<br>(peak pain & post peak pain) | yes (20mins) |
| ***Schabrun (2012)*** | first dorsal interosseous | first dorsal interosseous & abductor digiti minimi | rest | 120% RMT | figure 8 | hotspot | no | yes (0 mins) |
| ***Schabrun (2013)*** | first dorsal interosseous | first dorsal interosseous | rest | 120% RMT | figure 8 | hotspot | yes | yes (0 mins) |
| ***Svensson (2003)*** | first dorsal interosseous | first dorsal interosseous & flexor carpi ulnaris | rest | 95% RMT to150% RMT | figure 8 | hotspot | no | yes (0 mins) |
| ***Del Santo (2007)*** | abductor digiti minimi<br>biceps brachii | abductor digiti minimi<br>biceps brachii | active (30% MVC) | 120% AMT | figure 8 | hotspot | yes | yes (0 mins) |
| ***Martin (2008)*** | biceps brachii | biceps brachii & triceps brachii | rest & active (20% MVC) | MEP amp 10-15% Mmax | circular | vertex | yes | yes (0 mins) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Rittig-Rasmussen (2014)*** | neck tissue 2cm lateral to C3 | trapezius & abductor pollicis brevis | active (trap only, % MVC NR) & rest (APB only) | MEP max amp (120-140% RMT or AMT) | figure 8 | hotspot | no | yes (30mins, 1hour) |
| ***Romaniello (2000)*** | masseter | masseter | active (15%, 30%, 45% MVC) | 110% RMT | circular | hotspot | yes | yes (20 mins) |

Abbreviations, TMS: transcranial magnetic stimulation; RMT: resting motor threshold; AMT: active motor threshold; MVC: maximum voluntary contraction; NR: not reported.

## Appendix A.5

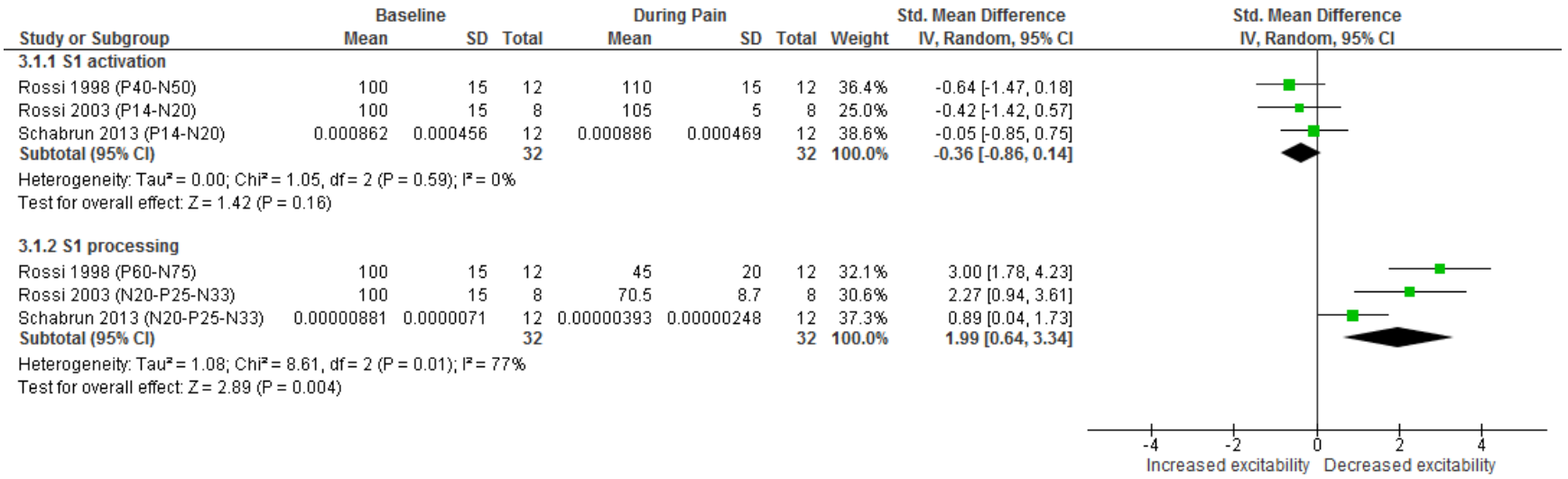


**Figure S1.** The effect of acute muscle pain on S1 excitability: a forest plot of baseline vs. during pain data. The standardized mean difference and confidence interval for the area of SEP components reflective of S1 activation and processing is indicated with a box and horizontal line for each study. The pooled effect estimates for each subgroup is denoted by a diamond

## Appendix A.6

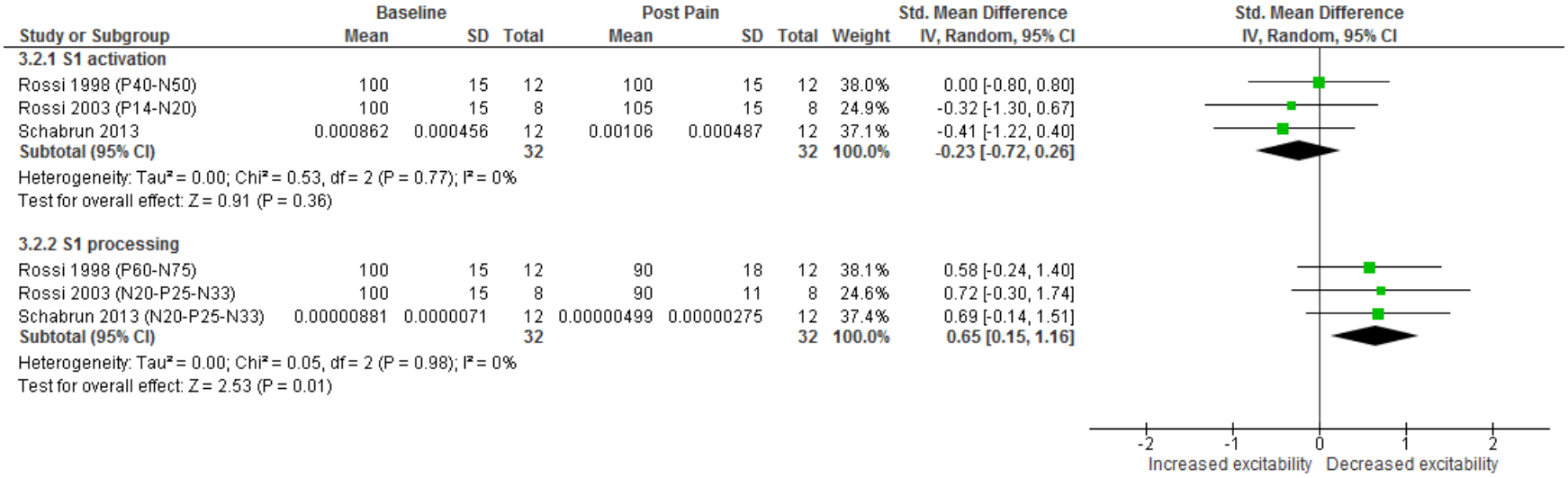


**Figure S2.** The effect of acute muscle pain on S1 excitability: a forest plot of baseline vs. post pain data. The standardized mean difference and confidence interval for the area of SEP components reflective of S1 activation and processing is indicated with a box and horizontal line for each study. The pooled effect estimates for each subgroup is denoted by a diamond.

## Appendix A.7

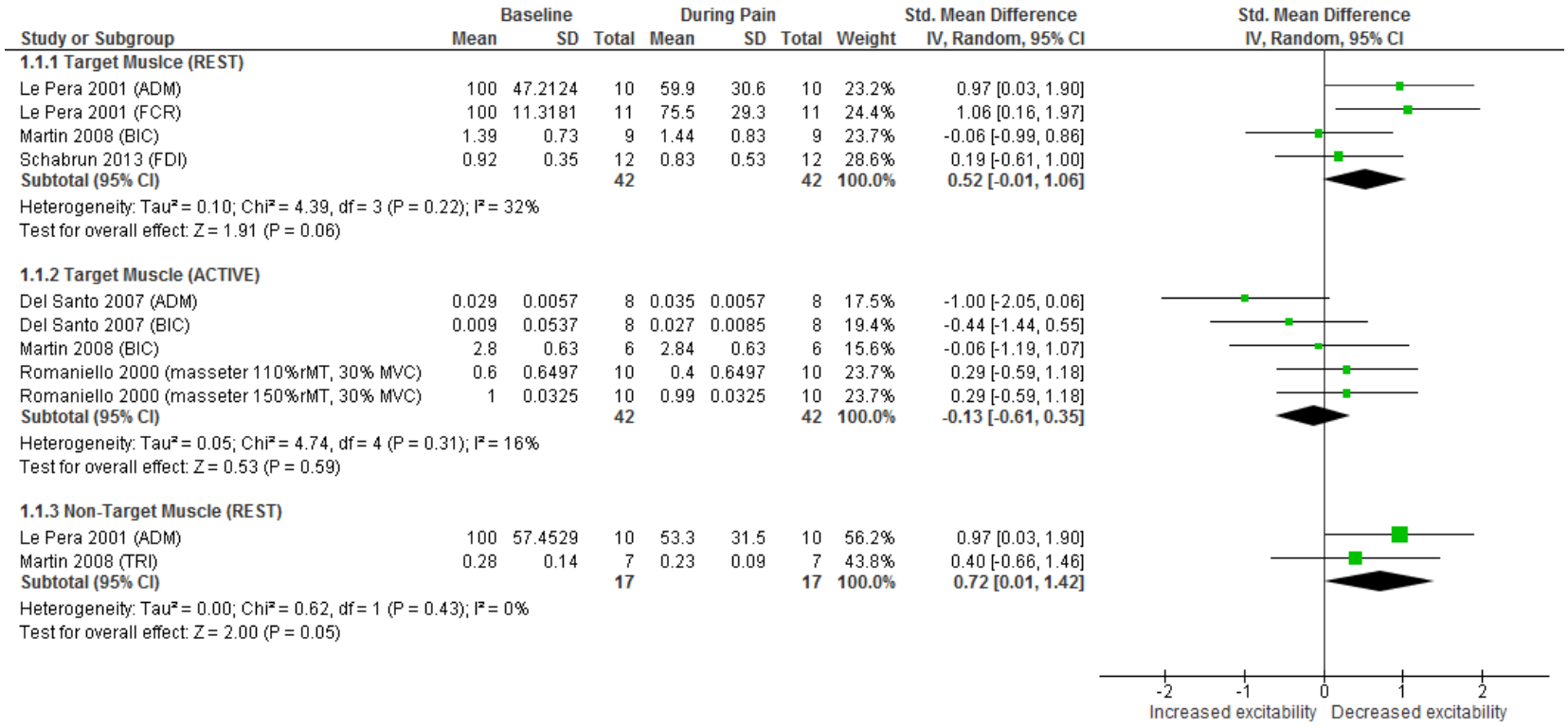


**Figure S3.** The effect of acute muscle pain on corticomotor excitability: a forest plot of baseline vs. during pain data for resting and actively contracting muscles. The standardized mean difference and confidence interval for MEP amplitude/area recorded from 'target' and 'non-target' muscles is indicated with a box and horizontal line for each study. The pooled effect estimates for each subgroup is denoted by a diamond.

## Appendix A.8

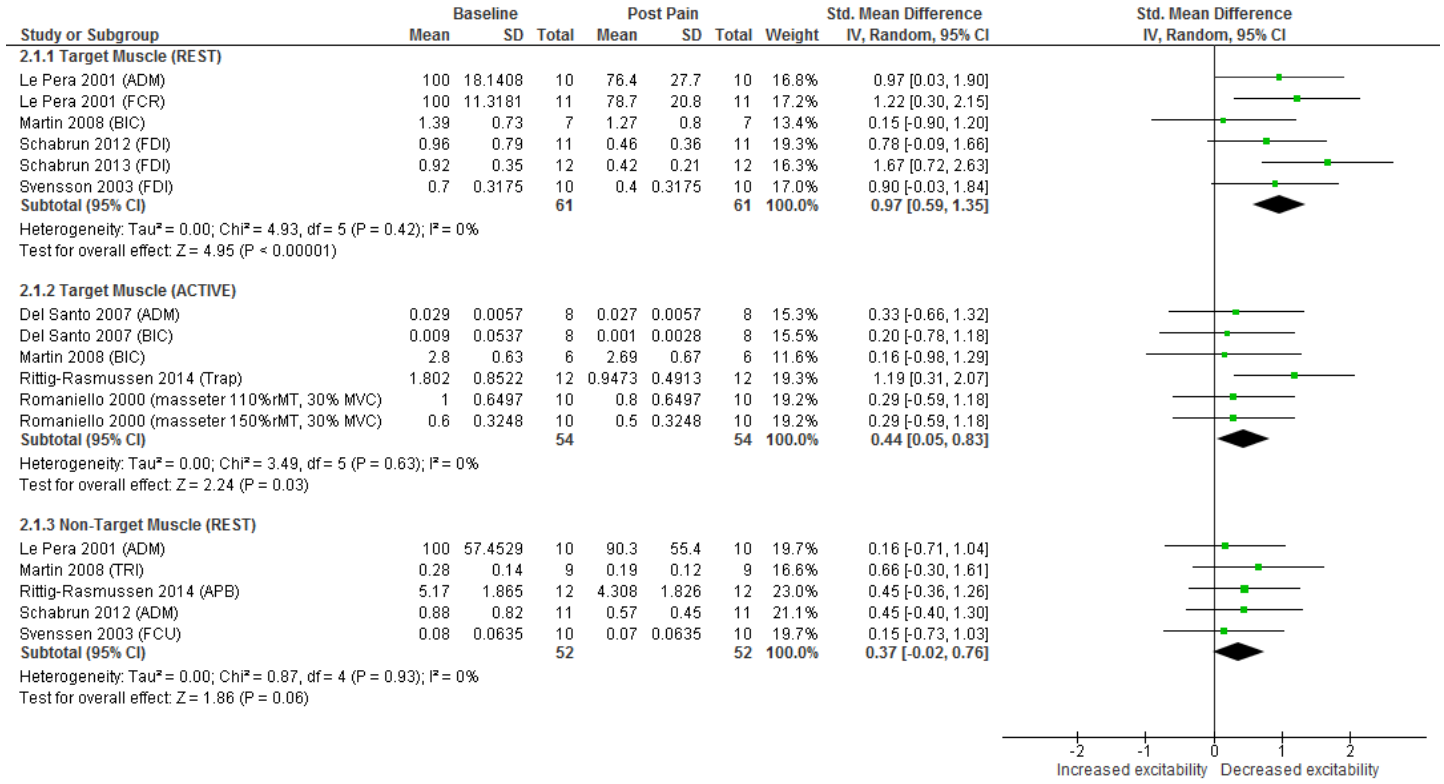


**Figure S4.** The effect of acute muscle pain on corticomotor excitability: a forest plot of baseline vs. post pain data for resting and actively contracting muscles. The standardized mean difference and confidence interval for MEP amplitude/area recorded from 'target' and 'non-target' muscles is indicated with a box and horizontal line for each study. The pooled effect estimates for each subgroup is denoted by a diamond.

# Appendix B

*Pain Medicine 2016; 17: 1343–1352*
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OXFORD

# REHABILITATION SECTION

## *Original Research Article*

# Reduced Short- and Long-Latency Afferent Inhibition Following Acute Muscle Pain: A Potential Role in the Recovery of Motor Output

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Conflicts of interest: There are no conflicts of interest to declare.

### Abstract

**Objective. Corticomotor output is reduced in response to acute muscle pain, yet the mechanisms that underpin this effect remain unclear. Here the authors investigate the effect of acute muscle pain on short-latency afferent inhibition, long-latency afferent inhibition, and long-interval intra-cortical inhibition to determine whether these mechanisms could plausibly contribute to reduced motor output in pain.**

**Design. Observational same subject pre-post test design.**

**Setting. Neurophysiology research laboratory.**

**Subjects. Healthy, right-handed human volunteers (n = 22, 9 male; mean age ± standard deviation, 22.6 ± 7.8 years).**

**Methods. Transcranial magnetic stimulation was used to assess corticomotor output, short-latency afferent inhibition, long-latency afferent inhibition, and long-interval intra-cortical inhibition before, during, immediately after, and 15 minutes after hypertonic saline infusion into right first dorsal interosseous muscle. Pain intensity and quality were recorded using an 11-point numerical rating scale and the McGill Pain Questionnaire.**

**Results. Compared with baseline, corticomotor output was reduced at all time points ($p = 0.001$). Short-latency afferent inhibition was reduced immediately after ($p = 0.039$), and long-latency afferent inhibition 15 minutes after ($p = 0.035$), the resolution of pain. Long-interval intra-cortical inhibition was unchanged at any time point ($p = 0.36$).**

**Conclusions. These findings suggest short- and long-latency afferent inhibition, mechanisms thought to reflect the integration of sensory information with motor output at the cortex, are reduced following acute muscle pain. Although the functional relevance is unclear, the authors hypothesize a reduction in these mechanisms may contribute to the restoration of normal motor output after an episode of acute muscle pain.**

**Key Words. Experimental Muscle Pain; Long-Interval Intracortical Inhibition; Long-Latency Afferent Inhibition; Sensorimotor Integration; Short-Latency Afferent Inhibition; Transcranial Magnetic Stimulation**

### Introduction

Pain affects sensorimotor function. When pain is present, the capacity of a muscle to generate force is

diminished [1–3], muscle co-ordination is altered [4–7], proprioception is distorted [8–10], and the ability to integrate sensory information with motor commands (sensorimotor integration) is impaired [11]. A number of authors have shown reduced corticomotor output during and after the resolution of muscle pain [12–16], and these changes are hypothesized to contribute to altered sensorimotor function. However, the mechanisms that underpin reduced corticomotor output in pain are unclear.

Changes occurring across multiple levels of the nervous system (peripheral, spinal, cortical) could reasonably contribute to altered motor output in pain. Previous studies have excluded changes at the muscle, as the peripheral M-wave (muscle compound action potential), contractile properties, and conduction velocity of action potentials along the muscle fiber membrane are unchanged [3,16–18]. At the level of the spinal cord, findings have been contradictory. One study demonstrated suppression of motoneurons in the late phase of pain [14], while another demonstrated facilitation [15]. This discrepancy could be explained by the use of different measures (H-reflex vs cervicomedullary-evoked potentials) to record spinal excitability. Regardless of the direction of spinal effects, both studies reported depression of motor cortical excitability, suggesting cortical mechanisms may contribute to the reduced motor output observed in pain.

A recent study has provided the first evidence for altered activity in motor cortical inhibitory and facilitatory networks in response to pain [12]. That study used transcranial magnetic stimulation (TMS) paired pulse paradigms to show a reduction in intracortical facilitatory (glutamate-mediated) and an increase in intracortical inhibitory (gamma-aminobutyric acid $[GABA]_A$ receptor mediated) networks during and immediately after the resolution of pain. The authors hypothesized that these changes act to restrict activity of painful muscles and limit the range and velocity of movement in pain, protecting the painful part from further pain and/or injury [19]. However, the impact of acute muscle pain on other intracortical networks such as those mediated by $GABA_B$ (long-interval intracortical inhibition;[ LICI]), or circuits involved in the integration of sensory information with motor execution (sensorimotor integration, measured using short- [SAI] and long-latency [LAI] afferent inhibition protocols) has not been investigated. Thus, here we aimed to examine the effect of acute experimental muscle pain on intracortical inhibitory networks, specifically those mediated by $GABA_B$, and networks associated with sensorimotor integration.

## Methods

### *Participants*

Twenty-two healthy, right-handed volunteers participated (mean ± standard deviation; 9 male, age = 22.6 ± 7.8 years). Handedness was determined using the Edinburgh Handedness inventory (laterality quotient, 88.8 ± 19.6) [20]. All volunteers completed a TMS safety screening questionnaire [21] and were excluded if they had a recent history of arm pain or injury; a personal or family history of epilepsy; major neurological, respiratory, orthopaedic, or circulatory disorders; were pregnant; had metal in their head or jaw; or were taking central nervous system acting medication [21]. The study was approved by the institutional human medical research ethical committee and performed in accordance with the Declaration of Helsinki. All participants provided written, informed consent. All testing took place in a University laboratory.

### *Electromyography (EMG)*

Surface EMG was recorded from FDI of the right hand. Dual silver-silver chloride disposable electrodes (spacing 2.0cm; Noraxon Inc, USA) were positioned over the muscle in a belly-tendon arrangement and remained in place for the duration for the experiment. The ground electrode was positioned over the right olecranon. EMG signals were amplified x1000, band-pass filtered 20-1000 Hz (Neurolog System Pre–amplifier; Digitimer Ltd, UK), and sampled at 2000 Hz using a Micro3 1401 Data Acquisition System and Signal5 software (Cambridge Electronic Design, Cambridge, UK).

### *Corticomotor Output*

Corticomotor output was investigated using single pulse TMS delivered to the primary motor cortex (M1) using a Magstim $200^2$ stimulator (Magstim Co. Ltd., Dyfed, UK) connected to a figure-of -eight coil (70mm wing diameter). TMS was performed over the left hemisphere (contralateral to the side of pain) for all participants. The coil was oriented at a 45 degree angle to induce posterior-anterior current flow and positioned over the motor 'hotspot' of FDI [22]. The motor hotspot was determined via systematic application of TMS to the scalp until the site that produced the largest motor evoked potential (MEP) was identified. A Brainsight neuronavigation system (Rogue Research Inc, Quebec, Canada) was used to target this site and ensure accurate coil placement for the duration of the experiment. All procedures adhered to the TMS checklist for methodological quality and were performed with the muscle at rest [23]. Fifteen MEPs (rate of 1 every 6 s) were recorded using a constant stimulator intensity sufficient to evoke a MEP of approximately 1mV peak-to-peak amplitude in FDI at baseline.

### *Long-Interval Intra-Cortical Inhibition*

A standard paired-pulse TMS protocol, using a suprathreshold conditioning stimulus delivered 160 ms before a suprathreshold test stimulus [24], was used to evaluate LICI. The intensity for both stimuli was set to evoke a peak-to-peak response of 1mV in FDI. As the amplitude of the test MEP is known to influence the LICI response [25], TMS intensity was adjusted where

necessary, to maintain a consistent 1mV test response at all time points. Twelve trials were recorded in random order at an inter-stimulus interval (ISI) of 160 ms and for the test stimulus alone (rate of 1 every 6 s; total of 24 trials).

*Short- and Long-Latency Afferent Inhibition*

SAI and LAI were assessed by pairing a conditioning electrical stimulus with a suprathreshold TMS test stimulus at ISIs of 20 ms and 200 ms, respectively [26]. Electrical stimuli were delivered via a Digitimer Constant Current stimulator (DS7A; Digitimer Ltd, UK) connected to surface electrodes placed 2 cm proximal to the wrist crease. Single electrical stimuli (100μs duration, 400V) were applied to the ulnar nerve of the right arm at an intensity sufficient to elicit a visible muscle contraction (motor threshold) [27]. Intensity of the TMS test stimulus was adjusted to evoke a peak-to-peak response of 1mV in FDI. Where necessary, the amplitude of the test stimulus was adjusted to maintain a consistent response at all time points [28]. Twelve trials were recorded in random order for each ISI (20 ms, 200 ms) and for the test stimulus alone (rate of 1 every 6 s; total of 36 trials).

*Compound Muscle Action Potentials (M-Waves)*

To control for changes in excitability occurring at the muscle and neuromuscular junction, electrical stimuli (100μs duration, 400V, amplified x100) were applied to the right ulnar nerve using the set-up outlined for SAI/LAI above. Five trials were recorded at a stimulus intensity 50% above that required to elicit a maximal compound muscle action potential (M-wave) in the FDI muscle at rest.

*Hypertonic Saline Infusion*

Hypertonic saline was infused into right FDI following a standard procedure [12,13]. A 25-gauge disposable cannula (Winged Infusion Set, Terumo, Japan) was placed into right FDI with the tip of the cannula at a depth of approximately 0.5 cm. A single bolus of 0.2 ml of sterile saline (5% NaCl) was infused over 20 seconds using an infusion pump (Harvard Apparatus, SDR Scientific, USA), a 10 ml plastic syringe (Terumo, Japan), and a low sorbing extension tube (140cm length; Braun, Germany). Following the bolus, a steady infusion rate of 6 ml/hour was maintained for 10 minutes. Pain intensity and quality were evaluated using an 11-point numerical rating scale (NRS) anchored with '0' as no pain and '10' as worst pain imaginable and the McGill short form pain questionnaire [29].

*Experimental Protocol*

As the LICI response has been shown to be influenced by concurrent recording of SAI and LAI measures [30,31], data collection was performed in two experiments: the effect of acute muscle pain on LICI was examined in Experiment 1 and the effect of acute muscle pain on SAI and LAI was assessed in Experiment 2. All 22 participants completed both experiments, separated by at least 36 hours, and experimental order was randomized. The experimental protocol was as follows: participants sat upright in a chair with their head and neck supported and their arms resting comfortably on a pillow. Data were recorded at four time points: i) baseline, ii) during pain (measurement commenced once pain reached a moderate intensity of NRS 5/10), iii) post pain (once pain had returned to 0/10), and iv) follow up (15 minutes after pain had returned to 0/10). Measures at each time point were performed in the following order: 15 MEPs, either 24 LICI trials (Experiment 1) or 36 SAI/LAI trials (Experiment 2) and 5 M-waves. Following the completion of baseline assessments, a cannula was inserted into FDI and remained in situ for the duration of the 'during pain' time period. Immediately after the infusion, the cannula was removed. Participants were asked to verbally rate their pain using the NRS after the completion of each measure within the 'during pain' time period and for every minute after the removal of the cannula, until NRS 0/10 was achieved. At the conclusion of each experiment, participants described the location, quality and intensity (subjective average) of muscle pain using the McGill short form pain questionnaire. The protocol for Experiments 1 and 2 is outlined in Figure 1.

*Data and Statistical Analyses*

MEP and Mwave data were measured as peak-to-peak amplitudes (mV) and averaged at each time point. To account for any activity-dependent changes in muscle fiber action potentials resulting from the induction of pain, statistical analyses for corticomotor output were performed with MEP amplitude expressed as a proportion of Mwave amplitude (MEP/Mwave amplitude ratio) [22]. To determine whether acute muscle pain induced a change in LICI (Experiment 1) or SAI and LAI (Experiment 2), peak-to-peak conditioned MEP amplitudes for these data were expressed as a percentage of the unconditioned (test) response. It has been shown that approximately 30% of individuals fail to respond to afferent inhibition protocols at baseline under uniform test conditions [32], thus, consistent with previous work [32], it was elected to exclude participants who did not display inhibition at baseline from further analyses.

All neurophysiological outcomes (MEP/Mwave amplitude ratio, LICI, SAI, and LAI) were assessed for normality and compared between time points (baseline, during pain, post pain, follow up) using a one-way repeated measures analysis of variance (ANOVA) or where data was not normally distributed, a Friedman's repeated measures analysis on ranks. Post hoc analyses were performed using the Student Newman Keuls (SNK) test that corrected for multiple comparisons. Significance was set at 5%. All data in text is presented as mean ± standard deviation.

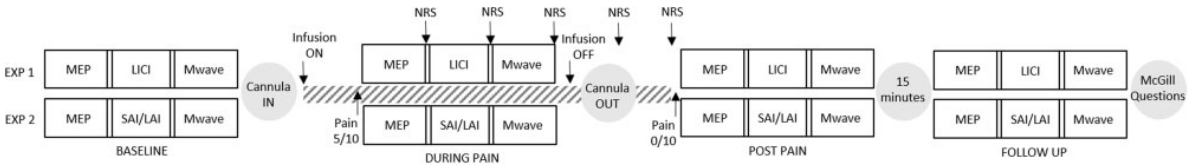



**Figure 1** Experimental protocol. Measures of corticomotor output (MEP), long-interval intra-cortical inhibition (LICI), short- and long-latency afferent inhibition (SAI/LAI), and peripheral excitability (Mwave) were made at i) baseline, ii) during pain (once pain reached 5/10 intensity), iii) immediately post pain (pain 0/10), and iv) 15 minutes following the resolution of pain (follow up). LICI and SAI/LAI were assessed in separate experiments (EXP 1 and 2) to avoid inhibitory interactions. Cannulae were inserted after completion of baseline measures and were removed once the infusion was terminated. During and following infusion of hypertonic saline, pain intensity was monitored using a numerical rating scale (NRS). The McGill short form pain questionnaire (McGill Questions) was completed at the conclusion of EXP 1 and 2.

## Results

### *Pain Characteristics*

Infusion of hypertonic saline induced an average pain intensity of 5.2 ± 1.7 cm for Experiment 1 and 5.0 ± 1.7 cm for Experiment 2. The most frequent words used to describe pain were aching (82%), throbbing (73%), sharp (62%), and cramping (60%). The majority of participants reported symptoms localised to the dorsal surface of the hand. One participant reported numbness localised to the thumb and an additional two participants reported pain that extended beyond the wrist into the proximal forearm.

### *Corticomotor Output Is Reduced During and After Acute Muscle Pain*

As the effect of acute muscle pain on corticomotor output (MEP/Mwave amplitude ratio) was similar for Experiments 1 and 2 (ANOVA main effect of interaction, $p = 0.67$), these data were pooled for analyses. Corticomotor output was reduced during pain (ANOVA main effect of time, $p = 0.001$) relative to baseline (SNK $p = 0.003$) and this reduction persisted immediately (SNK $p = 0.003$) and 15 minutes following the resolution of pain (SNK $p = 0.047$; Figure 2).

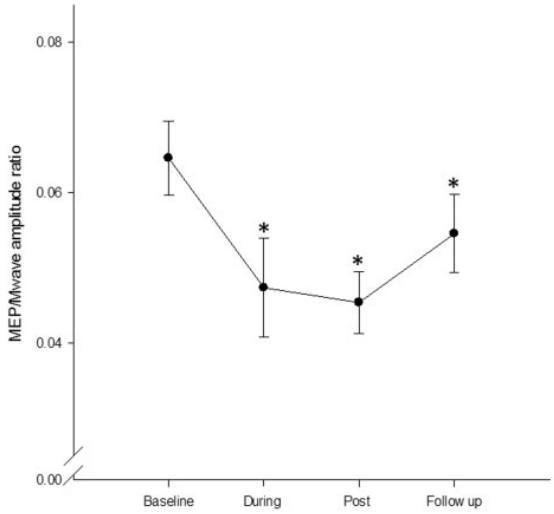


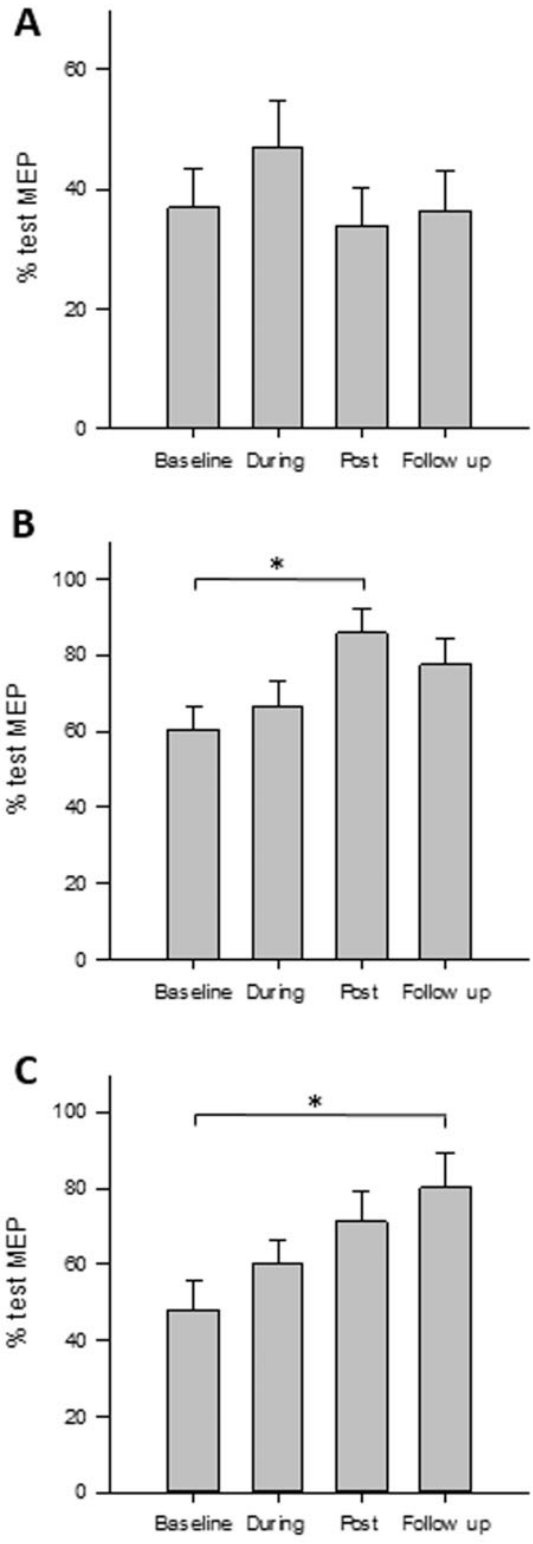

**Figure 2** Pooled group data from both experiments ($n = 22$ in each experiment) for corticomotor output before, during, immediately post, and 15 minutes following the resolution of experimental muscle pain. Data are expressed as MEP/Mwave amplitude ratio. Corticomotor output is reduced during, immediately post, and 15 minutes following the resolution of muscle pain. *$p < 0.05$.

### *Long-Interval Intra-Cortical Inhibition Is Unchanged by Muscle Pain*

Two participants did not display inhibition at baseline (two females, aged 19 years) and these individuals were excluded from further analysis of LICI [32]. Thus, data from 20 participants (nine male, age = 23.0 ± 8.4 years) were analyzed. It was necessary to adjust the intensity of TMS output at each time point to maintain test responses of approximately 1mV peak-to-peak amplitude for all participants. The intensity at baseline was 57.3 ± 9.6%, 61.4 ± 9.5% during pain, 64.0 ± 10.2% post pain, and 61.8 ± 12.2% at follow up. The induction of acute muscle pain did not alter LICI at any time point (ANOVA main effect of time, $p = 0.36$; Figure 3A). The amplitude of the test MEP remained stable over time (baseline: 1.10 ± 0.42 mV, during: 0.96 ± 0.40 mV, post: 1.02 ± 0.37 mV, follow up: 0.99 ± 0.37 mV; ANOVA main effect of time, $p = 0.54$).

### *Short-Latency Afferent Inhibition Is Reduced Immediately After the Resolution of Acute Muscle Pain*

Six participants did not display inhibition in response to the SAI protocol at baseline (two male, age = 26.0 ± 12.1 years) and were excluded [32]. Thus, data




from 16 participants were included in the analysis (seven male, age = 21.4 ± 4.9 years). It was necessary to adjust the intensity of TMS output at each time point to maintain test responses of approximately 1mV peak-to-peak amplitude for all participants. The intensity at baseline was 53.2 ± 10.0%, 57.6 ± 12.1% during pain, 60.9 ± 12.3% post pain, and 59.9 ± 12.1% at follow up. Acute muscle pain reduced SAI (ANOVA main effect of time, $p = 0.039$) relative to baseline immediately following the resolution of muscle pain (baseline vs immediate, SNK $p = 0.044$; Figure 3B). This effect was not maintained at 15 minutes follow-up (baseline vs follow-up, SNK $p = 0.16$). There was no change in SAI during pain (baseline vs during, SNK $p = 0.55$). The amplitude of the test MEP remained constant over time (baseline: 1.00 ± 0.13 mV, during: 0.93 ± 0.19 mV, post: 0.95 ± 0.13 mV, follow up: 0.95 ± 0.16 mV; ANOVA main effect of time, $p = 0.16$).

### *Long-Latency Afferent Inhibition Is Reduced 15 Minutes After the Resolution of Acute Muscle Pain*

Six participants did not display inhibition in response to the LAI protocol at baseline (two male, age = 20.0 ± 1.7 years) and were excluded. Thus, data from 16 participants were included in the analysis (seven male, age = 24.5 ± 10.4 years). It was necessary to adjust the intensity of TMS output at each time point to maintain test responses of approximately 1mV peak-to-peak amplitude for all participants. The intensity at baseline was 58.8 ± 10.1%, 57.3 ± 12.1% during pain, 60.6 ± 12.6% post pain, and 59.7 ± 12.8% at follow up. As data was not normally distributed, comparisons were performed using Friedman's repeated measures analysis on ranks. Acute muscle pain reduced LAI (Friedman: main effect of time, $p = 0.035$) 15 minutes after pain had resolved (follow-up vs baseline SNK $p < 0.05$; Figure 3C). There was no change in LAI during (baseline vs during, SNK $p > 0.05$) or immediately after the resolution of pain (baseline vs immediate, SNK $p > 0.05$). The amplitude of the test MEP remained constant over time (baseline: 0.99 ± 0.26 mV, during: 0.91 ± 0.29 mV,

**Figure 3** Group data (mean ± standard error) for (A) long-interval intracortical inhibition (LICI, n = 20), (B) short-latency afferent inhibition (SAI, n = 16), and (C) long-latency afferent inhibition (LAI, n = 16) at baseline, during, immediately post, and 15 minutes following the resolution of experimental muscle pain. Individuals who did not show inhibition at baseline were excluded from analyses and are not shown in the Figure. LICI, SAI, and LAI were determined by expressing the conditioned MEP as a percentage of the unconditioned test MEP (% of test MEP). LICI was not affected by acute muscle pain at any time point (A). Compared with baseline, SAI was reduced (less inhibition, higher proportion of the test MEP) immediately post pain (B) and LAI was reduced at follow up (C). *$p < 0.05$.

post: 0.86 ± 0.28 mV, follow up: 0.89 ± 0.32 mV; ANOVA: main effect of time, $p = 0.25$).

## Discussion

To our knowledge, this study is the first to examine circuits involved in sensorimotor integration (SAI, LAI) and intracortical networks mediated by $GABA_B$ (LICI) in response to acute muscle pain. The novel finding is that experimental muscle pain reduces SAI and LAI (indicative of less inhibition) but only once pain has resolved, whereas intra-cortical networks mediated by $GABA_B$ appear unaffected by acute muscle pain. Our data also confirm previous reports of reduced corticomotor output during and following the resolution of pain. Taken together, our findings provide new insight into mechanisms that may contribute to recovery of motor output following acute muscle pain.

Short- and long-latency afferent inhibition is investigated by coupling peripheral nerve stimulation at the wrist with TMS at M1 [26]. When paired at short (~20ms) and long (~200ms) inter-stimulus intervals, inhibition of the MEP is observed, an effect thought to reflect the interaction between sensory input and motor output at the level of the cortex (sensorimotor integration) [26,33]. The latencies at which inhibition is induced suggest that SAI and LAI reflect the activation of distinct sensorimotor pathways and thus are likely to represent separate indices of sensorimotor integration. For instance, SAI is postulated to involve direct S1-M1 pathways [26,31,34], whereas LAI is thought to involve indirect basoganglia-thalamocortical pathways [31,33–35]. This interpretation is further supported by evidence that SAI and LAI display unique interactions with other intracortical circuitry [30,31,36], and are differentially affected by pathology [34,35,37] and drug formulations [38–40]. In the present study we show reduced SAI immediately following, and reduced LAI 15 minutes following, an episode of acute muscle pain. Hence, the different cortical pathways that underpin these mechanisms are likely to explain these differential time effects. Although it is beyond the scope of the present study to infer causation, it is possible that the timing of these effects may relate to the relative temporal sensitivity of the cortical generators of SAI and LAI to salient cues (i.e., the cessation of nociceptive input) [41].

Although the functional significance of reduced SAI and LAI in response to acute muscle pain is unclear, evidence suggests that appropriate sensorimotor integration constitutes an essential component of fluid, coordinated motor control [42]. Work performed in non-pain conditions indicate that SAI and LAI support the operation of 'surround inhibition', a mechanism that facilitates fine motor control via regulation of the inhibitory drive to specific muscles [43]. Indeed, it has been shown during selective movement of the index finger that SAI/LAI to muscles necessary for movement is decreased, whilst inhibition to redundant muscles is increased [44,45]. One explanation for our data is that reduced afferent inhibition (SAI and LAI) following resolution of muscle pain acts to facilitate motor recovery, promoting a return to normal motor output of the painful part as the threat of pain and injury subsides. Indeed, although motor output remains suppressed compared with baseline in the post pain time period, our data demonstrates that motor output has undergone recovery of approximately 50% 15 minutes after pain has resolved (Figure 2). Further work is necessary to determine the time course of recovery of motor output and the relationship between motor output and afferent inhibition to confirm this hypothesis.

We contend that motor output in the post-pain period may reflect a balance between the need for recovery of motor output and protection from the threat of further pain and injury. This is supported by evidence of altered activity in the cortical circuits thought to support each of these functions. For instance, although SAI and LAI are reduced (less inhibition) in the post-pain period, possibly to facilitate motor recovery, other inhibitory mechanisms are increased. Short-interval intracortical inhibition (SICI), an index of M1 intracortical activity mediated via $GABA_A$ receptors on inhibitory interneurons [46,47], is increased immediately following the resolution of muscle pain and this is thought to maintain a protective motor strategy in the post-pain period by continuing to limit motor output [12]. As SICI is known to inhibit the expression of SAI [36,48], and interacts in an additive manner with LAI when activated concurrently [31], it is possible that SICI is the dominant mechanism during this early post-pain time period, ensuring a protective strategy persists until the threat of pain is completely removed. Once all threat has dissipated, SICI may reduce, and mechanisms such as SAI and LAI may become dominant, facilitating recovery of motor output. However, SICI has only been investigated in the period immediately following the resolution of acute pain [12], and it is unknown how the temporal profile of this mechanism is altered in relation to SAI, LAI and motor output over an extend time frame. Future work should seek to examine the interaction of these mechanisms over a prolonged time period once pain has resolved.

Our observation that LICI remains unchanged in response to pain is further evidence that the response to pain varies across different inhibitory networks. Unlike SICI, the effects of LICI are mediated via $GABA_B$ receptors located on M1 interneurons [49,50], therefore the results of the present study suggest that the effect of pain differs among GABA receptor subtypes. Although it is unclear why $GABA_A$, but not $GABA_B$ mediated inhibition is affected by pain, one possible explanation may be that LICI is more robust than SICI and less sensitive to transient changes in afferent input. Indeed, since reductions in LICI have been observed in chronic pain conditions such as fibromyalgia and rheumatoid arthritis [51], it is likely that the effect of pain on LICI may depend upon symptom duration. Alternatively, as LAI has been demonstrated to interact with LICI in an intensity dependent manner [31], it is possible that LICI remained

unaffected during or immediately post pain due to the stability of LAI at these time points. However, since LAI was reduced 15 minutes post pain but LICI was unchanged, further work is needed to determine whether LICI/LAI interactions are relevant during and/or following a painful episode.

A vehicle control was not included in the current study for several reasons. First, use of such a control is common in both animal and human studies of experimental pain and rarely, if ever, shows an effect [52,53]. Indeed, previous studies of similar methodology have demonstrated no effect of intramuscular isotonic saline solution on the amplitude or latency of MEPs to muscles of the upper limb [14,15]. Second, numerous studies demonstrate that measures of M1 organization and function are stable and reliable over time [54–56]. For example, corticomotor output has been shown to be stable during 30 minutes of controlled, quiet sitting [16] and is reliable over short (0-4 days) and long (0-14 days, 0-1 months) intersession intervals [54,55]. Similarly, LICI and SAI have been shown to be stable over testing intervals of 1 and 7 days, respectively [27,57]. Taken together, these observations indicate that the measures used in this study are not sensitive to time effects and our findings are unlikely to be replicated in a no pain control condition.

This study has several limitations that should be acknowledged. First, we did not collect data from muscles other than FDI and thus, this study is not able to determine whether reductions in SAI and LAI are muscle specific. However, results from previous studies suggest a non-specific, but localised, effect of hand pain on corticomotor output. For example, muscle pain induced in FDI has been shown to reduce corticomotor output of distant hand muscles (abductor digiti minimi) [12,14], but not of proximal forearm muscles (flexor carpi ulnaris) [16]. Further research is required to determine whether reductions in SAI and LAI are confined to the muscle in pain, or extend to other local hand and forearm muscles. Second, SAI, LAI, and LICI were not tested using a range of ISIs. In early studies of SAI, the ISIs tested were based on calculations of the specific latency of the N20 component of the somatosensory evoked potential observed following median nerve stimulation for each participant [26]. Following ulnar nerve stimulation, the latency of this component is known to occur within the range of $19.93 \pm 1.1$ ms [27] to $20.7 \pm 0.7$ms [36]. As inter-individual variability for SAI has been shown to be high within the optimal test range [27,58] and SAI is decreased or absent if afferent stimulation fails to correspond with the latency of N20, it is possible that that the stimulus parameters used in this present study may have been suboptimal for some participants. As this limitation necessitated the exclusion of six participants from SAI and LAI analysis, future research should seek to use stimulus parameters specific to induce inhibition in each individual participant. Two participants who responded with MEP facilitation to the LICI protocol were also excluded as there is evidence that such facilitation is due to the interference of intracortical facilitatory mechanisms [59]. Future work concerning LICI should seek to investigate the effect of pain at intervals $<160$ ms as recent evidence suggests that LICI evoked using early ($\sim$100ms) and late ($\sim$150 ms) test intervals [60,61] may differentially reflect pre- and postsynaptic inhibitory processes [62].

## Conclusions

This study is the first to provide evidence of reduced SAI and LAI following acute muscle pain. We hypothesize that these responses may reflect the early activation of mechanisms that restore normal motor function after the resolution of pain. It is possible that a protective motor strategy (reduced corticomotor output) prevails early after the resolution of pain, despite reductions in SAI and LAI, due to the existence of competitive interactions between SAI/LAI and other inhibitory networks. Further studies are required to confirm this hypothesis. If confirmed, these data may have relevance for the design of clinical interventions that aim to restore motor output in musculoskeletal pain conditions.

## Acknowledgements

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# Appendix C

EJP
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ORIGINAL ARTICLE

# Altered function of intracortical networks in chronic lateral epicondylalgia

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None declared.

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## Abstract

**Background:** Lateral epicondylalgia (LE) is a musculotendinous condition characterized by persistent pain, sensorimotor dysfunction and motor cortex reorganization. Although there is evidence linking cortical reorganization with clinical symptoms in LE, the mechanisms underpinning these changes are unknown. Here we investigated activity in motor cortical (M1) intracortical inhibitory and facilitatory networks in individuals with chronic LE and healthy controls.

**Methods:** Surface electromyography was recorded bilaterally from the extensor carpi radialis brevis (ECRB) muscle of 14 LE (4 men, 41.5 ± 9.9 years) and 14 control participants (4 men, 42.1 ± 11.1 years). Transcranial magnetic stimulation of M1 was used to evaluate resting and active motor threshold, corticomotor output, short- (SICI) and long-latency intracortical inhibition (LICI) and intracortical facilitation (ICF) of both hemispheres.

**Results:** In individuals with LE, SICI ($p = 0.005$), ICF ($p = 0.026$) and LICI ($p = 0.046$) were less in the M1 contralateral to the affected ECRB muscle compared with healthy controls. Motor cortical threshold (rest: $p = 0.57$, active: $p = 0.97$) and corticomotor output ($p = 0.15$) were similar between groups. No differences were observed between individuals with LE and healthy controls for the M1 contralateral to the unaffected ECRB muscle.

**Conclusions:** These data provide evidence of less intracortical inhibition mediated by both $GABA_A$ and $GABA_B$ receptors, and less intracortical facilitation in the M1 contralateral to the affected ECRB in individuals with LE compared with healthy controls. Similar changes were not present in the M1 contralateral to the unaffected ECRB. These changes may provide the substrate for M1 reorganization in chronic LE and could provide a target for future therapy.

**What does this study add:** Lateral epicondylalgia (LE) is a common musculoskeletal condition characterized by elbow pain and sensorimotor dysfunction. The excitability and organization of the motor cortical representation of the wrist extensor muscles is altered in LE, but the mechanisms that underpin these changes are unknown. evidence of less intracortical inhibition mediated by both $GABA_A$ and $GABA_B$ receptors, and less intracortical facilitation mediated by NMDA receptors, in the M1 contralateral to the affected extensor carpi radialis brevis muscle in chronic LE compared with healthy controls. Altered activity in intracortical networks may contribute to altered motor cortex organization in LE and could provide a potential target for future treatments.

## 1. Introduction

Lateral epicondylalgia (LE), commonly termed 'tennis elbow', is a disabling condition affecting the musculotendinous structures at the lateral epicondyle, characterized by symptoms of persistent pain and sensorimotor dysfunction (Bisset et al., 2006; Skinner and Curwin, 2007; Juul-Kristensen et al., 2008; Coombes et al., 2012). Thought to be triggered by repetitive, forceful use of the forearm extensor muscles (Fan et al., 2009), LE affects 1–3 per cent of the general population and 15% of workers, with high rates of symptom persistence and recurrence (Shiri et al., 2006). Recent evidence suggests that altered primary motor cortex (M1) organization could contribute to symptoms of pain and motor dysfunction in this condition. For instance, increased excitability, greater overlap and a reduced number of discrete cortical peaks have been demonstrated in the representations of the elbow extensor muscles in chronic LE, and these changes are associated with pain severity (Schabrun et al., 2014). As M1 representations are known to be maintained and adjusted by intracortical inhibitory (ICI) and facilitatory (ICF) networks (Liepert et al., 1998a), altered activity in these networks could underpin altered M1 organization in LE.

Intracortical networks can be probed in the human M1 using paired-pulse transcranial magnetic stimulation protocols. ICI measured at short (SICI) and long (LICI) latencies is thought to reflect activity in $GABA_A$ and $GABA_B$ receptor systems, respectively, while ICF is thought to reflect activity in the glutamatergic (NMDA receptor) system, with a relative spinal contribution (Ziemann et al., 1998; Werhahn et al., 1999; McDonnell et al., 2006). Studies have shown increased ICI, and reduced ICF, in response to acute muscle pain, and these findings are hypothesized to underpin reduced M1 excitability and restriction of motor output observed in acute pain (Hodges and Tucker, 2011; Schabrun and Hodges, 2012). Conversely, in persistent neuropathic pain, complex regional pain syndrome and low back pain, ICI is reduced, a finding that may underpin increased excitability and altered organization of M1 in these conditions (Schwenkreis et al., 2003, 2010; Eisenberg et al., 2005; Masse-Alarie et al., 2012). Similar alterations in intracortical networks could be present in LE, yet no study has investigated these mechanisms in this condition.

Understanding the mechanisms that contribute to chronic LE has the potential to provide new targets for therapy. Indeed, use of repetitive transcranial magnetic stimulation to restore ICI in chronic neuropathic pain is associated with a reduction in pain severity, suggesting that therapies designed to target intracortical networks may be effective in ameliorating persistent pain (Lefaucheur et al., 2006). Here we aimed to investigate the function of M1 intracortical networks mediated by $GABA_A$ (short-interval intracortical inhibition, SICI), $GABA_B$ (long-interval intracortical inhibition; LICI) and NMDA receptors (ICF) in individuals with persistent LE and healthy controls. Consistent with findings in other persistent pain conditions, we hypothesized a reduction in intracortical inhibition in individuals with LE.

## 2. Method

### 2.1 Participants

Fourteen individuals with LE (mean ± standard deviation; 4 men, aged 41.5 ± 9.9 years) and 14 age- and gender-matched healthy controls (4 men, aged matched ± 5 years: 42.1 ± 11.1) participated. As M1 intracortical mechanisms are yet to be investigated in LE, sample size calculations were based on effect sizes from a previous study examining cortical excitability (motor evoked potential amplitude) in LE (Schabrun et al., 2014). Based on these data (difference of means between patients and controls: 0.59 mV, standard deviation: 0.625 mV), it was calculated that a minimum sample size of nine participants in each group were needed to observe a statistically significant difference (80% power, alpha 0.05) should one exist (Kadam and Bhalerao, 2010). Individuals with LE were included if they had experienced elbow pain over the lateral epicondyle for greater than 6 weeks that was provoked by palpation, gripping, resisted wrist and/or middle finger extension (Linaker et al., 1999). Exclusion criteria included: (1) use of oral or topical pain-relief medication in the preceding 48 h; (2) concomitant neck or arm pain that prevented participation in usual work or recreational activities; (3) corticosteroid injections in the last 6 months; and (4) evidence of sensory disturbances, history of fractures, elbow surgery, arthritic or inflammatory disorders or pain localized to the radiohumeral joint (Coombes et al., 2009, 2013). In addition, participants completed a transcranial magnetic stimulation (TMS) safety screening questionnaire and were excluded from enrolment if they had a personal or family history of epilepsy, major neurological, respiratory, orthopaedic or circulatory disorders, if they were pregnant, had

metal in their head or jaw or were taking central nervous system acting medications (Keel et al., 2001). The study was approved by the institutional human medical research ethical committee and performed in accordance with the Declaration of Helsinki. All participants provided written, informed consent.

### 2.2 Clinical measures of LE

#### 2.2.1 Pain and disability

The Patient Rated Tennis Elbow Evaluation (PRTEE) was used to assess pain and disability for the week preceding the experiment (Macdermid, 2005). Scores for pain (sum of five items out of 50) and function (sum of 10 items, divided by 2, out of 50) were combined to give a total score ranging from 0 (no pain and no functional impairment) to 100 (worst pain imaginable with significant functional impairment). Current pain intensity was recorded using an 11-point numerical rating scale (NRS) anchored with '0' as no pain and '10' as worst pain imaginable.

#### 2.2.2 Pressure pain thresholds (PPT)

A hand-held pressure algometer (Commander probe size 1 $cm^2$, JTECH Medical, Midvale, Utah, USA) was applied perpendicular to the extensor carpi radialis brevis (ECRB) muscle at a steadily increasing rate of pressure. ECRB was located via palpation during resisted middle finger and wrist extension. The location of the muscle belly was marked on the skin with an oily-tipped pen to ensure accurate probe placement between trials. Participants were instructed to vocalize at the exact moment they perceived the sensation of pressure first turn to pain. Pressure pain threshold (*N*) was assessed for each arm and defined as the average of five consecutive trials (rate of 1 trial every 10 s).

#### 2.2.3 Pain-free grip strength and maximum grip strength

Pain-free and maximum grip strength were assessed using a hand-held dynamometer (Baseline Digital Hand dynamometer, Chattanooga Group, Chattanooga, Tennessee, USA). Participants assumed a seated position and were assisted in placing their arm in 90° shoulder flexion, full elbow extension and neutral forearm pronation (De Smet et al., 1998). For pain-free grip strength, participants were instructed to squeeze the dynamometer, but to cease immediately at the onset of pain. To assess maximal grip strength, participants were instructed to squeeze the dynamometer as hard as possible, regardless of pain. Grip strength (kg) of each arm was determined based on the average of three consecutive trials (rate 1 trial every 30 s).

### 2.3 Neurophysiological measures

#### 2.3.1 Electromyographic recordings

Surface electromyography (EMG) was recorded from ECRB using dual silver–silver chloride disposable electrodes (spacing 2.0 cm). Electrode position was determined following palpation of ECRB during resisted wrist and middle finger extension. The ground electrode was positioned over the olecranon. EMG signals were amplified ×1000 (NL844, Digitimer Ltd, Welwyn Garden City, UK), band-pass filtered: 20–1000 Hz and sampled at 2000 Hz using a Micro3 1401 Data Acquisition System and Signal 5 software (Cambridge Electronic Design, Cambridge, UK).

#### 2.3.2 Motor threshold and corticomotor output

Transcranial magnetic stimulation (TMS) was delivered to the primary motor cortex using a Magstim 200 stimulator (Magstim Co. Ltd., Dyfed, UK) connected to a circular coil (90 mm diameter) and all procedures adhered to the TMS checklist for methodological quality (Chipchase et al., 2012). The coil was oriented to induce posterior–anterior current flow (left hemisphere: anticlockwise current direction; right hemisphere: clockwise current direction), and positioned over the optimal scalp position for ECRB (Groppa et al., 2012). This position was determined by systematic application of TMS to the scalp until the site that produced the largest motor evoked potential (MEP) was identified. A Brainsight neuronavigation system (Rogue Resolutions Ltd., Cardiff, UK) ensured accurate coil placement throughout each experiment. Resting and active motor threshold were determined at the optimal position via the maximum-likelihood protocol (Motor threshold Assessment Tool, version 2.0: http://www.clinicalresearcher.org/software.htm) (Awiszus, 2003). Resting motor threshold (RMT) was defined as the minimum stimulus intensity that evoked a MEP greater than 50 μV in the target muscle at rest (Rossini et al., 1994). Active motor threshold (AMT) was defined as the minimum stimulus intensity that evoked a MEP greater than 200 μV during isometric contraction of ECRB [10% maxi-

mum voluntary contraction (MVC)] (Rossini et al., 1994). Thirty MEPs were recorded from resting ECRB at 120% RMT to assess corticomotor output (rate of 1 pulse every 6 s). Trials containing muscle activity within 50 ms preceding the TMS pulse were discarded (Groppa et al., 2012).

#### 2.3.3 Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF)

A standard paired-pulse TMS protocol that consisted of a sub-threshold conditioning stimulus delivered 2 or 10 ms before a supra-threshold test stimulus, was used to evaluate SICI and ICF, respectively (Kujirai et al., 1993). Magnetic stimulation was delivered at the optimal scalp site to evoke a response in ECRB with the muscle at rest. Conditioning intensity was based on a percentage of AMT to decrease the likelihood of SICI and ICF measurements being influenced by other intracortical mechanisms, such as short-interval intracortical facilitation (sICF) (Ortu et al., 2008; Peurala et al., 2008). However, as individual variability for SICI thresholds is known to be high (Orth et al., 2003), testing SICI at a single conditioning intensity is unlikely to reflect the true maximum inhibition for all individuals. Thus, to account for between-subject variability to paired-pulse TMS (Boroojerdi et al., 2000; Maeda et al., 2002; Wassermann, 2002) and to investigate the stimulus–response profile of SICI and ICF, three conditioning intensities were used in this experiment. The intensities 70, 80, 90% of AMT were selected as these are known to elicit reliable SICI and ICF in healthy individuals (Ortu et al., 2008; Peurala et al., 2008). As MEPs from proximal upper limb muscles are typically smaller and less defined than those evoked from distal muscles (Chen et al., 1998; Groppa et al., 2012), test stimulus intensity was set to evoke a test MEP with a peak-to-peak amplitude of approximately 0.3–0.5 mV in all subjects (Perez and Cohen, 2008; Schwenkreis et al., 2011). Twelve trials were recorded in pseudorandom order at each interstimulus interval (ISI; 2 ms, 10 ms) and for the test stimulus alone (rate of 1 every 6 s; total of 36 trials per conditioning intensity).

#### 2.3.4 Long-interval intracortical inhibition (LICI)

Long-interval intracortical inhibition (LICI) was evaluated using a supra-threshold conditioning stimulus delivered 160 ms before a supra-threshold test stimulus (Claus et al., 1992). Stimulations were performed at the optimal scalp site to evoke a response in ECRB at rest. The test and conditioning intensities were equal and set to evoke a peak-to-peak response of approximately 0.3–0.5 mV in ECRB. Twelve trials were recorded in pseudorandom order at ISI 160 ms and for the test stimulus alone (rate of 1 every 6 s; total of 24 trials).

### 2.4 Experimental protocol

All experimental procedures were conducted in a single test session. Data collection was performed in the following order: (1) clinical outcome measures (LE participants only); (2) resting and active motor threshold; (3) corticomotor output (MEPs); (4) SICI and ICF (three conditioning intensities: 70, 80, 90% of AMT); and (5) LICI. TMS measures were repeated over both hemispheres for consenting participants. Rest intervals of at least 2 min were provided between each measure.

### 2.5 Data and statistical analysis

#### 2.5.1 Clinical measures of LE

Measures of PPT, maximum grip strength and pain-free grip strength were compared between sides (affected vs. unaffected arm) for individuals with LE. Pearson correlation coefficients were used to evaluate relationships between (1) pain (NRS) and disability (PRTEE), sensorimotor function (PPT, max grip strength, pain-free grip strength); and (2) SICI, ICF and LICI of the corresponding hemisphere.

#### 2.5.2 Neurophysiological measures

MEP data were measured as peak-to-peak amplitudes and averaged for each trial. Intracortical inhibition (SICI, LICI) and facilitation (ICF) were calculated by expressing the mean peak-to-peak conditioned MEP amplitudes as a percentage of the unconditioned (test) response.

All neurophysiological outcomes (RMT, AMT, MEP, SICI, ICF and LICI) were assessed for normality via the Shapiro–Wilk test, transformed if necessary, and compared between groups (LE vs. control) for each hemisphere using separate one-way ANOVAs. To account for the influence of the amplitude of the unconditioned (test) MEP on the magnitude of SICI, ICF and LICI (Chen et al., 1998; Udupa et al., 2010), test MEP amplitude was also compared between groups with one-way ANOVAs. One-way repeated measures ANOVA were used to compare contralateral and ipsilateral data within the LE group. *Post hoc* analyses were corrected for multiple

comparisons using the Holm–Sidak method. Statistical significance was set at $p < 0.05$. Data in text are expressed as mean ± standard deviation unless stated otherwise.

## 3. Results

### 3.1 Clinical measures of LE

Patient characteristics are summarized in Table 1. Average current pain intensity was 3.5 ± 2.8 on the NRS and the average combined score for the pain and function subscale of the PRTEE was 38.4 ± 19.0. Individuals with LE demonstrated reduced strength for the affected arm compared to the unaffected arm in the pain-free (affected: 18.6 ± 18.2 kg; unaffected: 32.1 ± 12.8 kg; main effect, $p = 0.004$) and maximal grip strength tasks; however, results for the latter failed to reach statistical significance (affected: 30.3 ± 15.0 kg; unaffected: 34.7 ± 11.2 kg; main effect, $p = 0.075$). Pressure pain thresholds were less for ECRB of the affected arm compared to the unaffected arm (affected: 17.8 ± 8.7 kg; unaffected: 21.4 ± 7.4 kg; main effect, $p = 0.017$), suggesting increased sensitivity to mechanical stimuli in the affected limb.

### 3.2 Neurophysiological measures

#### 3.2.1 TMS of the hemisphere contralateral to the 'affected' arm in LE and the matched hemisphere in healthy controls

There was no difference in resting motor threshold (LE: 48.6 ± 8.5; Control: 50.7 ± 10.4; main effect, $p = 0.57$), active motor threshold (LE: 45.6 ± 9.3; Control: 45.4 ± 8.9; main effect, $p = 0.97$) or MEP amplitude (LE: 0.30 ± 0.14 mV; Control: 0.53 ± 0.46 mV; main effect, $p = 0.15$) between individuals with LE and healthy controls.

The magnitude of SICI and ICF observed for ECRB of healthy controls was consistent with previous investigations of similar methodology (circular coil, 2 ms, 10 ms ISI) (Shimizu et al., 1999). SICI was less in individuals with LE compared to healthy controls at a conditioning intensity of 90% AMT (main effect, $p = 0.005$), but was similar between groups at conditioning intensities of 70% and 80% AMT (70%: main effect, $p = 0.24$; 80%: main effect, $p = 0.50$; Fig. 1A). Similarly, LICI was less in individuals with LE when compared with controls (main effect, $p = 0.046$; Fig. 2A). Intracortical facilitation was less in LE participants compared with healthy controls at a conditioning intensity of 80% AMT (main effect, $p = 0.026$; Fig. 1C), but not at 70% (main effect, $p = 0.95$) or 90% AMT (main effect, $p = 0.43$). Test MEP amplitudes were comparable between groups for LICI (main effect, $p = 0.30$) and for each SICI and ICF conditioning intensity (70% AMT: main effect, $p = 0.30$; 80% AMT: main effect, $p = 0.96$; 90% AMT: main effect, $p = 0.58$). The magnitude of SICI, ICF and LICI did not correlate with pain, disability or sensorimotor function in the affected arm (all $p > 0.16$).

#### 3.2.2 TMS of the hemisphere ipsilateral to the 'affected' arm in LE and the matched hemisphere in the healthy controls

Ten LE (3 men, aged 40.2 ± 11.8 years) and 10 control participants (3 men, aged 42.0 ± 12.3 years) consented to TMS of the ipsilateral hemisphere. Of

**Table 1** Demographic and clinical characteristics for individuals with lateral epicondylalgia ($n = 14$).

| Subject | Gender | Age (years) | Symptom duration (months) | Dominant arm | Affected arm | PRTEE (/100) | NRS (/10) |
|---|---|---|---|---|---|---|---|
| 1 | F | 52 | 288 | R | R | 35 | 2 |
| 2 | F | 38 | 3 | R | R | 79 | 7 |
| 3 | M | 44 | 12 | R | R | 14 | 0.5 |
| 4 | F | 45 | 7 | R | R | 45 | 6 |
| 5 | F | 48 | 7 | R | R | 31.5 | 2 |
| 6 | F | 43 | 3 | R | R | 17 | 0 |
| 7 | F | 50 | 24 | L | R | 24 | 0.5 |
| 8 | M | 46 | 60 | R | R | 22 | 2 |
| 9 | F | 45 | 3 | R | R | 67 | 7.5 |
| 10 | M | 20 | 3 | R | R | 32.5 | 1 |
| 11 | M | 37 | 4 | R | R | 34.5 | 6 |
| 12 | F | 21 | 24 | R | L | 56 | 7 |
| 13 | F | 50 | 60 | R | L | 30 | 3 |
| 14 | F | 42 | 24 | R | R | 50.5 | 5 |

PRTEE, patient rated tennis elbow evaluation (pain and disability); NRS, numerical rating scale.

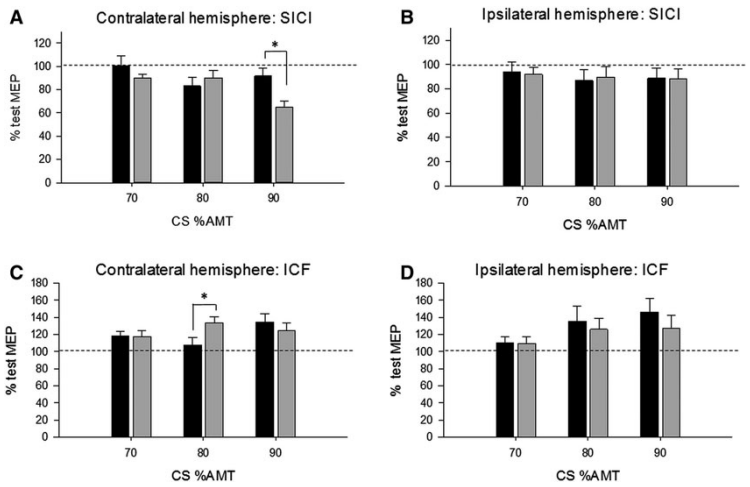


**Figure 1** Group data (mean ± standard error) for SICI and ICF over the hemisphere contralateral (LE, $n = 14$; control, $n = 14$) and ipsilateral (LE, $n = 10$; control, $n = 10$) to the affected arm of LE participants (black bars) and the matched arm of healthy controls (grey bars). Trials were performed using conditioning stimulus (CS) intensities 70%, 80% and 90% of AMT. SICI and ICF were determined by expressing the conditioned MEP as a percentage of the unconditioned test MEP (percentage of test MEP). LE participants displayed less SICI (less inhibition, higher percentage of the test MEP) for the contralateral hemisphere compared to healthy controls at CS 90% AMT (A). There was no difference in SICI between groups for the ipsilateral hemisphere (B). LE participants displayed less ICF (less facilitation, lower percentage of the test MEP) for the contralateral hemisphere compared to healthy controls at CS 80% AMT (C). There was no difference in ICF between groups for the ipsilateral hemisphere (D). *$p < 0.05$. AMT, active motor threshold; ICF, intracortical facilitation; LE, lateral epicondylalgia; MEP, motor evoked potential; SICI, short-interval intracortical inhibition.

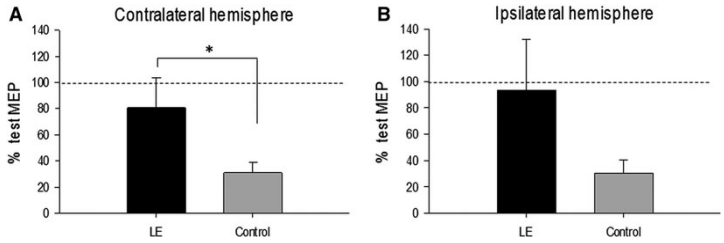


**Figure 2** Group data (mean ± standard error) for LICI over the hemisphere contralateral (LE, $n = 14$; control, $n = 14$) and ipsilateral (LE, $n = 10$; control, $n = 10$) to the affected arm of LE participants (black bars) and the matched arm of healthy controls (grey bars). LICI was determined by expressing the conditioned MEP as a percentage of the unconditioned test MEP (percentage of test MEP). LE participants displayed less LICI (less inhibition, higher percentage of the test MEP) for the contralateral hemisphere compared to healthy controls (A), however no difference was detected between groups for the ipsilateral hemisphere (B). *$p < 0.05$. LE, lateral epicondylalgia; LICI, long-interval intracortical inhibition; MEP, motor evoked potential.

the participants who chose to withdraw from this part of the study, all cited poor tolerability to TMS as the reason for their decision. Similar to data obtained from the contralateral hemisphere, there was no difference in resting motor threshold (LE: 47.8 ± 10.7; Control: 49.5 ± 13.7; main effect, $p = 0.76$), active motor threshold (LE: 45.6 ± 10.0; Control: 46.0 ± 9.9; main effect, $p = 0.93$) or MEP amplitude (LE: 0.28 ± 0.14 mV; Control: 0.33 ± 0.22 mV; main effect, $p = 0.54$) between groups.

The magnitude of SICI was similar between those with and without LE under all test conditions (70%: main effect, $p = 0.91$; 80%: main effect, $p = 0.95$; 90%: main effect $p = 0.89$; Fig. 1B), and this was the same for measures of LICI (main effect, $p = 0.14$; Fig. 2B) and ICF (70% AMT: main effect, $p = 0.99$; 80%: main effect, $p = 0.71$; 90%: main effect, $p = 0.43$; Fig. 1D). The amplitude of the test MEP was comparable between groups for LICI (main effect, $p = 0.15$) and for each SICI and ICF conditioning

intensity (70% AMT: main effect, $p = 0.37$; 80% AMT: main effect, $p = 0.07$; 90% AMT: main effect, $p = 0.19$).

#### 3.2.3 Comparison of TMS of the hemisphere contralateral and ipsilateral to the 'affected' arm in LE

There was no difference in resting motor threshold ($p = 0.78$), active motor threshold ($p = 0.47$) or MEP amplitude ($p = 0.50$) between the motor representations of ECRB of the affected or unaffected arm for individuals with LE consenting to TMS of both hemispheres ($n = 10$). Similarly, there was no difference in SICI (70%: $p = 0.21$, 80%: $p = 0.99$, 90%: $p = 0.80$) or ICF (70%: $p = 0.40$, 80%: $p = 0.15$, 90%: $p = 0.16$) at any conditioning intensity. LICI was also comparable between hemispheres ($p = 0.86$) as were test MEP amplitudes for the paired-pulse protocols (SICI/ICF 70%: $p = 0.93$, 80%: $p = 0.11$, 90%: $p = 0.68$; LICI: $p = 0.81$).

## 4. Discussion

This study is the first to investigate M1 intracortical networks in individuals with chronic LE. We demonstrate less intracortical inhibition mediated by both $GABA_A$ and $GABA_B$ receptors, and less intracortical facilitation, in the M1 contralateral to the affected ECRB in individuals with LE compared with healthy controls. Similar changes were not present in the M1 contralateral to the unaffected ECRB. These changes may provide the substrate for altered M1 organization in chronic LE and could provide a target for future therapy.

Individuals with LE displayed on average, 27% less inhibition in networks mediated by $GABA_A$ (SICI) and 50% less inhibition in networks mediated by $GABA_B$ (LICI) for the motor representation of the affected ECRB muscle compared with healthy controls. These data are suggestive of cortical disinhibition (shift towards greater excitability) in LE. However, individuals with LE also displayed 26% less ICF than healthy controls, suggesting that ICI and ICF are differentially affected by the presence of chronic elbow pain. This finding is not surprising given that ICI and ICF are mediated by different receptor systems and are thought to act independently (Ziemann et al., 1996a, 1998; Werhahn et al., 1999; Ilic et al., 2002; McDonnell et al., 2006; Di Lazzaro et al., 2007). Indeed, despite similar conditioning requirements, ICI and ICF are differentially affected by current direction and intensity (Ziemann et al., 1996b), and evoke different patterns of cerebral blood flow (Strafella and Paus, 2001).

Previous studies investigating intracortical networks in chronic musculoskeletal pain have produced mixed findings. Although some studies report less SICI in fibromyalgia (Mhalla et al., 2010; Schwenkreis et al., 2011), low back pain (Masse-Alarie et al., 2012) and complex regional pain syndrome (Schwenkreis et al., 2003; Eisenberg et al., 2005) compared with healthy controls, others report normal levels of inhibition (Salerno et al., 2000). Similarly, less ICF has been demonstrated in fibromyalgia (Salerno et al., 2000; Mhalla et al., 2010) compared with controls, whereas studies of people with osteoarthritis and complex regional pain syndrome report no difference (Schwenkreis et al., 2003, 2010; Eisenberg et al., 2005). Only one study has examined the effect of musculoskeletal pain on LICI. That study reported less inhibition in individuals with fibromyalgia than controls (Salerno et al., 2000). Since M1 disinhibition has been demonstrated in conditions characterized by pain of neuropathic (neuralgia), but not nociceptive (osteoarthritis) origin (Schwenkreis et al., 2010), it has been suggested that discrepancies between studies might reflect differences in the pathological process of different chronic conditions. Alternatively, it is possible that symptom duration is a significant determinant of the direction and extent of M1 cortical change. Indeed, although there is currently little data detailing the progression of cortical change during the transition from the acute to the chronic pain state, there is evidence to suggest that the direction of these effects differ for acute experimental pain (e.g. SICI increased), compared to clinical chronic pain (e.g. SICI decreased or unchanged) (Schabrun and Hodges, 2012). In addition to differences in patient characteristics, discrepancies between studies in identical pain conditions may also relate to methodological factors. For example, in fibromyalgia, ICI is reduced when TMS pulses are separated by 2 ms (Mhalla et al., 2010; Schwenkreis et al., 2011), but not at intervals of 4 ms (Salerno et al., 2000) and in the present study, between-group differences were only detected using a conditioning intensity of 90% active motor threshold for SICI and 80% active motor threshold for ICF. These data indicate that studies investigating M1 intracortical networks should use a range of conditioning stimulus intensities and interstimulus intervals.

The conditioning stimulus intensity required to observe differences between people with LE and healthy controls provides further information on the

integrity of intracortical networks in this condition. Data from healthy individuals indicate that SICI is strongest at a conditioning intensity of 90% active motor threshold and ICF is strongest at a conditioning intensity of 80% active motor threshold (Ortu et al., 2008). However, individuals with LE displayed the strongest SICI and ICF at 80% and 90% active motor threshold, respectively. As the threshold for evoking ICI/ICF is hypothesized to reflect the threshold for stimulating axons belonging to GABAergic and glutamatergic interneurons, respectively (Ziemann et al., 1998; Ilic et al., 2002; Orth et al., 2003), these findings suggest that the electrophysiological properties of circuits involving these populations may be altered in LE (Di Lazzaro et al., 1998, 2006; Hanajima et al., 1998). Changes to the structure of these circuits, such as the proximity of each population to the stimulating coil and the orientation of their axons with respect to the induced current, may also contribute to altered ICI/ICF thresholds in this condition (Orth et al., 2003).

The results of the present study provide insight into mechanisms that may contribute to the development of altered M1 organization and motor dysfunction in LE. Rapid reorganization of M1 is thought to depend on changes in synaptic efficacy that rely on GABAergic disinhibition and NMDA receptor-dependent long-term potentiation-like mechanisms (Liepert et al., 1998a). Altered function of intracortical networks is therefore a plausible mechanism to explain increased map volume, greater MEP amplitude and less separation in the cortical representations of the ED and ECRB muscles in LE (Schabrun et al., 2014). In addition, a key function of intracortical networks is to facilitate contraction of muscles required for a motor task while preventing unwanted movements, muscle overflow and co-contraction of surrounding muscles (Liepert et al., 1998b). Motor dysfunction characterized by altered muscle synergies between ECRB and other extensor/flexor muscles of the wrist (Alizadehkhaiyat et al., 2007), adoption of a flexed wrist posture (Bisset et al., 2006), diminished ability to generate force (De Smet and Fabry, 1997; Slater et al., 2005) and reduced fine motor performance (Skinner and Curwin, 2007) have been reported in LE. Finally, LE is common in people who perform manual tasks with repeated, rapid movements of the wrist and forearm (Fan et al., 2009; Descatha et al., 2013) and repetitive movement training has been shown to reduce expression of SICI and ICF (Nordstrom and Butler, 2002; Cirillo et al., 2011). One possibility is that repetitive movements drive intracortical changes (reduced ICI and reduced ICF as observed in the present study) leading to altered M1 organization, motor dysfunction and pain persistence in LE. However, as this study was exploratory in nature and not designed to determine causality, further work using longitudinal study designs are needed to confirm this hypothesis. Studies investigating larger cohorts of participants and studies including individuals in the acute (<6 weeks) and chronic (>3 months) stage of disease are required to determine the time-course of altered cortical organization and function in LE.

We did not observe a relationship between ICI/ICF and measures of pain or disability. However, previous studies of M1 organization in LE have shown a relationship between the degree of cortical reorganization (specifically the degree of overlap between the representations of ED and ECRB) and pain severity in the last 6 months (Schabrun et al., 2014). It is conceivable that any relationship between ICI/ICF and outcomes of pain and disability is non-linear, and depends on the relative contribution of changes in ICI and ICF to M1 organization. This hypothesis would explain why features of altered M1 organization are associated with pain severity in LE while intracortical mechanisms are not. Furthermore, although SICI has been shown to correlate with pain measures in myofascial pain syndrome (Volz et al., 2013) and complex regional pain syndrome (Schwenkreis et al., 2003), similar findings have not been reported for other conditions such as low back pain or fibromyalgia (Mhalla et al., 2010; Schwenkreis et al., 2011; Masse-Alarie et al., 2012). Despite these findings, it remains possible that therapies that normalize activity in intracortical networks could have a role in the treatment of LE and other chronic pain conditions by preventing maladaptive reorganization of M1. Future studies should seek to investigate whether therapies capable of targeting intracortical networks, such as repetitive TMS, are of benefit in persistent LE and whether normalization of these networks is associated with improved M1 organization.

Finally, there are several limitations of the present study that should be acknowledged. First, it is unclear whether the present findings are typical of the general LE population as data were collected from a small sample of convenience. Studies involving a larger number of participants are required to confirm our results and to further examine potential relationships between neurophysiological outcomes and clinical characteristics. Larger trials would also be better able to control for confounding variables

such as 'handedness' – a factor not accounted for in the in the present study due to the small proportion of LE participants ($n$ = 3) experiencing symptoms in the non-dominant arm. Second, since we did not assess muscle representations other than ECRB, it is not clear whether the observed changes are restricted to the 'painful' muscle. Indeed, since the M1 representation of a muscle adjacent to ECRB (extensor digitorum) has been found to be similarly altered in LE in a previous study (Schabrun et al., 2014), it may be anticipated that altered inhibitory and/or facilitatory network activity may extend to other local muscles. Further research is required to determine whether M1 cortical change is confined to muscles located within the immediate vicinity of pain in LE or whether these effects also extend to muscles distal and/or proximal to ECRB.

## 5. Conclusion

This study is the first to provide evidence of reduced intracortical activity mediated by $GABA_A$ (SICI), $GABA_B$ (LICI) and NMDA (ICF) receptors in individuals with chronic LE. We hypothesize that these mechanisms may drive altered M1 organization and aspects of motor dysfunction in this condition. However, longitudinal trials on larger subject numbers are required to confirm this relationship. If confirmed, therapies that restore intracortical function may have the potential to normalize cortical abnormalities and improve outcomes in LE.

### Author contributions

E.B., L.C. and S.S. designed the study. E.B. collected and analysed the data, interpreted the results and drafted the manuscript. E.B., L.C. and S.S. discussed the results, commented on the manuscript and approved the final version.

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# Appendix D

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ORIGINAL ARTICLE



## Temporal and spatial characteristics of post-silent period electromyographic bursting in low back muscles: comparison between persons with and without low back pain

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**ABSTRACT**

**Purpose/aim:** Recently, a novel measure of cortical disinhibition was identified using transcranial magnetic stimulation (TMS). This measure, described as post-silent period electromyographic (EMG) bursting, may inform on the corticomotor control of movement in health and disease; however, it has not been investigated for muscles outside the hand or in musculoskeletal conditions. Thus, the aim of this study was to investigate the temporal and spatial characteristics of "EMG bursting" in individuals with and without low back pain (LBP).

**Materials and Methods:** TMS was used to map the motor cortical representation of paraspinal muscles in 11 individuals with LBP and 11 pain-free controls. The latency, duration and magnitude of bursting, number of active burst sites, map volume and coordinates of the burst "hotspot" were compared between the groups.

**Results:** In pain-free controls, the latency, duration and magnitude of bursts were similar to the hand; however, bursts occurred earlier and were of smaller magnitude in LBP. Bursting was widespread throughout the cortical representation in both groups; however, there was a trend towards smaller mean EMG burst and map volume in LBP.

**Conclusions:** We confirm the presence of EMG bursting in back muscles and provide a description of the spatial profile of this mechanism. Our observations in LBP suggest that cortical disinhibition may be altered in this condition.

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Transcranial magnetic stimulation; low back pain; cortical disinhibition; primary motor cortex; brain mapping

### Introduction

Transcranial magnetic stimulation (TMS) can be used to assess features of the corticomotor control of movement in health and disease. In particular, paired pulse paradigms have been used to investigate inhibitory activity mediated by gamma-aminobutyric acid (GABA) in healthy individuals and in a range of pathological conditions. In low back pain (LBP), for example, there is evidence of reduced inhibition in networks mediated by $GABA_A$ receptors, suggesting changes in these networks might be involved in this condition. Recently, a novel single-pulse measure of intracortical activity has been identified. This response, described as post-silent period "electromyographic (EMG) bursting", has been hypothesised to reflect the activation of $GABA_B$ receptors on inhibitory interneurons and represent a measure of corticomotor disinhibition [1]. However, despite recurrent observations [2–5], few studies have specifically examined this measure in healthy individuals and there have been no studies in musculoskeletal pain conditions.

EMG burst responses following the cortical silent period have been demonstrated following magnetic stimulation of the cortical territory devoted to the hand [1,5]. In a recent investigation, TMS delivered over the motor hotspot during volitional contraction consistently evoked transient (~60 ms) but distinct bursts of muscle activity up to three times the amplitude of the background EMG. A GABAergic origin for this response was proposed since bursts were the largest following longer cortical silent periods ($GABA_B$-mediated) and occurred at latencies corresponding with a known period of reduced corticomotor inhibition (also $GABA_B$-mediated) [6]. Hence, measurement of EMG bursting may provide further insight into cortical processes involving GABA-mediated networks in pathological conditions such as LBP. However, the temporal characteristics and the cortical distribution of EMG bursts have yet to be investigated for muscles outside the hand. It is also unknown whether EMG bursting differs between healthy individuals and those with musculoskeletal pain. Thus, the aims of the present study were to (1) confirm the presence of post-

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silent period EMG bursting in low back muscles and (2) examine the spatial and temporal profile of EMG bursting using TMS mapping, in persons with and without LBP. We hypothesised that the examination of burst characteristics in individuals with LBP would reveal further evidence of reduced cortical inhibition in this condition.

## Methods

### Participants

Eleven right-handed individuals with a history of recurring episodes of non-specific LBP (6 males, aged 29 ± 7 years) and 11 age- and gender-matched pain-free controls (6 males, 27 ± 5 years) participated. Individuals with LBP were recruited during an active episode of LBP (with or without buttock pain). To be eligible for inclusion, the average pain intensity was required to be greater than 3 on an 11-point numerical rating scale (NRS) anchored with "no pain" at 0 and "worst pain imaginable" at 10, and of sufficient intensity to interfere with at least three important activities of daily living (assessed via a Patient-Specific Functional Scale) [7]. All participants completed a TMS safety screening questionnaire and were excluded from enrolment if they had a personal or family history of epilepsy, major neurological, respiratory, orthopaedic or circulatory disorders, if they were pregnant, had metal in their head or jaw or were taking central-nervous-system-acting medications [8]. Additional exclusion criteria for LBP participants included previous spinal surgery, the use of analgesic or anti-inflammatory medication in the last month or the receipt of treatment from a health professional in the last month. The study was approved by the institutional human medical research ethical committee and performed in accordance with the Declaration of Helsinki. All participants provided written, informed consent.

### Electromyography (EMG)

Surface electromyography (EMG) was recorded bilaterally from the paraspinal muscles 3 cm lateral to the spinous process of L3 and 1 cm lateral to the spinous process of L5 via dual silver–silver chloride disposable electrodes (spacing 2.0 cm, Noraxon USA Inc, Scottsdale, AZ, USA). These sites record EMG from deep and superficial back muscles including the multifidus and erector spinae [9]. Ground electrodes were positioned over the anterior superior iliac spine of the same side. EMG signals were amplified x1000 (NL844, Digitimer Ltd., Welwyn Garden City, UK), band-pass filtered: 20–1000 Hz and sampled at 2000 Hz using a Micro1401 data acquisition system and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

### Transcranial magnetic stimulation (TMS)

TMS mapping of the cortical representation of the lumbar paraspinal muscles was performed according to the procedures outlined previously [10–12]. In brief, TMS was delivered to the primary motor cortex contralateral to the side of worst pain (or the matched side for pain-free controls) using a Magstim 200 stimulator (Magstim Co. Ltd., Dyfed, UK) connected to a figure-of-eight coil (70 mm wing diameter), oriented with the handle facing posteriorly with respect to the midline. The location of the vertex (Cz) was determined using the 10/20 International EEG Electrode Placement system and registered using a Brainsight neuronavigation system (Rogue Research Inc., Quebec, Canada). Starting at the vertex, five magnetic stimuli were delivered at 1-cm intervals on a 6 cm × 7 cm grid with the aid of the neuronavigation instrument. The stimuli were applied at 100% of the stimulation output with an inter-stimulus interval of 6 s, while participants performed a low-level voluntary contraction (20% maximum) of the paraspinal muscles. The target amplitude was determined based on the largest root mean square (RMS) EMG achieved during three 3-s maximal trunk extension efforts performed against manual resistance in sitting [11]. During testing, the participants maintained the appropriate level of muscle contraction by sitting forward with the back straight [11,13]. Visual feedback was provided on a computer monitor to ensure symmetrical pre-activation. To ensure that the prolonged sitting and high TMS stimulator output required during the mapping procedure did not exacerbate LBP symptoms, the pain severity was monitored verbally throughout, and evaluated on completion of TMS mapping using an 11-point numerical rating scale (NRS). All procedures adhered to the TMS checklist for methodological quality [14].

### Data analyses

Analysis of TMS map data was performed using MATLAB 7 (The Mathsworks, Natick, MA). EMG was full-wave-rectified and trials (five) at each scalp site were averaged. Five parameters were extracted from these data: (1) motor-evoked potential (MEP) amplitude, (2) cortical silent period, (3) percentage burst ratio (PBR), (4) burst duration and (5) burst silent period. To account for pre-activation, the MEP amplitude (uV) was calculated by subtracting the RMS EMG recorded 55–5 ms prior to stimulation (background EMG) from the RMS EMG between MEP onset and offset [15–18]. The duration of the cortical silent period (ms) was determined as the time between MEP offset and the resumption of EMG equivalent to or greater than that present pre-stimulus. If a clear burst in EMG activity was identified following

the cortical silent period, the RMS of the burst was calculated by manually cursoring burst onset and offset. Where a burst was not apparent in a trial, but could be identified in other trials within the set of five, the mean of those bursts was used to calculate the RMS EMG for that map site [1]. If no bursts were identified within the set of five trials, the RMS EMG for that map site was calculated from the background EMG 0–50 ms after the cortical silent period. The PBR (%) was calculated by expressing the mean burst RMS EMG as a percentage of the mean background EMG [1]. The duration of the burst silent period (ms) was determined to be the period between burst offset and the resumption of RMS EMG equivalent to or greater than that observed pre-stimulus. To examine the spatial profile of EMG bursting in M1, the PBR was superimposed over the respective scalp sites to generate a "PBR map" for each participant. The number of active burst sites, the mean PBR of the map (%), the PBR map volume, and the anterior–posterior and the medial–lateral location (cm) of the largest MEP (the "motor hotspot") and the PBR ("burst hotspot") were identified for each map. For a map site to be considered "active", at least one trial out of five was required to display the evidence of EMG bursting. The mean PBR of the map (%) and the PBR map volume were calculated as the average or the sum of the PBRs recorded at each active site, respectively.

### *Statistical analyses*

Data for MEP amplitude, cortical silent period, PBR, map volume, burst duration and burst silent period were assessed for normality via the Shapiro–Wilk test and compared between the groups (Control vs. LBP) using separate one-way analyses of variance (ANOVA). To ensure that pre-activation during TMS testing did not aggravate pain in the LBP group, pain intensity before and after TMS were compared using one-way repeated measures ANOVA. Separate two-way ANOVA was used to compare map hotspots (MEP vs. PBR) between the groups (Control vs. LBP) in the anterior–posterior and the medial–lateral directions. *Post hoc* analyses were corrected for multiple comparisons using the Holm–Sidak method. Eta-squared was calculated as a measure of the effect size for each outcome. Pearson correlation analyses were performed to examine the relationship between (1) the cortical silent period duration and PBR in both groups and (2) the pain severity and neurophysiological outcomes in LBP. Statistical significance was set at $P < 0.05$. Data in text are expressed as mean ± standard deviation unless stated otherwise.

## Results

As there was no difference between the responses at L3 and L5 within the LBP or pain-free groups ($P > 0.42$), data generated from both recording sites were pooled and compared between the groups. Normative data and data from individuals with LBP are presented in Table 1.

### *EMG bursting is present in the low back muscles of pain-free individuals*

#### *Burst characteristics of the map in pain-free individuals*

The present data indicate that EMG bursting can be elicited by stimulating multiple scalp sites overlying the cortical territory of the low back muscles. Example traces of EMG bursting are presented in Figure 1 and average PBR maps are presented in Figure 2. In pain-free controls, approximately 92% of map sites showed evidence of EMG bursting. In this group, the largest burst was more than double the amplitude of the background RMS EMG (PBR ~229%) and was located 1.3 ± 1.8 cm anterior and 1.7 ± 1.7 cm lateral to Cz. The mean PBR of the map was 140% ± 13% and the map volume was 5406% ± 734%.

#### *Burst characteristics at the motor hotspot in pain-free individuals*

At the motor hotspot, normative data indicated that the burst amplitude was approximately 1.5 times larger than that of the background RMS EMG. On average, the burst activity commenced 123 ± 54 ms following magnetic stimulation and lasted 57 ± 33 ms. This was immediately followed by a period of EMG silence in 10 out of 11 control participants (Figure 1(b)). In these participants, the duration of the burst silent period was 54 ± 34 ms. A positive correlation between the PBR and the duration of the cortical silent period was detected at the motor hotspot ($r = 0.54$, $P = 0.0096$).

**Table 1.** Group data (mean ± SD) for controls and individuals with LBP.

| | Control ($n = 11$) | LBP ($n = 11$) |
|---|---|---|
| Active burst sites ($n$/42) | 38.6 ± 3.5 | 36.5 ± 7.6 |
| Map volume (%) | 5406 ± 734 | 4794 ± 1327 |
| Mean PBR of map (%) | 140 ± 13 | 131 ± 18 |
| Max PBR of map (%) | 229 ± 59 | 209 ± 77 |
| MEP at motor hotspot (mV) | 0.019 ± 0.013 | 0.010 ± 0.009* |
| Cortical silent period at motor hotspot (ms) | 81.4 ± 49.3 | 53.7 ± 21.3* |
| PBR at motor hotspot (%) | 150 ± 62 | 117 ± 46* |
| Burst onset at motor hotspot (ms) | 123 ± 54 | 88 ± 23* |
| Burst duration at motor hotspot (ms) | 57.0 ± 33.6 | 61.2 ± 25.4 |
| Burst silent period duration at motor hotspot (ms) | 54.3 ± 34.2 | 54.7 ± 33.1 |

*$P < 0.05$.

**Abbreviations:** PBR: Percentage burst ratio; MEP: motor-evoked potential.

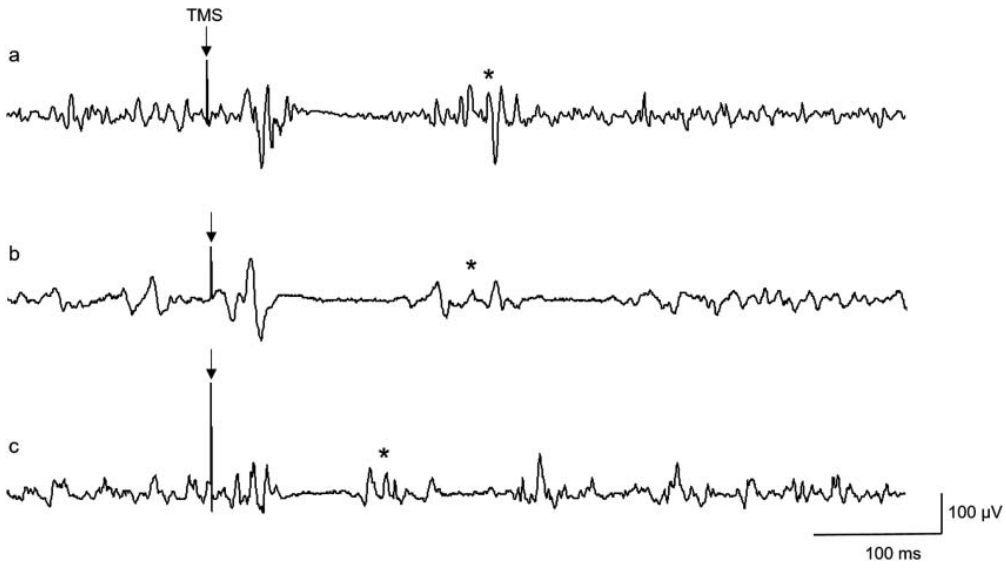


**Figure 1.** Representative waveforms demonstrating EMG bursting in the lumbar paraspinal muscles of two pain-free subjects (a,b) and one LBP subject (c) during sustained 20% maximum voluntary contraction (MVC). Note that two types of responses were typically observed: (a) the cortical silent period is terminated by a burst of EMG activity, or (b,c) the cortical silent period is interrupted by a burst of EMG activity. The MEP amplitudes were typically smaller and bursts occurred earlier in persons with LBP compared to controls. * indicates the position of the burst.

### *Burst characteristics differ for individuals with low back pain compared to pain-free controls*

Patient characteristics are summarised in Table 2. The average pain intensity on the day of testing was 4.0 ± 2.0 on the NRS and the time elapsed since the first pain episode was 56 ± 40 months. The procedure of TMS mapping did not alter the pain intensity ($F_{(1,10)}$ = 2.41, $P$ = 0.15, $\eta^2$ = 0.028). At the motor hotspot, EMG bursts occurred earlier ($F_{(1,42)}$ = 7.62, $P$ = 0.009, $\eta^2$ = 0.15) and were of smaller magnitude in individuals with LBP compared to controls ($F_{(1,42)}$ = 4.09, $P$ = 0.050, $\eta^2$ = 0.091). There was, however, no detectable correlation between pain severity and PBR ($r$ = 0.27, $P$ = 0.22) or pain severity and burst onset ($r$ = 0.26, $P$ = 0.24). Other characteristics including the duration of the burst ($F_{(1,42)}$ = 0.23, $P$ = 0.64, $\eta^2$ = 0.005) and the burst silent period did not differ ($F_{(1,30)}$ = 0.001, $P$ = 0.97, $\eta^2 < 0.001$) between the groups. The number of active burst sites was also similar for

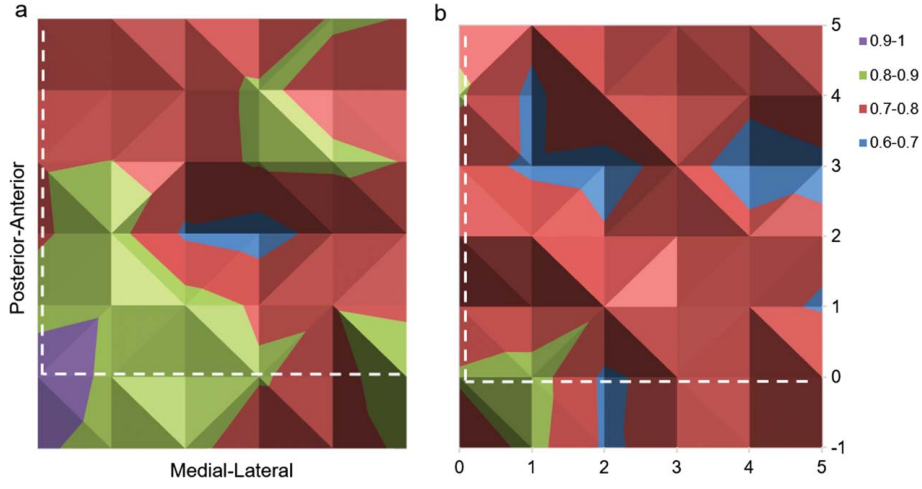


**Figure 2.** Averaged and normalised PBR maps obtained from the lumbar paraspinal muscles of (a) pain-free individuals and (b) individuals with LBP. The horizontal dashed line represents the inter-aural line and the vertical dashed line represents the line from the nasion to the inion. Cz is located at the coordinate (0,0). Note the difference in the magnitude and distribution of EMG bursting between the groups.

**Table 2.** Demographic and clinical characteristics for individuals with LBP ($n$ = 11).

| Subject | Gender | Age (years) | Side of pain | Current pain (NRS/10) | Pain duration (months) |
|---|---|---|---|---|---|
| 1 | F | 27 | L | 1 | 84 |
| 2 | M | 36 | R | 7.5 | 60 |
| 3 | M | 39 | R | 4 | 100 |
| 4 | F | 19 | R | 2 | 60 |
| 5 | M | 27 | R | 6 | 18 |
| 6 | M | 41 | L | 3 | 60 |
| 7 | F | 31 | R | 5 | 6 |
| 8 | M | 31 | R | 6 | 108 |
| 9 | M | 24 | L | 5 | 6 |
| 10 | F | 27 | L | 2 | 100 |
| 11 | F | 19 | R | 3 | 17 |

**Abbreviation:** NRS: numerical rating scale.

individuals with and without LBP ($F_{(1,42)}$ = 1.43, $P$ = 0.24, $\eta^2$ = 0.033; Figure 2(a,b)), as was the maximum PBR of the map ($F_{(1,42)}$ = 1.03, $P$ = 0.32, $\eta^2$ = 0.024). A trend towards smaller mean PBR and map volume was detected for LBP but failed to reach significance (mean: $F_{(1,42)}$ = 3.48, $P$ = 0.069, $\eta^2$ = 0.076; volume: $F_{(1,42)}$ = 3.58, $P$ = 0.065, $\eta^2$ = 0.079). The PBR hotspot was located 1.2 ± 1.7 cm anterior and 1.8 ± 1.7 cm lateral to Cz. Two-way ANOVA revealed that the burst hotspot was located significantly closer to the midline compared to the MEP hotspot in both groups (Figure 3; *post hoc*: $P$ = 0.007), however, did not identify an interaction between groups (medial–lateral: $F_{(1,84)}$ = 0.38, $P$ = 0.54, $\eta^2$ = 0.004).

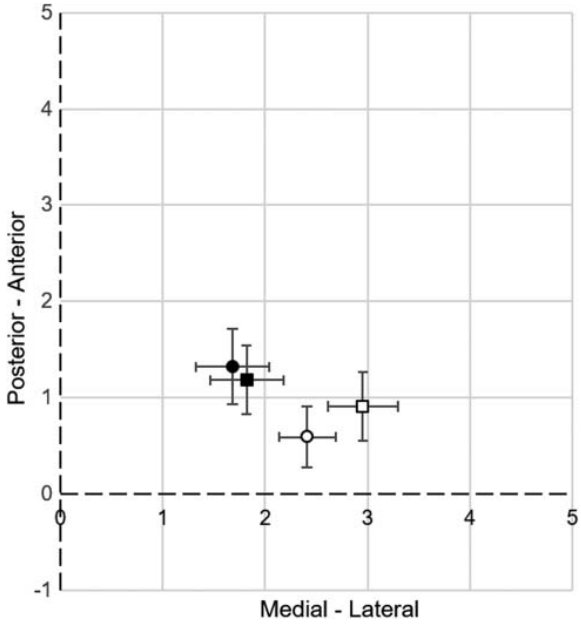


**Figure 3.** Topographical location (mean ± standard error) of MEP hotspot (open markers) and PBR hotspot (filled markers) for LBP (squares) and pain-free controls (circles). The horizontal dashed line represents the inter-aural line and the vertical dashed line represents the line from the nasion to the inion. Cz is located at the coordinate (0,0). Each grid square represents 1 cm × 1 cm. Note that the PBR hotspot is positioned medially to the MEP hotspot in both groups.

### *MEP amplitude and the duration of the cortical silent period are less in LBP compared to pain-free controls*

At the motor hotspot, MEP amplitudes were smaller ($F_{(1,42)}$ = 5.90, $P$ = 0.019, $\eta^2$ = 0.12) and the cortical silent period was shorter ($F_{(1,42)}$ = 5.87, $P$ = 0.02, $\eta^2$ = 0.12) for individuals with LBP compared to controls. In contrast to pain-free controls, there was no correlation between PBR and cortical silent period duration at this site for individuals with LBP ($r$ = 0.21, $P$ = 0.34). There was also no significant correlation between pain severity and MEP amplitude ($r$ = −0.20, $P$ = 0.37) or pain severity and cortical silent period duration ($r$ = 0.074, $P$ = 0.75) in this group.

## Discussion

The results of the present study confirm the presence of post-silent period EMG bursting in muscles of the low back. We also provide a description of the spatial distribution of bursting in M1, and the first account of differences between healthy individuals and those with musculoskeletal pain.

### *Spatial and temporal characteristics of EMG bursting in the back*

This study utilised TMS mapping techniques to examine the spatial profile of post-silent period EMG bursting in M1. Our findings show that, like MEPs, EMG bursts may be elicited from a number of scalp sites overlying the cortical representation of the lumbar paraspinal muscles in individuals with and without LBP. While the magnitude of bursting varied across sites, we found the largest responses to be distributed within the posterior half of the map. Here, we observed a clear peak in PBR approximately 2 cm medial to the location of the motor hotspot (Figure 3). In pain-free individuals, PBR at the burst hotspot was 53% greater than that recorded at the motor hotspot. Interestingly, this difference was more pronounced in LBP (~77%). Differences in PBR at the burst and motor hotspots could be evidence that the burst and MEP responses are generated by independent cortical networks and may explain previous accounts of bursting in the absence of an evoked potential [2,5]. These findings also suggest that the motor hotspot may not be the optimal location to assess EMG bursting, particularly in patient populations.

In pain-free individuals, EMG bursts at the motor hotspot demonstrated a similar latency, duration and magnitude to those reported in the hand under similar test conditions [1]. Similarly, a positive correlation between the cortical silent period and PBR was detected in the present study. Taken together, these findings suggest that the burst characteristics may be similar across the distal and axial muscles. If confirmed, responses recorded from the hand could be used to provide a global estimate of EMG bursting in M1. This method would be particularly advantageous in patient studies where the cortical representation of the affected muscle is positioned deep in the cortex and is difficult to target with TMS (e.g. low back or leg muscles) or where volitional contraction aggravates pain. However, as we only investigated EMG bursting at the site of pain in LBP, further work is needed to determine whether the burst characteristics are identical for painful and pain-free muscles in this and other musculoskeletal pain conditions.

By confirming the latency and duration of the burst and its affiliation with the cortical silent period, our data further support the hypothesis that the post-silent period EMG bursting represents a measure of the depth and magnitude of disinhibition in M1 [1]. As was the case in the hand, the latency (~120 ms) and duration (~50 ms) of bursting in the back muscles are consistent with that of a recently described period of late cortical disinhibition [6]. In that study, a period of raised corticomotor excitability was noted following the evocation of a form of $GABA_B$-mediated inhibition known as long-interval intracortical inhibition (LICI). This effect was attributed to the action of pre-synaptic $GABA_B$ receptors which function to negatively regulate GABA release and facilitate excitatory post-synaptic potentials [19,20]. This, taken together with the evidence linking the EMG bursts with the cortical silent period (also $GABA_B$-mediated), suggests that this mechanism may reflect the depth and magnitude of disinhibition in M1. However, as LICI and late cortical disinhibition have yet to be investigated in muscles of the trunk, further work is necessary to determine whether these mechanisms are present in the low back and whether this hypothesis also holds for our results.

A novel outcome of the present study was identification of the "burst silent period". While previous studies show the evidence of EMG bursts terminating the cortical silent period or trains of bursts interspersed with low-level EMG in a proportion of test subjects, the EMG bursts in the present study were followed by a clear period of EMG silence in 20 of 22 participants [1,2]. In pain-free individuals, this episode appeared 200 ms post-stimulus and persisted for ~50 ms. Since cortical silent periods longer than 85 ms have yet to be demonstrated in muscles of the low back [2,16], we suggest that this response represents a separate entity, rather than a continuation of the cortical silent period. It is possible that this "burst silent period" may signify a return of post-synaptic GABAergic activity following the conclusion of the EMG burst; however, further work is necessary to establish the causation and identify the mechanisms involved.

### *EMG bursting in low back pain*

At the location of the motor hotspot, bursts occurred earlier and were smaller in magnitude in individuals with LBP compared to pain-free controls. In keeping with current hypotheses, we interpret smaller bursts in LBP as an evidence of reduced cortical disinhibition in this condition. Previous estimates of disinhibition in LBP have been based on chance observations in paired pulse studies investigating inhibitory networks in M1. In contrast to the present study, those studies cite lower levels of short interval intracortical inhibition (SICI) in the abdominal and paravertebral muscles in LBP as evidence of increased disinhibition in this condition [21,22]. Although it is common to interpret a reduction in SICI as a form of disinhibition, pharmacological evidence indicates that this mechanism reflects inhibitory processes mediated by post-synaptic $GABA_A$ receptors [23,24], which, unlike the pre-synaptic $GABA_B$ receptors proposed to underlie EMG bursting [1], do not have the innate capacity to negatively regulate GABAergic transmission. Furthermore, a number of competitive interactions have been documented between SICI and other inhibitory and excitatory mechanisms in M1 [25–28]. Therefore, we suggest that the present findings may provide a more accurate account of the depth and duration of M1 disinhibition in LBP. However, as we found no evidence of a relationship between the burst characteristics and pain, the clinical significance of these findings remains unclear.

Despite no statistical difference between the location of hotspots between the groups, there was a trend towards smaller mean PBR and smaller map volume in individuals with LBP. These findings are further evidence that the topography and mean excitability of the motor representation of low back muscles are altered compared to the controls [11,12,15]. As these changes are hypothesised to underpin the altered trunk muscle coordination and postural control in LBP, it is possible that an abnormal burst mechanism may similarly contribute to motor dysfunction in this condition [15].

In addition to the altered burst characteristics, we also report reduced MEP amplitudes and shorter cortical

silent periods at the motor hotspot in LBP. While these findings complement previous observations of decreased corticomotor excitability [11,16,29] and dysfunctional GABAergic disinhibition in LBP [21,22], they are not entirely compatible with our conclusion of reduced cortical disinhibition. Discrepant responses to TMS are not uncommon in observational studies and serve to highlight the intricate nature of M1; however, methodological factors also have the potential to affect the study outcomes. For example, in the present study, TMS mapping was performed with maximum stimulator output (100% intensity) in all the participants. While in line with previous studies using surface EMG over muscles of the low back during volitional contraction [2,10–12], this methodology may give rise to erroneous results if differences in corticomotor excitability exist between the groups. Indeed, since the magnitude of the MEP, the cortical silent period and EMG burst are reportedly intensity-dependent [1,30], it is possible that our observation of smaller MEPs, smaller bursts, shorter silent periods and earlier burst onset in LBP may be a consequence of raised motor threshold. However, as motor threshold was not an outcome of the present study and there is a lack of consensus regarding this aspect of cortical excitability for low back muscles in LBP [16,21], the impact of this factor on our results remains unclear. To prevent such ambiguity in the future, we recommend that future studies, requiring maximum stimulator output to generate M1 maps, include a measure of active motor threshold to validate their findings, especially if abnormal motor threshold is suspected.

## Conclusion

This study confirms the presence of post-silent period EMG bursting in low back muscles. We found that the latency, duration and magnitude of bursting in healthy persons were similar to that reported previously in the hand; however, our novel observation of a "burst silent period" suggests that this feature may be specific to the back. TMS mapping revealed a graded distribution of EMG bursting throughout M1, culminating in one definitive "hotspot". Our observation of spatial and temporal differences between individuals with and without LBP may be evidence of altered cortical disinhibition in this condition; however, further work is necessary to confirm this hypothesis.

## Disclosure statement

The authors report no conflicts of interest.

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