Investigating neuroplasticity in burn injury in older adults

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This thesis is presented for the degree of Master of Philosophy of The University of Western Australia

School of Surgery
The University of Western Australia
Year of submission 2018
THESIS DECLARATION

I, Casey John Whife, certify that:

This thesis has been substantially accomplished during enrolment in the degree.

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The research involving human data reported in this thesis was assessed and approved by The University of Western Australia Human Research Ethics Committee. Approval #: RA/4/1/8354.

Written patient consent has been received and archived for the research involving patient data reported in this thesis.

The following approvals were obtained prior to commencing the relevant work described in this thesis:
• Murdoch University Human Research Ethics Committee (MU HREC Reference: 2016-166)

• East Metropolitan Health Service (EMHS HREC Reference: 16-012)

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• Governance approval from East and South Metropolitan Health Services

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This thesis contains published work and/or work prepared for publication, some of which has been co-authored.

Date: 21st May 2018
Rehabilitation from an acute burn injury is a challenging process, in which physical function and quality of life are often impacted long after the initial injury (Klein et al., 2011; Renneberg et al., 2014; Wasiak et al., 2014). Older adult burns survivors are known to experience decreased mobility, reduced levels of independence, limited function and depressed mood (Abu-Sittah et al., 2016). Prior research from the State Adult Burn Unit in Western Australia has shown that a deterioration in physical function post-burn injury is greater in older adults than younger adults, with the deterioration more apparent from approximately 45 – 50 years of age (Edgar et al., 2013).

Neuroplasticity, the ability of the brain to change structurally and functionally with experience, underlies the ability to acquire, consolidate and retain motor skills (Pascual-Leone et al., 2005). Neuroplastic change has been demonstrated to occur in a variety of musculoskeletal injuries and the capacity for neuroplastic adaptation likely impacts the functional recovery from these injuries (Boudreau et al., 2010; Snodgrass et al., 2014). To date, there is no literature regarding the neuroplastic response to an acute burn injury in older adults. The broad aim of this thesis is to investigate changes in markers of neuroplasticity and functional recovery over twelve weeks post-burn injury in older adults.

This thesis is presented as a series of papers. Chapter One provides a general introduction to neuroplasticity, transcranial magnetic stimulation (TMS), burn injury and older age.
Chapter Two presents the manuscript, “Neuroplasticity in musculoskeletal injury”. This provides a narrative review of the role of neuroplasticity in musculoskeletal injury and the potential impact on functional recovery from injury.

Chapter Three presents the manuscript “Decreased neuroplasticity in older burn injury survivors compared to non-injured older adults”. The aim of this manuscript was to investigate whether there are differences in the capacity for repetitive TMS (rTMS) -induced neuroplastic responses between burn injured older adults and non-injured older adults. Additionally, the manuscript investigated whether the capacity for neuroplastic adaptation was associated with functional and quality of life measures following burn injury. The results showed that non-injured older adults demonstrate a delayed rTMS-induced neuroplastic response, whereas burn injured participants did not demonstrate an rTMS-induced neuroplastic response. Burn injured participants who demonstrated a typical neuroplastic response also demonstrated better outcomes in some functional measures.

Chapter Four presents the manuscript, “No difference in short-interval intracortical inhibition in older burn injury survivors compared to non-injured older adults”. The aim of this manuscript was to investigate whether rTMS-induced differences in neuroplastic responses between older burn injured adults and non-injured adults demonstrated in the previous manuscript were secondary to short-interval intracortical inhibition. The results suggest that the differences between groups are not mediated by short-interval intracortical inhibition.
Chapter Five provides a summation of discussion for Chapters Two – Four, while Chapter Six provides clinical recommendations and conclusions. The evidence presented in this thesis suggests that minor burn injury can affect neuroplasticity (as measured by rTMS-induced neuroplastic changes) in older adults and that the change in neuroplasticity post-burn injury may impact functional recovery.
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**AUTHORSHIP DECLARATION: CO-AUTHORED PUBLICATIONS**

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Location in thesis:

Chapter Four

Student contribution to work:

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Feedback, data analysis assistance and editing provided by co-authors.

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Fiona Wood  Ann-Maree Vallence  Dale Edgar

30/05/2018  30/05/2018  30/5/2018

Student signature:

Date: 21st May 2018

I, W. Prof. Fiona Wood, certify that the student statements regarding their contribution to each of the works listed above are correct

Coordinating supervisor signature:

Date: 30/05/2018
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<tr>
<td>ACL</td>
<td>Anterior cruciate ligament</td>
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<tr>
<td>ADM</td>
<td>Abductor digiti minimi</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APB</td>
<td>Abductor pollicis brevis</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood-oxygen-level-dependent</td>
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<tr>
<td>BSHS-B</td>
<td>Burn Specific Health Survey – Brief</td>
</tr>
<tr>
<td>C-CF</td>
<td>Cranio-cervical flexion</td>
</tr>
<tr>
<td>CIT</td>
<td>Constraint-induced therapy</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CS</td>
<td>Conditioning stimulus</td>
</tr>
<tr>
<td>cTBS</td>
<td>Continuous theta-burst stimulation</td>
</tr>
<tr>
<td>DCF</td>
<td>Deep cervical flexor muscle</td>
</tr>
<tr>
<td>ECR</td>
<td>Extensor carpi radialis</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
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</tr>
<tr>
<td>EMG</td>
<td>Electromyographic</td>
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<tr>
<td>EMG-BF</td>
<td>Electromyographic biofeedback</td>
</tr>
<tr>
<td>FCR</td>
<td>Flexor carpi radialis</td>
</tr>
<tr>
<td>FDI</td>
<td>First dorsal interosseous</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FPB</td>
<td>Flexor pollicis brevis</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>ICF</td>
<td>Intracortical facilitation</td>
</tr>
<tr>
<td>ICI</td>
<td>Intracortical inhibition</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1 beta</td>
</tr>
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<td>IL-6</td>
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<tr>
<td>IPI</td>
<td>Inter-PAS interval</td>
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<tr>
<td>ISI</td>
<td>Inter-stimulus interval</td>
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<tr>
<td>iTBS</td>
<td>Intermittent theta-burst stimulation</td>
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<td>Abbreviation</td>
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<tr>
<td>LICI</td>
<td>Long-interval intracortical inhibition</td>
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<td>LLFI</td>
<td>Lower limb functional index</td>
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<tr>
<td>LTD</td>
<td>Long-term depression</td>
</tr>
<tr>
<td>LTP</td>
<td>Long-term potentiation</td>
</tr>
<tr>
<td>MEP</td>
<td>Motor-evoked potential</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>MSO</td>
<td>Maximal stimulator output</td>
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<tr>
<td>M1</td>
<td>Primary motor cortex</td>
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<tr>
<td>NIBS</td>
<td>Non-invasive brain stimulation</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<tr>
<td>PAS</td>
<td>Paired associative stimulation</td>
</tr>
<tr>
<td>PES</td>
<td>Peripheral electrical stimulation</td>
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<tr>
<td>pITG</td>
<td>Posterior inferior temporal gyrus</td>
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<tr>
<td>pre-SMA</td>
<td>Presupplementary motor area</td>
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<td>RM-ANOVA</td>
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<tr>
<td>RMT</td>
<td>Resting motor threshold</td>
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<td>rTMS</td>
<td>Repetitive transcranial magnetic stimulation</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<td>SICI</td>
<td>Short-interval intracortical inhibition</td>
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<td>SIIp</td>
<td>Posterior secondary somatosensory area</td>
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<td>Theta-burst stimulation</td>
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<tr>
<td>TBSA</td>
<td>Total body surface area</td>
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<td>tDCS</td>
<td>Transcranial direct current stimulation</td>
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<td>Transcranial magnetic stimulation</td>
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<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor alpha</td>
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<tr>
<td>TS</td>
<td>Test stimulus</td>
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<tr>
<td>TUG</td>
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CHAPTER ONE – General Introduction

Post-burn morbidity continues to be a challenge for burns survivors, with long-term sequelae often persisting for years after the initial injury (Jeschke et al., 2011; Williams et al., 2009). Although burn injury primarily affects the skin, it is associated with a variety of systemic effects such as sustained oxidative stress (Liu et al., 2008), prolonged hypermetabolic states and elevation of stress hormones (Atiyeh et al., 2008; Williams et al., 2009). Systemic responses are induced by both minor and moderate size burn injury (Anderson et al., 2010; Rea et al., 2009), and likely contribute to the increased long-term morbidity secondary to these injuries (Duke et al., 2015a). Burn injury is a major cause of mortality and morbidity, particularly among older adults (Duke et al., 2012; Duke et al., 2011; Klein et al., 2011; Pham et al., 2009). Despite the poorer associated outcomes in older compared to younger burn injury survivors, the mortality due to burn injury in Western Australia has been decreasing by a mean of three percent each year from 1983 to 2008 (Duke et al., 2012). The decrease in mortality has resulted in an increase in the number of older burns survivors, which is associated with prolonged hospital stays (Duke et al., 2011) and increased resource allocation (Pham et al., 2009) compared to younger adults. Additionally, approximately one quarter of older burns survivors will not be discharged back to their pre-admission home status (Duke et al., 2012). Age is known to negatively impact burn injury outcomes (Abu-Sittah et al., 2016). Evidence from Western Australia shows that adults over the age of 45 with a burn injury have a 1.4-fold higher all-cause mortality than non-injured individuals (Duke et al., 2015a). In addition to increased mortality, older burns survivors have increased physical impairment, reduced quality of life and loss of independence compared to younger individuals (Klein et al., 2011; Lundgren et al., 2009). Burn injury continues to be associated with physical dysfunction and impaired quality of life long after the initial injury (Wasiak et al., 2014). Evidence from the State Adult Burn Unit in Western Australia has shown the
deterioration in physical function post-burn is greater in older adults than younger adults, and that this is apparent from 45-50 years of age (Edgar et al., 2013).

Neuroplasticity refers to the intrinsic ability of the brain to adapt in response to environmental changes, physiological modifications and experiences (for review see Pascual-Leone et al., 2005). Neuroplasticity is a continuously occurring process as a result of changing inputs and demands of the brain. Neuroplasticity can occur both over short and prolonged periods, and is mediated by both structural and functional changes in the brain (Pascual-Leone et al., 2005). Functional neuroplasticity (changes in strength (or efficacy) of existing structural networks) plays a fundamental role in motor learning (Dayan and Cohen, 2011). There is strong evidence to suggest that motor learning is mediated by activity- or use-dependent modification of synaptic strength. Specifically, long-term potentiation (LTP), which is an increase in synaptic strength, and long-term depression (LTD), which is a weakening in synaptic strength (Sanes and Donoghue, 2000).

Non-invasive brain stimulation (NIBS) techniques, such as transcranial magnetic stimulation (TMS), can be used to measure and induce neuroplasticity in the human primary motor cortex (M1) (Di Lazzaro et al., 2008; Groppa et al., 2012; Reis et al., 2008; Rothwell, 2010; Vallence and Ridding, 2014). Thus, TMS is a valuable technique for investigating the role of neuroplasticity in motor learning and recovery of motor function following injury. Single-pulse TMS can be used to measure excitability of the M1. By delivering a single TMS pulse to the M1, a motor response can be elicited and recorded in a muscle contralateral to the stimulated M1. This motor response is known as a motor-evoked potential (MEP); the MEP is a result of the activation of motor cortical output cells and the subsequent descending
volley in the corticospinal tract, and thus, provides a measure of corticospinal excitability. The MEP is influenced by changes in synaptic efficacy within the stimulated cortical network and therefore a change in the amplitude of the MEP can be used as a marker of neuroplasticity (Di Lazzaro et al., 2008; Groppa et al., 2012).

The application of trains of TMS pulses over short time periods is known as repetitive TMS (rTMS). Trains of stimuli applied in appropriate temporal patterns are capable of inducing changes in cortical excitability that outlast the period of stimulation, which is indicative of modification of synaptic efficacy (i.e. functional neuroplasticity). rTMS can therefore be used to induce neuroplastic changes in the M1, which are thought to be mediated by mechanisms similar to LTP and LTD (which underlies motor learning and impacts recovery from injury or disease) (Berardelli et al., 1998; Rothwell, 2010; Vallence and Ridding, 2014; Wang et al., 1996; Ziemann et al., 2004). rTMS-induced neuroplasticity is dependent upon the interaction between stimulation frequency, intensity and train duration. Patterned stimulation protocols have emerged, in which high frequency (30 – 50 Hz) bursts are applied at theta frequency (theta-burst stimulation; TBS). Continuous theta-burst stimulation (cTBS) involves the continuous application of bursts of stimuli (three stimuli at 50 Hz every 200 milliseconds (ms) (5 Hz)) for 20 – 40 seconds. cTBS has been shown to induce a lasting reduction in cortical excitability (Huang et al., 2005). The capacity for neuroplastic adaptation has been shown to impact functional motor outcomes (Muellbacher et al., 2002; Reis et al., 2009; Robertson et al., 2005). rTMS is therefore a useful tool that allows the investigation of changes in the capacity for neuroplasticity, as well being a potential therapeutic tool that can induce functionally meaningful neuroplastic adaptation that may help promote the recovery of motor function (Hulme et al., 2013; Ridding and Rothwell, 2007).
There is strong evidence to suggest that inhibition plays an important role in motor cortical neuroplasticity (Chen et al., 2002; Sanes and Donoghue, 2000). Motor cortical inhibition is largely mediated by the activation of gamma-aminobutyric acid (GABA) receptors (Jacobs and Donoghue, 1991; Kujirai et al., 1993). Intracortical inhibition (mediated by cortical GABA-ergic inhibitory interneurons) plays an important role in modulating cortical excitability. For example, a reduction in intracortical inhibition may result in a more permissive state within the cortex that enables strengthening or weakening of synaptic connections to occur (Chen et al., 1998). Deficiencies or abnormalities in GABA-ergic activity are known to occur in movement disorders (Hallett, 2011) and may be associated with a reduced capacity for functional neuroplasticity.

TMS is a useful tool to investigate the activity of GABA-ergic inhibitory interneurons. Paired-pulse TMS can be used to obtain a measure of excitability of the inhibitory interneurons within the M1 (Kujirai et al., 1993), such as short-interval intracortical inhibition (SICI). A subthreshold conditioning stimulus (below the sufficient intensity to cause a descending influence on the spinal cord) is followed by a suprathreshold test stimulus to elicit a MEP. Intracortical influences that have been initiated by the conditioning stimulus modulate the amplitude of the MEP produced by the test stimulus (Hallett, 2007). Short inter-stimulus intervals (ISIs) of less than 5 ms result in decreased amplitude of the MEP (relative to that from a single pulse), and therefore reflects SICI (Dayan et al., 2013; Di Lazzaro et al., 2008; Hallett, 2007). There is pharmacological evidence that SICI is mediated by GABA_A receptor activity (Di Lazzaro et al., 2000; Illic et al., 2002; Ziemann et al., 2006).
Given that neuroplastic adaptation occurs continuously in response to changes in sensory input and motor output/demands, neuroplastic adaption is an unescapable consequence of injury. Changes in corticospinal excitability and intracortical inhibition have been documented in response to amputation and deafferentation (both transient and permanent) (Chen et al., 2002). Cortical changes in this population have shown that central nervous system neuroplastic adaptation occurs in response to a peripheral nerve insult or injury and that the mechanism of neuroplastic adaptation likely differs depending on the time frame (Chen et al., 2002). Neuroplasticity after central nervous system injury such as stroke is well documented, and the extent of functional recovery is understood to be positively correlated to the extent of neuroplastic change during rehabilitation (Di Lazzaro et al., 2010; Hamdy et al., 1998; Jones, 2017). Within this population, rehabilitation strategies have been identified that maximise the capacity for neuroplastic change to improve clinical outcomes (Oujamaa et al., 2009). The role of neuroplasticity in musculoskeletal injury and rehabilitation is increasingly being recognised, however data regarding the capacity for neuroplastic changes within this population is limited, especially in acute injuries. While there is some research to show that burn injury can cause nervous system adaptation (Anderson et al., 2010; Hamed et al., 2011; Lim et al., 2014; Morellini et al., 2012), there are currently no studies investigating the capacity for neuroplasticity following an acute burn injury.

The impact that neuroplasticity in older adults has on burn injury rehabilitation is currently unknown. The ability of the healthy motor cortex to reorganise in response to training has been shown to decrease with age (Sawaki et al., 2003), although these age-related differences may be subtler than initially thought (Berghuis et al., 2017). Normal ageing is associated with physiological changes in structure and function of the brain. Relative to younger adults, older adults exhibit motor performance deficits that include coordination difficulty, increased
variability of movement and difficulties with balance and gait (Seidler et al., 2010). Declines in these areas affect the ability of older adults to perform activities of daily living and maintain their independence. Older adults have demonstrated impaired relative motor skill acquisition compared to younger adults, including differing neuronal mechanisms during skill acquisition and skill retention (Berghuis et al., 2016). In order to address and potentially improve functional outcomes in the older burn injury population with targeted rehabilitation strategies more information regarding neuroplasticity post-burn injury is required. The aim of this thesis is to review the current literature regarding neuroplasticity following musculoskeletal injury (including the impact of injury on the peripheral nervous system) and investigate changes in markers of neuroplasticity in acute burn injury in older adults as an at-risk target population. This thesis aims to investigate both how neuroplasticity is impacted after an acute burn injury in older adults compared to non-injured older adults, and how the capacity for neuroplastic adaptation post-injury is associated with functional outcomes and quality of life. This thesis presents three manuscripts that have either been submitted or are to be submitted for publication.

Chapter Two introduces and includes the first manuscript, “Neuroplasticity in musculoskeletal injury”. This manuscript provides a review of neuroplasticity in the musculoskeletal system. Specifically, the manuscript reviews the evidence for neuroplasticity in motor learning (use-dependent neuroplasticity), disuse-dependent neuroplasticity, neuroplasticity secondary to musculoskeletal injury and the relationship between neuroplasticity and pain. The manuscript then focuses on how neuroplastic adaptations occur and evolve post-injury and how these neuroplastic changes can be addressed in injury rehabilitation. This manuscript provides context for the investigation of neuroplasticity in acute burn injury. This manuscript is awaiting submission for publication.
Chapter Three presents the second manuscript, “Decreased neuroplasticity in older burn injury survivors compared to non-injured older adults”. This manuscript investigated the relationship between acute burn injury, markers of neuroplastic change (at six and twelve weeks post-injury) and functional and quality of life outcomes at twelve weeks post-injury in older adults. This manuscript is currently under review in Clinical Neurophysiology. The aims of this study were:

- To establish whether there are differences in the capacity for neuroplastic adaptation between older adult burns survivors and non-injured older adults, and;
- To investigate whether the capacity for neuroplastic adaptation is associated with functional and quality of life measures following burn injury.

To investigate these aims both burns survivors (Burns Group) and non-injured participants (Control Group) were recruited to undergo non-invasive brain stimulation (rTMS) to induce functional neuroplasticity approximately six and twelve weeks post-burn injury (or six weeks apart in the Control Group). Functional and Quality of Life assessment was performed at the second experimental session. It was hypothesised that burns survivors would show a smaller change in MEP amplitude following rTMS than non-injured, age-matched control participants. It was also hypothesised that burn survivors who showed a greater change in MEP amplitude following rTMS would have the highest scores on the Functional and Quality of Life measures.

Chapter Four presents the third manuscript, “No difference in short-interval intracortical inhibition in older burn injury survivors compared to non-injured older adults”. This
manuscript investigated the relationship between acute burn injury, motor cortex inhibition (specifically, SICI, at six and twelve weeks post-injury) and functional and quality of life outcomes at twelve weeks post-injury in older adults. This manuscript is awaiting submission for publication. The aims of this study were:

- To establish whether there are differences in baseline SICI between older adult burns survivors and non-injured older adults,

- To establish whether there are differences in SICI post-rTMS between older adult burns survivors and non-injured older adults,

- To investigate whether SICI is associated with functional and quality of life measures following burn injury.

The motor cortical inhibition data were collected in the same TMS experimental sessions as the MEP amplitude data in *Chapter Three*, in the same burns survivors and non-injured control sample. The same Functional and Quality of Life measurements from *Chapter Three* were used in this study to provide a measure of rehabilitation and recovery from burn injury. It was hypothesised that burns survivors would have less intracortical inhibition than non-injured, age-matched controls, as this has been demonstrated in deafferentation injuries (Chen et al., 2002).

The Forewords to *Chapters Two, Three* and *Four* serve to introduce aspects of the manuscripts that require clarification or introduction, as the manuscripts have been presented in the form intended/submitted for publication. *Chapter Five – Summation of Discussion* follows the three manuscript *Chapters* to summarise and discuss the relevant findings, followed by *Chapter Six – Clinical Recommendations and Conclusions*. Given that the three
manuscripts presented in this thesis are intended to provide the reader with a comprehensive background as a stand-alone paper there is some unavoidable repetition, especially describing Methods in Chapter Three and Chapter Four. References are presented in the Bibliography at the end of each manuscript for Chapters Two, Three and Four. References for all remaining Chapters (including this General Introduction) are presented in the Bibliography at the end of the thesis. The Bibliography is then followed by Appendices, which have been referred to in Chapters Three and Four.
CHAPTER TWO – Neuroplasticity in Musculoskeletal Injury

Foreword

This Chapter presents a review manuscript, “Neuroplasticity in musculoskeletal injury”. This manuscript is to be submitted for publication and is presented in the form that is required upon submission for publication. This includes an Abstract, Keywords and Highlights. All references from this manuscript are presented in the Bibliography at the end of the manuscript, not in the Bibliography at the end of the thesis (although they may be in the final Bibliography as well).
Neuroplasticity in musculoskeletal injury.

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Abstract

Neuroplasticity is the ability of the brain to change structurally and functionally with experience. While the role of neuroplasticity in chronic pain conditions and in the recovery from stroke has received increasing interest, the role of neuroplastic adaption in the primary motor cortex in the recovery and rehabilitation from musculoskeletal injuries remains underappreciated. Despite this, there is evidence for primary motor cortex neuroplastic adaptation to a variety of musculoskeletal conditions other than chronic pain. These include amputation, nerve injury and deafferentation, joint and ligament injury, fracture and immobilisation and acute musculoskeletal pain. Neuroplastic adptions evolve over time and as an injury evolves there may be a shift from beneficial to maladaptive changes. The capacity for neuroplastic adaption has been shown to influence the extent of functional recovery from injury. Multiple rehabilitation strategies have emerged with the aim of maximising neuroplastic adaption to improve functional outcomes. Other techniques, such as non-invasive brain stimulation, may also play a role in modulating neuroplasticity to facilitate motor learning in rehabilitation. This article reviews the evidence for neuroplasticity in motor learning, disuse, and neuroplastic adaption secondary to injury. The role of neuroplasticity in injury rehabilitation is discussed. Finally, rehabilitation techniques that address the underlying capacity for neuroplasticity are presented.
**Keywords:** Neuroplasticity, primary motor cortex, musculoskeletal injury, rehabilitation, non-invasive brain stimulation

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**Highlights:**

- Neuroplastic adaption in the primary motor cortex occurs in a variety of musculoskeletal injuries, both acute and chronic.
- Neuroplastic adaptations may be beneficial or maladaptive, and the extent of functional recovery may be correlated with the extent of neuroplastic change.
- A range of rehabilitation techniques, including non-invasive brain stimulation, are available to target neuroplastic adaption to improve functional outcomes from musculoskeletal injury.
1. Introduction

Prior to the 1990s, musculoskeletal injury was thought to be the sole driver of subsequent musculoskeletal dysfunction (Pelletier et al., 2015a; b; Snodgrass et al., 2014). Rehabilitation had been largely focused on movement specific performance or exercises. However, growing evidence strongly suggests that the central nervous system changes in response to musculoskeletal injury (Boudreau et al., 2010; Pelletier et al., 2015b; Snodgrass et al., 2014). Neuroplasticity—the ability of the brain to change structurally and functionally with experience—has been demonstrated in both animals and humans (Sanes and Donoghue, 2000). The concept of neuroplasticity has existed for centuries and gained popularity in the latter part of the nineteenth century, with ongoing research throughout the twentieth century further defining and providing insight to the nature of neuroplasticity (for review see Markram et al., 2011). Central nervous system neuroplasticity not only occurs in response to use (Pascual-Leone et al., 2005), but also in response to disuse (Rosenkranz et al., 2014), central nervous system injuries such as stroke (Jones, 2017), and in peripheral (i.e. not central nervous system) injuries such as amputation (Chen et al., 1998), nerve injuries (Kaas, 2000), joint and ligament injuries (Ward et al., 2015), fracture and immobilisation (Zanette et al., 2004), and in response to pain (Burns et al., 2016a; Parker et al., 2016).

The role of neuroplasticity in musculoskeletal injury pathology is now emerging. Neuroplasticity in response to musculoskeletal injury may be functionally beneficial or maladaptive (Boudreau et al., 2010; Pelletier et al., 2015b; Snodgrass et al., 2014). Initial adaptation to musculoskeletal injury is often beneficial as this serves to protect the injured structure from insult and therefore facilitates healing (e.g. quadriceps inhibition in an acute knee injury) (Gabler et al., 2013; Pelletier et al., 2015b). However, maladaptive
neuroplasticity can occur for a variety of reasons, including ongoing pain perception (Moseley and Flor, 2012) and altered afferent sensory input (Chen et al., 2002; Osborne et al., 2018; Ward et al., 2015). Altered sensory input secondary to injury is capable of driving specific neuroplastic adaptations such as altered corticospinal excitability and reorganisation of the primary motor cortex (M1) (Chen et al., 2002; Wall et al., 2002; Ward et al., 2015). When coupled with persisting pain or reinforced learning patterns this can result in long-term changes in muscle activation and recruitment that are detrimental to an individual’s recovery (Hodges and Tucker, 2011; Moseley and Flor, 2012; van Dieen et al., 2017). Neuroplastic adaptations to injury can occur rapidly though (over minutes to hours) and may continue to evolve over weeks to months (Navarro et al., 2007; Wall et al., 2002). The timing of neuroplastic adaption to injury likely reflects different neuroplastic mechanisms (Chen et al., 2002). Therefore, a better understanding of neuroplasticity following musculoskeletal injury and the impact on functional outcomes will promote targeted interventions that enhance recovery following injury.

Most traditional rehabilitation approaches to musculoskeletal injury do not sufficiently take into consideration the knowledge that the mature central nervous system can undergo adaptation in response to use, disuse, and injury. While these adoptions are being addressed in some chronic pain patients, the role of neuroplasticity has been underappreciated in other areas of musculoskeletal injury. Indeed, it is reasonable to suggest the capacity for neuroplasticity would influence the extent of functional recovery from an acute musculoskeletal injury. Rehabilitation efforts that maximise beneficial neuroplastic change provide the greatest potential for maximal functional recovery (Boudreau et al., 2010; Snodgrass et al., 2014). Motor rehabilitation strategies are emerging in which underlying cortical mechanisms are targeted with the aim of improving functional outcomes beyond
what a traditional structural-pathology approach could achieve (Pelletier et al., 2015b; Snodgrass et al., 2014).

The following sections of this review outline evidence for neuroplasticity in the musculoskeletal system, the relationship between injury and neuroplasticity, and potential rehabilitation strategies that may be employed to facilitate recovery from these injuries while allowing for, or utilising, neuroplasticity. It should be noted that animal data (in addition to data from human studies) is also presented and this is not necessarily generalizable to a human population. The quality of studies available is variable, ranging from literature reviews or meta-analysis to small cohort and case-control studies.

2. Neuroplasticity

Contemporary belief is that the human brain and nervous system are in a constant state of change, with neuroplasticity playing an integral role (Buonomano and Merzenich, 1998; Pascual-Leone et al., 2005). The intrinsic ability of the brain to change is the underlying mechanism by which we adapt to new experiences, physiological and environmental stressors and behavioural changes (Pascual-Leone et al., 2005; Pascual-Leone et al., 2011). Changes in the brain can be functional, such as the strengthening and weakening of existing connections, and structural, such as neurogenesis and the formation of new synaptic connections (Pascual-Leone et al., 2011). Neuroplasticity is a fundamental process by which learning and memory occur (Pascual-Leone et al., 2005; Pascual-Leone et al., 2011; Sanes and Donoghue, 2000).
Associative plasticity, introduced by Hebb in 1949, is an important mechanism mediating neuroplasticity at the cellular level (Ilic et al., 2011). Synaptic connections between neurons are strengthened if the pre-synaptic neuron firing is repetitively and persistently paired with firing of the post-synaptic neuron (Buonomano and Merzenich, 1998; Hebb, 1949; Ilic et al., 2011). Associative plasticity is often described as, “neurons that fire together wire together” (Gabler et al., 2013), although contemporary theory suggests that the initial understanding of Hebb was somewhat simplistic (Ilic et al., 2011).

Neuroplastic changes can be transient, however neuroplasticity can result in long lasting changes when stimulus-driven and external (or internal) pressures are repetitive, meaningful and require sustained attention (Jenkins et al., 1990; Kleim and Jones, 2008; Pascual-Leone et al., 1995; Pelletier et al., 2015b; Remple et al., 2001; Tyc et al., 2005). Repetitive activity, such as practising a skill, modulates the strength of existing synapses and may drive synapse formation (Kleim et al., 2002; Remple et al., 2001). A persistent increase in synaptic efficacy is known as long-term potentiation (LTP) (Cooke and Bliss, 2006; Sanes and Donoghue, 2000); a persistent decrease in synaptic efficacy is known as long-term depression (LTD) (Massey and Bashir, 2007; Sanes and Donoghue, 2000). Evidence from animal work shows that LTP and LTD can occur throughout the central nervous system, including the cortex (Navarro et al., 2007). Strong evidence suggests that LTP and LTD underlie learning and memory across the lifespan, including a fundamental role in learning motor skills (Buonomano and Merzenich, 1998; Massey and Bashir, 2007; Rioult-Pedotti et al., 2000; Rioult-Pedotti et al., 1998; Sanes and Donoghue, 2000; Ziemann et al., 2004). These cellular changes are not selective, instead reinforcement of existing synaptic connections will occur as a consequence of demand (both afferent and efferent) and environmental influences (Pascual-Leone et al., 2005). Consequently, neuroplastic adaptations have been associated
with not only physiologically beneficial situations such as motor learning, but also in pathological situations such as the development of chronic pain (Boudreau et al., 2010; Flor, 2003; Pelletier et al., 2015b).

In humans, use-dependent neuroplasticity is often quantified using transcranial magnetic stimulation (TMS). TMS involves the use of a magnetic coil placed against the scalp to generate an electrical field within the targeted cortex. The electrical field causes current to flow in loops parallel to the plane of the coil, which induces current flow in the underlying cortical tissue. If applied to the primary motor cortex, the depolarisation of neurons elicits a response in the targeted muscle, known as a motor-evoked potential (MEP). The amplitude of a MEP reflects the excitability within the corticospinal system (Di Lazzaro et al., 2008; Groppa et al., 2012; Hallett, 2007; Vallence and Ridding, 2014). By applying TMS at numerous sites over the motor cortex and recording the MEPs researchers can determine the size the cortical representation of the target muscle. Neurophysiological measures obtained by TMS, such as corticospinal excitability, can provide a measure of neuroplasticity. TMS can also be used to induce neuroplasticity when applied in trains of pulses, known as repetitive TMS (rTMS). Applying trains of pulses at a particular frequency and intensity can induce changes in cortical excitability that outlast the period of stimulation, which is indicative of neuroplasticity (Hallett, 2007). By varying the frequency and temporal pattern of rTMS bidirectional changes in cortical excitability can be induced (Hallett, 2007).

Neuroplasticity has the potential to drive rehabilitation outcomes. It has been well demonstrated that the extent of neuroplastic change positively correlates with the extent of functional recovery in individuals with a central nervous system injury such as stroke (Caleo,
Given that a stroke directly affects the central nervous system it is reasonable that central nervous system adaption will directly impact recovery. However, there is now substantial evidence that injuries to peripheral nerves and the musculoskeletal system is capable of driving central nervous system neuroplastic adaptation, and that the extent to which beneficial neuroplastic changes can be implemented through rehabilitation will influence the extent of recovery from these injuries.

2.1. Use-dependent neuroplasticity

Use-dependent neuroplasticity has been shown in the motor cortex in both non-human primate studies and human studies. In non-human primates, for example, use-dependent neuroplastic change in the motor cortex has been demonstrated in a study of squirrel monkeys engaging in a motor learning task (Nudo et al., 1996). The monkeys were trained in the retrieval of small pellets from a well, which required the skilled use of their digits. Electrophysiological mapping of the cortex was performed by intracortical microstimulation prior to training and again after the training period. After training, there was an increase in the size of the motor cortical representation of the trained digit and a concurrent decrease in the size of the motor cortical representation of the untrained wrist representation. The results show that motor cortical representations can undergo task-specific changes in response to motor training, which is supported in the literature (Sanes and Donoghue, 2000).

There is also evidence of use-dependent neuroplasticity in the human motor cortex in response to motor training tasks (for review see: Dayan and Cohen, 2011). In a seminal
study, Pascual-Leone et al. (1995) investigated use-dependent neuroplastic adaptation; subjects completed two-hour practice sessions of a five-finger sequence on a piano keyboard daily for five days. Following training on the piano keyboard motor performance improved, evidenced by a decrease in reaction time. Furthermore, the motor cortical representation of the trained muscles increased in size (as measured by TMS), providing evidence of a rapid (within one hour) modulation of motor cortex in response to motor training (Pascual-Leone et al., 1995). The results suggest that the acquisition of motor skills is associated with modification of cortical motor representations (Pascual-Leone et al., 1995). In both animal and human models the acquisition of skills modulates cortical representation, reflecting use-dependent neuroplasticity, thought to be mediated by increased synaptic efficiency in existing connections and the unmasking of existing connections due to disinhibition (Pascual-Leone et al., 1995; Sanes and Donoghue, 2000).

2.2. Disuse-dependent neuroplasticity

Neuroplastic changes in the motor cortex also occur in response to disuse. Neuroplasticity secondary to disuse has the potential to impact rehabilitation from injuries that require immobilisation for treatment of the underlying condition. Limb immobilisation has been used to examine disuse-dependent neuroplasticity in both non-human primates and humans. For example, Milliken et al. (2013) used intracortical microstimulation to investigate changes in the M1 representation of the distal forelimb in adult squirrel monkeys after “short-term” (as defined by study authors; 38-85 days) and “long-term” (cumulative time of 125-169 days) immobilisation of the preferred distal forelimb in a soft cast. Although the total distal forelimb motor representation and movement thresholds remained constant across the forelimb restriction, there was a redistribution of the digit and wrist/forearm areas.
Specifically, the digit representations progressively decreased in size and the wrist/forearm representations increased in size. After a recovery period, motor mapping indicated a reversal of immobilisation-induced changes. Finally, one monkey had recasting for an additional 79 days (with no intervening recovery period), after which motor mapping was again repeated. This monkey exhibited chronic motor impairment, that is, it did not return to use of the restricted (preferred) forelimb, even after an additional recovery period of 78 days. However, when the previously unrestricted forelimb was casted to encourage use of the previously restricted hand, this rehabilitation resulted in restoration of normal motor maps and a return to use of the limb that underwent the original restriction. Together, these results show that both training and restriction of a limb can induce motor cortex neuroplasticity. Furthermore, the restricted-use motor cortex changes are reversible with the aid of interventions, even with an initial adverse motor outcome.

In humans, Rosenkranz et al. (2014) used TMS to measure changes in the excitability of the motor cortex following short-term upper limb immobilisation (eight hours). Motor cortical excitability was measured using a stimulus-response curve that showed MEP amplitude change with increasing stimulus intensity. Cortical excitability in the immobilised muscle (abductor pollicis brevis muscle) was reduced immediately following short-term immobilisation. In addition, paired-pulse TMS was used to measure changes in GABA-ergic (GABA: gamma-aminobutyric acid) inhibition following immobilisation. When a subthreshold conditioning stimulus (i.e. not sufficient to elicit a MEP) precedes a suprathreshold test stimulus (that does elicit a MEP) by 1 – 5 milliseconds (ms), the amplitude of the MEP elicited by the test stimulus is suppressed due to the activation of short-interval intracortical inhibitory (SICI) circuits (Di Lazzaro et al., 1998; Kujirai et al., 1993). Pharmacological studies provide strong evidence that SICI is mediated by GABA\_A
receptor activity (McDonnell et al., 2006; Müller-Dahlhaus et al., 2008; Sanger et al., 2001; Werhahn et al., 1999). Following short-term immobilisation, Rosenkranz et al. (2014) showed a reduction in SICI, however a more recent similar study by Opie et al. (2016) found no change in SICI after eight hours of immobilisation of the index finger. Together these findings suggest that short-term disuse leads to a reduction in the excitability of motor cortical circuits, however the role of short-acting intracortical inhibition is unclear. These findings are supported by similar studies, which have also demonstrated a decrease in corticospinal excitability in different muscles exposed to short-term immobilisation (Facchini et al. (2002) – abductor digiti minimi (ADM) (immobilised) and first dorsal interosseous (FDI) (non-immobilised) after three and four days; Huber et al. (2006) – FDI after arm in sling immobilisation for twelve hours; Karita et al. (2017) – flexor pollicis brevis (FPB) from three hours immobilisation; Ngomo et al. (2012) – FDI after four days).

In contrast to short-term immobilisation, long-term immobilisation (i.e. ≥ seven days) has been shown to increase MEP amplitude in the immobilised muscles. Clark et al. (2008) used TMS to measure the excitability of the motor cortex following long-term upper-limb immobilisation (three weeks) in healthy adults compared to a control group. Both groups underwent nerve stimulation and TMS testing at three weeks, assessing for changes in central activation of muscles, spinal reflexes and corticospinal excitability. The immobilised group also had testing at week one and two during immobilisation, and at one week after immobilisation was removed. Immobilisation resulted in an increase in the MEP amplitude within one week of immobilisation and remained elevated for the following two weeks and for the week after the cast was removed. The study illustrates the profound effect that long-term immobilisation can have on the neuromuscular system, with corticospinal excitability changes persisting beyond one week after removal of immobilisation. These changes in
excitability may be secondary to a change in the motor map area or a neurotransmitter driven increase in corticospinal excitability within a specific projection area, similar to that seen in deafferentation (Avendano et al., 1995; Pelled et al., 2007).

A study by Roberts et al. (2007) investigated the effect of lower limb immobilisation on cortical excitability in healthy participants. Eight healthy adults had TMS to assess motor cortex excitability prior to and after wearing a full-length cast of the left leg for ten days. Eight other adults, who also underwent TMS testing but did not have casting, served as a control group. While no significant changes were observed immediately after cast removal, there was an increased stimulus-response curve slope at 24 hours after removal of casting. This reflects increased corticospinal excitability in these participants after 24 hours compared to those who did not undergo casting. As there was no change immediately at the time of cast removal, the authors hypothesise that this increase in corticospinal excitability at 24 hours may have represented subsequent use of the limb in the preceding 24 hours and reflect neuroplastic adaptations related to recovery and/or relearning limb use (Roberts et al., 2007).

In contrast to the study by Clark et al. (2008), MEP amplitudes were elevated 24 hours after cast removal and returned to normal after a further 48 hours (increased recruitment curve slope in the Clark et al. (2008) study was seen after one week of immobilisation and persisted for up to a week after cast removal). These studies demonstrate cortical hyperexcitability secondary to long-term immobilisation, and the variation seen between studies may be related to muscle groups tested (i.e. upper or lower limb), time of immobilisation and testing conditions.
Disuse-dependent neuroplasticity has also been demonstrated in studies of patients who were immobilised predominantly for orthopaedic reasons (i.e. fractures), and therefore the effect of pain and inflammation could not be accounted for. Neuroplasticity in these patients will be discussed in 2.3.3 Fracture and immobilisation.

2.3. Musculoskeletal injury-induced neuroplasticity

The following section reviews the evidence regarding the impact of musculoskeletal injury on neuroplasticity. While peripheral nerves are generally not considered part of the musculoskeletal system they are included in this review as nerve injury may occur in conjunction with musculoskeletal injury and nerve injury has implications for neuroplastic changes and recovery.

2.3.1. Amputation, nerve injury and temporary deafferentation

Amputation has been shown to induce changes in the motor cortex in both animals and humans. In animals, Sanes et al. (1990) conducted a study in which rats were subjected to forelimb amputation. Cortical output mapping of the M1 by intracortical electrical stimulation in these rats between one week to five months after amputation was compared to that of a control group of rats. Those rats with forelimb amputation demonstrated an enlarged cortical area from which shoulder movements could be evoked by intracortical electrical stimulation compared to the control group. Likewise, transection of branches of the facial nerve (which supplies the vibrissa area) in rats in this study resulted in increased eye/eyelid output areas compared to a control group, with the enlargements occupying the area formally occupied by the vibrissa area. The results show that, following amputation or nerve
transection, cortical representation of the body part proximal to the amputation/transection increases at the expense of the cortical representation of the body part distal to the amputation/transection. This indicates that the M1 output relationships can reorganise in response to amputation or nerve injury (Chen et al., 2002; Sanes et al., 1990).

Evidence of motor cortex neuroplasticity in amputation in humans has also been demonstrated. Cohen et al. (1991) studied MEP responses to TMS in participants with a unilateral upper limb amputation and in normal subjects. Electromyographic (EMG) recordings were made from muscles (biceps and deltoid) immediately proximal to the stump and also from the same muscles in the contralateral intact limb. In those patients with an acquired amputation TMS evoked larger MEPs and elicited MEPs at a lower intensity in muscles proximal to the stump in the ipsilateral arm than in same muscles in the contralateral intact arm. Further, TMS of these ipsilateral muscles resulted in recruitment of a larger percentage of the motor neuron pool than the contralateral side, and muscles proximal to the stump could be recruited from a larger area. These findings are consistent with other research into upper limb amputation that also demonstrates an increase in size of muscle EMG amplitude in muscles proximal to the amputation and a wider area of cortical stimulation in humans (Chen et al., 2002; Kew et al., 1994; Ridding and Rothwell, 1995).

Similar motor cortex neuroplasticity in humans has also been demonstrated in lower limb amputation. Chen et al. (1998) studied the site and mechanisms of motor cortex reorganisation in sixteen subjects with traumatic lower limb amputation. Utilising TMS the authors demonstrated that the threshold for muscle activation (muscles proximal to the stump were mid-thigh for below knee amputees, mid-point between stump and inguinal ligament for
above knee amputees) on the amputation side was lower than the intact side. For a given TMS stimulation output there was activation of a higher percentage of the motor neuron pool on the amputated side than the intact side. Using paired-pulse TMS the authors also demonstrated that there was less intracortical inhibition (ICI) on the amputated side. This study suggests that decreases in ICI may allow a permissive state in which long-term adaptation (as demonstrated by changes in motor threshold) can occur in the motor cortex in response to amputation.

Mano et al. (1995) demonstrated that nerve injury is capable of inducing motor cortex neuroplastic adaptation. TMS mapping over three years was performed in patients with a traumatic cervical root avulsion and subsequent surgical anastomosis of intercostal to musculocutaneous nerves to restore function to the biceps brachii muscle. The study showed that the biceps representation in the M1 moved laterally from the intercostal area to the arm area. While the biceps muscle was initially controlled by the intercostal area of the M1 immediately after the operation, over time cortical reorganisation meant that the original biceps M1 area could activate the biceps muscle through the intercostal nerve. This cortical change was functionally significant, with these patients demonstrating improved elbow flexion control and independence of the biceps motor unit from respiration.

Ridding and Rothwell (1997) utilised TMS to investigate changes in stimulus-response curves in humans with temporary ischaemic anaesthesia to the upper limb. During ischaemia, the stimulus-response curves (measured in the biceps brachii muscle – proximal to the area of ischaemia) were steeper than they were before or after deflation of the cuff. The authors have commented that while this study indicates that short-term deafferentation is capable of
inducing motor cortex change they are unable to comment on whether these changes represent a change in organisation within the motor cortex or simply a change in excitability (Ridding and Rothwell, 1997). To better understand the mechanisms involved in motor cortex changes secondary to temporary deafferentation, Ziemann et al. (1998) tested the effects of forearm ischaemic nerve block combined with rTMS of the human motor cortex. Deafferentation alone resulted in increased MEP size in the biceps brachii muscle (proximal to the area of deafferentation) compared to baseline measurements. Deafferentation of the upper limb coupled with low frequency (0.1 Hz) rTMS (which would normally be subthreshold) to the contralateral motor cortex also resulted in increased MEP size in the biceps brachii muscle, to a greater extent than deafferentation alone. However, the effect of deafferentation on MEP size was blocked by low frequency rTMS to the ipsilateral motor cortex. The change in MEP size could represent a change in excitability or a change in the representation of these muscles at the level of the motor cortex. Given that the motor threshold of the biceps brachii muscle did not change in this study a change in excitability can be dismissed. Instead, the authors state that a change in MEP size that was further facilitated by rTMS to the contralateral cortex and blocked by rTMS to the ipsilateral cortex suggests that the neuroplastic changes are secondary to ICI and intracortical facilitation (ICF). The study also illustrates that it is possible to modulate cortical neuroplasticity in humans using non-invasive techniques such as rTMS (Ziemann et al., 1998).

Together, these studies illustrate that the motor cortex in mammals can be modified by amputation, nerve injury or temporary deafferentation, and that both the amount of cortex and strength of neural connections dedicated to a particular input or output is modifiable. While the focus of this review is on motor cortex adaptations, there is also considerable evidence that the sensory cortex is modifiable secondary amputation, nerve injury and deafferentation.
(Chen et al., 2002; Elbert et al., 1994; Flor et al., 1995; Knecht et al., 1996; Merzenich et al., 1984; Pons et al., 1991; Ramachandran, 1993; Rijntjes et al., 1997). This may be an important consideration in individuals who have other injuries, such as a burn injury in which peripheral nerves may be affected, as changes in sensory processing in the cortex may impact motor output.

2.3.2. Joint and ligament injury

As with immobilisation, amputation, nerve injury and temporary deafferentation, joint injury can result in human cortical neuroplasticity. These changes can be maladaptive and negatively impact patient recovery and function. There is evidence from knee and ankle injuries that a unilateral injury can induce bilateral cortical changes that has the potential to impact outcomes adversely (Needle et al., 2017). An example of this is arthrogenic muscle inhibition seen in quadriceps muscles after a knee injury. An acute knee injury will cause an increase in afferent sensory information to the spinal cord. This can activate inhibitory interneurons, which in turn decrease the excitability of the motor units supplying the joint musculature. This results in weakness to the affected muscles, such as the quadriceps muscles (Gabler et al., 2013). This weakness is initially beneficial as it protects the injured joint from further injury. However, arthrogenic muscle inhibition can continue beyond the presence of any joint pathology. Quadriceps activation deficits and weakness have been shown to remain years after knee injuries such as anterior cruciate ligament (ACL) rupture or procedures such as arthroscopic meniscectomy and ACL reconstruction (Gabler et al., 2013). Further, bilateral arthrogenic muscle inhibition of quadriceps muscles has been reported after a unilateral injury, with deficits in the uninjured limb nearly equal to that of the injured limb (Urbach and Awiszus, 2002). This suggests that there is a cross-over effect within the central nervous
system (Gabler et al., 2013). The literature regarding neuroplasticity in ACL injuries suggests that these unilateral injuries not only cause neuroplasticity at the level of the spinal cord, but also result in neuroplasticity at the level of the motor cortex (Ward et al., 2015).

ACL injury is known to cause decreased proprioception, a decline in functional performance and biomechanical variations (Grooms et al., 2015a; Kapreli and Athanasopoulos, 2006; Kapreli et al., 2009; Shi et al., 2012). However, the etiological factors driving this have been poorly understood. Recently it has been hypothesised that central nervous system adaptations secondary to injury might drive these detrimental outcomes (Grooms et al., 2015a; Shi et al., 2012). An injury to the ACL disrupts proprioceptive input to the central nervous system from ACL mechanoreceptors. This loss of peripheral input is comparable to that seen in amputations and motor neuropathies (Shi et al., 2012). The loss of ascending information then drives central nervous system (including motor cortex) neuroplasticity. Kapreli et al. (2009) investigated whether chronic ACL injury causes neuroplastic changes in brain activation patterns. Changes in blood-oxygen-level-dependent (BOLD) signal using functional magnetic resonance imaging (fMRI) allowed the authors to assess brain activation patterns in seventeen right leg dominant male participants with known chronic right ACL deficiency compared to eighteen healthy participants. While inside the MRI scanner participants were asked to perform unilateral extension-flexion movements of the right knee. Compared with controls, individuals with an ACL injury at least six months old were found to have less MRI signal change (brain activation) in key sensorimotor areas. Individuals with ACL injury had increased brain activation compared to the control group in the contralateral presupplementary motor area (pre-SMA), posterior secondary somatosensory area (SIIp) and the ipsilateral posterior inferior temporal gyrus (pITG). The authors theorise that the decreased activation of key sensorimotor areas in those with ACL injury could be due to
differentiation of the ascending pathways secondary to the ACL injury, as ACL rupture has been shown to lead to decreased proprioceptive information. Given that several sensorimotor cortical areas are affected, the study suggests that ACL rupture can influence both ascending and descending pathways, leading to cortical reorganisation. Increased activation in the ipsilateral pITG may represent the increased reliance on visualisation for movement feedback due to decreased proprioceptive information, as the pITG is located in the visual cortex and may play a role in recognition of biological movements. Increased activation of the SIIp may reflect increased pain or instability in the ACL rupture participants, as this area is believed to be responsible for the reception of painful stimuli. The pre-SMA is believed to be involved in preparation for movement, and typically activates before the primary motor cortex during normal movements. Increased pre-SMA activation usually reflects increased task complexity. In ACL-deficient participants, this increased activation compared to controls in a simple task may represent an increased need for movement planning, possibly due to articular deafferentation as a result of an ACL rupture. The study shows that ACL deficiency may cause reorganisation within the central nervous system, suggesting that ACL injury could be regarded as a neurophysiological dysfunction rather than a simple musculoskeletal injury (Kapreli et al., 2009; Ward et al., 2015). There is also evidence of decreased corticospinal excitability to the musculature surrounding knee and ankle joint injuries when assessed with TMS (McLeod et al., 2015; Pietrosimone and Gribble, 2012; Pietrosimone et al., 2015b). Notably, motor cortex changes have been documented bilaterally following unilateral injury, suggesting a functional reorganisation of the motor networks (McLeod et al., 2015; Needle et al., 2017; Pietrosimone and Gribble, 2012).

In addition to poor long-term outcomes to the injured knee, a prospective study by Paterno et al. (2012) found that young athletes returning to sport from an ACL reconstruction had a
25.3% chance of experiencing a second ACL injury in either knee within a year (compared to a 2.5% chance of an initial injury in controls). Further, the study found that the rate of ACL injury in the contralateral knee after ACL reconstruction was almost three times higher than the ipsilateral knee, however these findings were not significant ($p = .09$) (Grooms et al., 2015b; Paterno et al., 2012). This suggests that there are likely bilateral neurological alterations after injury (Grooms et al., 2015b). A study by Grooms et al. (2015b) into brain activation changes with fMRI post-ACL injury by chance happened to capture brain activation changes pre-ACL injury. The study was comparing an individual with a surgically repaired ACL injury to a healthy control. Approximately one month after imaging was completed the (ACL-injured) participant injured the contralateral ACL. This allowed a unique insight into brain activation changes in an individual with a contralateral ACL injury prior to injury of the uninjured knee. The study used a similar movement protocol to that described in Kapreli et al. (2009), and found similar changes in BOLD signal as described in Kapreli et al. (2009) for the injured knee. The knee with ACL that had yet to be injured demonstrated greater activity in the lingual gyrus than the healthy control, which is similar to the result seen in the injured knee. The authors theorise that this may represent a bilateral motor-control strategy that incorporates visualisation to assist movement after the initial injury. A decrease in activity in motor-action and planning areas (contralateral supplementary motor area and primary motor areas) relative to the healthy control (and in contrast to the injured knee) may have been secondary to extensive rehabilitation targeting the injured knee. The authors suggest that the injury, reconstruction or rehabilitation may have resulted in a functional reorganisation of the motor area to assist the ipsilateral (injured) knee, therefore decreasing activation when moving the contralateral side (Grooms et al., 2015b). This finding supports the hypothesis that a unilateral knee injury results in a cascade of altered
neuromuscular control, which can lead to bilateral cortical neuroplastic adaptations that may increase the risk of a subsequent, contralateral knee injury (Grooms et al., 2015b).

2.3.3. Fracture and immobilisation

Immobilisation can induce neuroplastic motor cortex change, as discussed in 2.2 Disuse-dependent Neuroplasticity. This section reviews whether similar changes are seen in immobilisation secondary to injury, such as splinting or casting for a wrist or ankle injury, as there are other factors (such as pain and inflammation) that could impact subsequent neuroplasticity. A study by Liepert et al. (1995) investigated cortical neuroplastic changes in twenty two patients who had unilateral immobilisation of an ankle joint due to injury (in most cases due to, “complicated fracture in distal parts of the tibia or due to talus fractures,” but without any peripheral nerve lesion). The average period of immobilisation was sixteen weeks but ranging from zero to 60 weeks. TMS was used to map the motor cortex bilaterally from which MEPs could be elicited in bilateral tibialis anterior muscles. TMS was conducted in participants while they were immobilised, with two of the participants only having been immobilised within 24 hours. A control group of ten healthy individuals also underwent TMS motor mapping and were found to have almost identical tibialis anterior motor area size bilaterally. In patients who had been immobilised within nine days there was no significant difference in motor cortex size. However, in patients who had been immobilised beyond four – six weeks there was a significant decrease in motor cortex size between the immobilised and unaffected sides on TMS mapping, with these changes becoming more distinct with more prolonged immobilisation. The authors found that these changes in motor cortex area were quickly reversed by voluntary muscle contraction, suggesting that the underlying changes were functional changes in synaptic thresholds rather than structural changes (Liepert et al.,
The authors did not assess MEP amplitude changes or change in recruitment curve slope to assess corticospinal excitability. Given the extensive period of immobilisation (beyond standard care for a fracture) in some participants it is possible that multiple issues may have influenced TMS results in this study.

Subsequent studies (Zanette et al., 2004; Zanette et al., 1997) have investigated the effect of long-term limb immobilisation due to unilateral wrist fracture on motor cortical neuroplastic change. Zanette et al. (1997) investigated the cortical representations of six participants with unilateral wrist fractures (and ten controls). The participants had been immobilised for one month and then had TMS to measure MEPs in four upper limb muscles bilaterally (two proximal – deltoid and biceps brachii, two distal – flexor carpi radialis (FCR) and abductor pollicis brevis (APB)) immediately upon removal (same day) of the splint. Three participants had further TMS testing conducted after one month. The authors found that MEP amplitudes on the immobilised side were elevated in participants immediately after splint removal, however these values returned to similar levels as the unaffected arm and in controls after one month. Further, these differences in MEP amplitude disappeared when evaluated with small voluntary contraction of the muscle being tested. The study shows that long-term immobilisation with injury can lead to a reversible enhancement in excitability of structures within the motor cortex. The authors did not find any change in the area of the motor map in immobilised participants, although the study by Liepert et al. (1995) may suggest that four weeks is not long enough to induce motor map area change (Zanette et al., 1997). There are also differences between deafferentation and immobilisation for four weeks, which may explain why a change in motor map area was not seen. The organisation of the cortical motor map may be regulated by inhibitory local circuit neurons (Jacobs and Donoghue, 1991). Activity of these inhibitory circuits may be decreased due to interruption of sensory feedback
from (for example) a deafferented body part, resulting in the unmasking of pre-existing intracortical excitatory connections (Zanette et al., 1997). This could allow a rapid shift in cortical representational maps in a functional manner, resulting in increased excitability and enlargement of cortical motor maps of adjacent territories (Zanette et al., 1997). The authors suggest that the maintenance of upper limb position through immobilisation resulting in restricted movements and decreased sensory experiences induces a deafferentation-like condition in all segments of the arm. However, it differs from deafferentation as there is maintenance of sensory and motor connections between the cortex and target muscles. This maintenance of connection might explain the absence of a motor map change, but may explain the increase in excitability to all muscles as a result of decreased intracortical inhibitory circuit activity. This study may therefore indicate that sustained restriction of volitional movements and reduced sensory input (as seen in an immobilised upper limb due to a fracture) can promote functional reorganisation of the motor cortex (Zanette et al., 1997). Further research has suggested these changes may occur as a result of synchronous activation of upper limb muscles, which could occur in a forced posture secondary to splinting, leading to altered upper limb sensory feedback (Coq and Xerri, 1999; Ridding et al., 2001; Zanette et al., 2004). A subsequent study by Zanette et al. (2004) supports the notion that decreased ICI in long-term immobilised participants contributes to cortical hyperexcitability and reversible neuroplastic changes. While Zanette et al. (2004) demonstrated a decrease in SICI in immobilised muscles at rest, a similar study by Clark et al. (2010) in non-injured immobilised participants only found an increase in long-interval ICI with muscle contraction (no change in inhibition at rest). The authors hypothesised that differences observed between studies may be secondary to injury-related factors such as pain and swelling associated with injury.
Together, these studies show that immobilisation with a fracture can cause similar changes in corticospinal excitability to immobilisation in non-injured individuals, although pain and swelling may affect ICI.

2.3.4. Musculoskeletal pain

Central nervous system neuroplastic changes are also seen in response to experimental (Bank et al., 2013; Burns et al., 2016a; Burns et al., 2016b) and chronic pain (Flor, 2003; Flor et al., 1997; Goossens et al., 2018; Moseley and Flor, 2012; Parker et al., 2016). In discussing the relationship between pain and neuroplasticity it is first worth mentioning the complex relationship between nociception and pain. Pain is considered a conscious experience that often is associated with nociception, however nociception itself is not sufficient and at times not necessary for a painful experience (Moseley and Vlaeyen, 2015). Neuroplastic changes can occur both in response to nociceptive stimuli and in response to the conscious experience of pain (Moseley and Vlaeyen, 2015). Unfortunately, much of the literature presented here refers simply to pain (acute, chronic or experimental), with little distinction between pain and nociception discussed. Therefore, to avoid confusion, the term “pain” will be used if this was the terminology predominantly used in the referenced research.

The effect of acute pain on motor cortex excitability was demonstrated in a study by Farina et al. (2001). The authors used TMS to measure MEPs in five hand/forearm muscles (FDI, APB, ADM, FCR, extensor carpi radialis (ECR)) before and after the painful application of capsaicin on the skin overlying the FDI and FCR muscles. The MEP amplitudes from FDI and FCR were significantly decreased at 20 minutes after application (while pain was still
present), before returning to baseline. Measures of peripheral nerve and spinal cord excitability did not change, suggesting that changes occurred at the level of the motor cortex. These findings are supported in a similar study by Le Pera et al. (2001) in which MEPs in healthy subjects were recorded from the right ADM muscle before (baseline), during hypertonic saline injection (to induce pain) into the right ADM and right FDI, left ADM or subcutaneous regions of the right ADM, and in recovery. Spinal motor neuron excitability was also assessed by measuring the H-reflex in the right FCR muscle at baseline, during pain (injection of hypertonic saline into the right FCR) and in recovery. The study found that tonic muscle pain resulted in an inhibition of the motor cortex initially, as demonstrated by reduced MEP amplitude during pain induced in the right ADM and FDI muscles. This is followed by decreased excitability of both cortical and spinal motor neurons in recovery, as measured by a delayed depression of H-reflex amplitude in the right FCR secondary to hypertonic saline injection into the right FCR and continued reduced MEP amplitude.

A systematic review and meta-analysis by Burns et al. (2016a) found evidence for reduced primary sensory cortex (S1) and corticomotor activity during and after the resolution of acute muscle pain, with research by Schabrun et al. (2015) demonstrating that there is a similar temporal profile of altered processing in the S1 and reduced corticomotor output secondary to acute muscle pain. Further research by Schabrun and Hodges (2012) has also demonstrated that intracortical interneuronal activity is altered (increase in SICI following pain, decrease in ICF both during and after pain) in the M1 during and after pain. Cortical excitability and intracortical interneuronal networks change when acute muscle pain persists (Schabrun et al., 2016). Sustained muscle pain over four days has demonstrated increased corticomotor excitability, reduced SICI and increased ICF by day four. These changes are the opposite to those demonstrated in acute pain. The increase in corticomotor excitability may reflect
processes similar to those seen in early motor learning, in which a new motor strategy is sought due to the sustained muscle pain (Pascual-Leone et al., 1995; Schabrun et al., 2016). A reduction in ICI has been reported in chronic pain conditions (Parker et al., 2016). Conversely, as mentioned, acute pain has demonstrated an increase in ICI (Schabrun and Hodges, 2012). The reduction of ICI at day four of sustained pain may represent a shift to chronicity and reflect a release on inhibition in order to support cortical reorganization and distribution of motor activity as alternative motor strategies are required (Schabrun et al., 2016).

These pain related cortical changes may contribute to protective motor control strategies that are responsible for altered movement patterns in chronic pain conditions (Boudreau et al., 2010). A study by Graven-Nielsen et al. (1997) investigated the influence of experimental pain on resting, static and dynamic muscle activity. In the dynamic experiment saline was injected into either the tibialis anterior or gastrocnemius muscles and then muscle activity and coordination were investigated during gait on a treadmill with EMG recordings from both the tibialis anterior and gastrocnemius muscles. The authors found that there was a decrease in EMG activity in the agonistic muscle (i.e. the muscle injected), but an increase of EMG activity in the antagonistic muscle during certain components of gait. The authors suggest that this may be a functional adaptation of muscle coordination to limit movement in the painful muscle (Graven-Nielsen et al., 1997). Motor cortex activity was not measured in this study and therefore the level at which adaptation is occurring is not clear. However, the theory that pain drives specific muscle adaptations is not supported by evidence from other studies shows that both painful and antagonistic muscles can demonstrate increases in excitability (Martin et al., 2008), and that pain can result in the redistribution of activity between many different muscles (van Dieen et al., 2003).
The review article, “The role of motor learning and neuroplasticity in designing rehabilitation approaches for musculoskeletal pain disorders”, by Boudreau et al. (2010) in Manual Therapy outlined the evidence for cortical neuroplastic change in response to chronic musculoskeletal pain disorders. While the mechanisms and neuroplastic adaptations underlying chronic pain states are different to those in acute pain, chronic pain states provide evidence that an acute injury may result in long-term, persistent cortical reorganisation (Apkarian, 2011). Compared to healthy individuals, patients with chronic recurrent lower back pain have been shown to have decreased corticospinal drive in the lumbar spinal muscles (Strutton et al., 2005), a shift in the somatosensory cortex of lower back muscle representation (Flor et al., 1997), a change in centre of gravity and increased representation of the transversus abdominis muscle in the primary motor cortex (Tsao et al., 2008). Central sensitisation (increased sensitivity of cortical and spinal neurons to sensory stimuli) and maladaptive reorganisation of pain networks in the brain are also implicated in persistent pain (Apkarian, 2011; Moseley and Flor, 2012; Nijs et al., 2014; Schabrun et al., 2014). A simplified, possible cause of chronic pain as discussed by Pelletier et al. (2015b) is outlined below in Figure 1:
These central adaptations in response to a peripheral injury may be advantageous initially, especially if they protect the injured area from further injury, as discussed previously. However, should these central changes in activation persist over a long period of time they may result in prolonged neuroplastic adaptation. These adaptations can then persist beyond any tissue injury, resulting in a chronic pain state where pain is perceived without any remaining structural pathology (Boudreau et al., 2010; Pelletier et al., 2015b).
Hodges and Tucker (2011) identified that previous motor adaption to pain models did not adequately explain many clinical and experimental observations. They noted that pain does not always have a uniform effect on excitability in the motor pathway, changes in motor control in pain are not predictable and previous theories could not account for changes in all classes of movement, nor could previous theories explain the maintenance of force when motor neuron discharge was reduced in pain. A new model was proposed, in which pain involves the redistribution of activity within and between muscles and adaptations to pain changes mechanical behaviour. The changes in mechanical behaviour lead to protective patterns to avoid actual or threatened pain or injury, of which the nervous system has multiple options to implement. Adaption to pain involves changes at multiple levels of the nervous system (including within the motor cortex), and while these adaptations likely have short-term benefit they ultimately have long-term consequences. More recently, it has also been suggested that reinforcement learning (e.g. repeated behaviour to avoid/minimise pain) likely contributes to cortical reorganisation in chronic pain conditions (van Dieen et al., 2017).

Interestingly, a recent systematic review and meta-analysis by Chang et al. (2018) found conflicting evidence for altered M1 structure, organisation and function for neuropathic and non-neuropathic pain conditions. While evidence for increased M1 long-interval intracortical inhibition was identified, evidence for other M1 changes in the chronic pain population was inconclusive. Studies with larger samples and rigorous methodology are required within the chronic pain population.
2.3.5. **Burn injury**

Evidence for acute neuroplasticity after burn injury in humans is lacking. However, limited research has investigated cortical neuroplasticity in chronic neuropathic pain following burn injury. Portilla et al. (2013) investigated neuroplastic changes in the M1 in three burn injury participants with injuries at least six months old and who reported neuropathic-like pain. Cortical excitability, ICI and ICF were assessed with TMS (single-pulse and paired-pulse with an inter-stimulus interval of 2 ms for ICI and 12 ms for ICF) before and after the application of anodal transcranial direct current stimulation (tDCS). MEP amplitude showed a modest decrease after tDCS. ICI increased in all subjects after tDCS and ICF showed a decreasing trend in all subjects after tDCS. The authors suggest that these findings are the opposite of what has been described in healthy adults (anodal tDCS causing decreased ICI and increased ICF (Nitsche et al., 2005)), however this is a very small study with no statistical analysis (Portilla et al., 2013).

There is some evidence for nervous system adaption secondary to burn injury in humans. Sensory deficits can persist in even good quality, matured scars after minor, partial thickness burn injury (Lim et al., 2014). Nerve fibre density in at twelve weeks post-injury has been shown to be reduced at both injured and uninjured sites, suggesting a systemic response to burn injury (Anderson et al., 2010). In contrast, burn survivors with chronic pain have been demonstrated to an increase in density of nociceptive nerve fibres both in scar sites and also in uninjured tissue when compared to a control group (Hamed et al., 2011). There is also evidence to show that burn injury is associated with increased nervous system-related morbidity for many years following the burn injury (Vetrichevvel et al., 2016). The mechanisms underlying this are not well understood, although it is possible that a systemic
inflammatory response contributes to changes in the central and peripheral nervous system (Vetrichevvel et al., 2016). Although the evidence for central neuroplasticity in burn injuries, especially in patients other than those with chronic pain, is lacking, it is reasonable to suggest that as burn injury can have a significant effect on cutaneous and nociceptive input there would likely be secondary adaptations occurring in the motor cortex.

There is emerging evidence to show that injury to musculoskeletal structures, including amputation, peripheral nerve injury, joint and ligament injury, trauma and immobilisation can result in central nervous system neuroplastic adaptation. The evidence suggests that this neuroplasticity likely occurs at both the cortical and subcortical level, and that both short- and long-term adaptation is possible. Persistent changes in central activation, such as those seen in ACL deficiency or in recurrent lower back pain, can result in maladaptive changes that persist beyond the recovery and repair of the injured tissues. Recovery and rehabilitation from musculoskeletal injury should require careful consideration of the central adaptations.

3. Neuroplasticity, time and functional recovery

There is now overwhelming evidence to show that the human central nervous system, including the human motor cortex, is able to adapt to changes in afferent information and modulate efferent output (Buonomano and Merzenich, 1998; Chen et al., 2002; Dayan and Cohen, 2011; Flor, 2003; Pascual-Leone et al., 2005; Sanes and Donoghue, 2000). The ability of the central nervous system to respond and adapt to change impacts both function and functional recovery from injury.
It has been suggested that neuroplasticity involves both the unmasking of pre-existing connections and the formation of new pathways (Dayan and Cohen, 2011; Pascual-Leone et al., 2005). Pascual-Leone et al. (2005) proposed that the unmasking of existing connections is a prerequisite for the formation of new connections. In the 2.1 Use-dependent neuroplasticity section, rapid neuroplastic change in response to motor training was discussed (Pascual-Leone et al., 1995). However, there is also evidence to suggest that neuroplastic changes can be not only rapid, but also short- or long-term, and reversible. Pascual-Leone et al. (2005) examined long-term changes in motor cortex representations following training on a five-finger keyboard-learning task. Subjects completed two hours of a one hand five-finger exercise each day. In the first week of daily practise no modification in cortical mapping was found prior to each motor training session. However, (as discussed in 2.1 Use-dependent neuroplasticity) subjects had both rapid changes (i.e. over the course of an individual practise session) and short-term changes (i.e. over the course of five days) in cortical representations of the trained muscles; this reflects the capacity of the motor cortex to reversibly increase the excitability of cortical representations. The authors suggested that these changes likely represent the unmasking of existing connections. Furthermore, one group of participants continued daily two-hour practise over the following four weeks (Group 1), while the other group did not (Group 2). Motor cortex mapping was performed on Mondays (prior to practise in Group 1) and on Fridays (after completing practise in Group 1). In Group 1, the Monday maps showed a small increase from baseline over the course of the study. The Friday maps showed an initial increase but then eventually a slow decrease in size despite ongoing practise. These cortical map changes represent the shift away from rapid reversible changes via pre-existing connections. Instead, the authors suggest that as the task becomes overlearned the pattern of automatic cortical activation for optimal performance shifts as other neural structures take a leading role. The authors state that the flexible, short-term
modulation of existing pathways represents a necessary step to facilitate longer-term structural changes within the intracortical and subcortical networks. The Group 2 cortical maps returned to baseline after the first week. These neuroplastic changes are supported by other studies that also demonstrate an initial, fast learning stage associated with the strengthening of connections and then a slow learning stage in which synaptic reorganisation may occur (Dayan and Cohen, 2011; Hayashi et al., 2002; Karni et al., 1998). The evidence suggests that motor skill training causes rapid cortical neuroplastic changes that will then continue to evolve with ongoing training (Dayan and Cohen, 2011; Hayashi et al., 2002; Karni et al., 1998).

As discussed in 2.2 Disuse-dependent neuroplasticity, immobilisation can induce changes in cortical excitability. Immobilisation has been shown to also impact subsequent motor function. In a recent study by Opie et al. (2016) participants underwent two experimental sessions on consecutive days in which TMS was used to assess corticospinal excitability before and after a grooved pegboard task. The pegboard task involved subjects selecting a peg from a well and then placing the peg into a hole, requiring precise alignment and a high degree of fine motor control. Prior to the second session one group of participants had their left index finger immobilised for eight hours while a control group were allowed normal limb use. Participants who had their index finger immobilised showed improvement within the second pegboard session similar to controls, however did not show any improvement at the start of the second session compared with the start of the first session. In comparison, control participants performed better at the start of the second session relative to the start of the first session. The results show that immobilisation, in addition to modulating use-dependent neuroplastic adaptation, can also affect the retention of motor skills (Opie et al., 2016).
In addition to modulation of use-dependent neuroplastic adaptation and motor skill retention, the study also found that short-term immobilisation resulted in decreased cortical excitability. However, changes in cortical excitability induced by training were greater in those who had been immobilised, suggesting increased use-dependent neuroplasticity following immobilisation. Similar changes in cortical excitability due to short-term immobilisation (typically less than 4 days) have been described in other studies and longer immobilisation (greater than 10 days) has been found to have the opposite effect (as discussed in 2.2 Disuse-dependent neuroplasticity and 2.3 Musculoskeletal injury-induced neuroplasticity).

As demonstrated by Opie et al. (2016), even short-term immobilisation can cause a reduction in cortical excitability and impair motor performance. Cross education (or contralateral) strength training refers to the maintenance (or increase) of a muscle’s size and strength due to unilateral training in the homologous muscle of the opposite limb (Carroll et al., 2006; Hendy and Lamon, 2017; Pearce et al., 2013). Multiple studies investigating cross-education when one limb is immobilised have found that the immobilised limb will maintain muscle size and strength when the other limb is engaged in strength training (Carroll et al., 2006; Goodwill et al., 2012; Kidgell et al., 2011; Pearce et al., 2013). The mechanism for this is likely secondary to central nervous system adaptation, as the cross transfer of strength occurs without muscle hypertrophy (Goodwill et al., 2012; Pearce et al., 2013). Pearce et al. (2013) used TMS to measure corticospinal responses following three weeks of unilateral arm training in a group with the contralateral arm immobilised, compared to a group with an immobilised arm with no training and a control group. The immobilised and trained group had no change in strength, muscle thickness or corticospinal excitability compared to
baseline. In contrast, the immobilised and untrained group showed a reduction in strength, muscle size and corticospinal excitability (as reflected by decreased MEP amplitude). The maintenance of normal corticospinal excitability in the training group provides evidence that M1 adaptation underpins the maintenance of contralateral strength (Pearce et al., 2013). This is consistent with other studies that have also demonstrated adaptive changes such as increased corticospinal excitability, reduced interhemispheric inhibition, reduced SICI and increased voluntary activation in the ipsilateral M1 as a result of unilateral strength training (Goodwill et al., 2012).

Im mobilisation, use-dependent neuroplasticity and cross-education all have implications in functional impairment and recovery. The role of neuroplasticity in functional recovery from a musculoskeletal injury is discussed in 4. Neuroplasticity in injury rehabilitation.

4. Neuroplasticity in injury rehabilitation

There is an increasing recognition not only of the neuroplastic changes that occur as a result of injury, but also the role that neuroplasticity plays in injury recovery and rehabilitation. While addressing neuroplastic changes in central nervous system injuries such as stroke is beyond the scope of this review, it is worth briefly discussing functional rehabilitation and neuroplasticity in this population. Multiple studies in both animals (particularly rodents) and humans have shown that the neuroplasticity after stroke plays a central role in functional recovery, and that the extent of functional recovery is positively correlated with the extent of neuroplastic change in stroke rehabilitation (Caleo, 2015; Di Lazzaro et al., 2010; Hamdy et al., 1998; Jones, 2017; Jones et al., 2009; Kleim et al., 2003; Ramanathan et al., 2006).
Further, there is significant evidence that rehabilitation strategies that address the potential for neuroplastic changes have an impact on functional recovery (Furlan et al., 2016; Jones et al., 2009). For example, constraint-induced therapy (CIT) has been developed for use in patients with chronic deficits due to stroke. CIT involves immobilising the unaffected limb in stroke patients to force them to use the affected limb in their daily activities (Hakkennes and Keating, 2005; Kunkel et al., 1999; Oujamaa et al., 2009; Peurala et al., 2012). CIT has been shown to improve the amount of use of the affected extremity in real world environments (Kunkel et al., 1999; Pollock et al., 2014), with cortical mapping by TMS showing ongoing modification of cortical activity (Liepert et al., 2000; Oujamaa et al., 2009). Rehabilitation in this population focuses on repetitive, meaningful and task-specific movements to maximise cortical neuroplastic change (Boudreau et al., 2010; Hakkenes and Keating, 2005). This approach may be useful when trying to address neurological adaptations secondary to musculoskeletal injury (Boudreau et al., 2010; Snodgrass et al., 2014).

This review has outlined that central neuroplastic changes occur, both rapidly and ongoing, in response to use (i.e. motor learning with repetitive, meaningful practise), disuse (both short- and long-term) and musculoskeletal injuries, including amputation, nerve injury, joint and ligament injury, fracture and subsequent immobilisation, musculoskeletal pain and burn injuries. The potential for neuroplastic changes to impact functional outcomes in musculoskeletal injuries is becoming evident, with neuroplasticity having an important role in the retention of motor skills in response to practise. Further, maladaptive neuroplasticity can predispose to impaired functional recovery and adverse outcomes, as discussed regarding ACL injuries and the risk for subsequent contra-lateral injury (2.3.2 Joint and ligament injury). Central neuroplastic changes are likely influenced by alterations in sensory processing, such as nociception, proprioception and cutaneous input, and result in changes to
motor planning and activation that may therefore play an important and at times obstructive role in rehabilitation from these injuries. Despite the likelihood of a central component in these injuries traditional rehabilitation in musculoskeletal injuries (with the exception of chronic pain) has targeted the affected area with little regard for neurological changes (Grooms et al., 2015a; Pelletier et al., 2015a; b; Snodgrass et al., 2014).

Given the importance of neuroplasticity in injury and motor learning, there is an ongoing call for the recognition of central neuroplasticity in musculoskeletal injuries and the need for rehabilitation strategies that address this. An their Manual Therapy article, “Recognising neuroplasticity in musculoskeletal rehabilitation: a basis for greater collaboration between musculoskeletal and neurological physiotherapists”, Snodgrass et al. (2014) commented that recognising and addressing neuroplasticity in patients with musculoskeletal dysfunction may not only lead to greater understanding of the neural mechanisms involved, but that addressing maladaptive neuroplasticity may improve the effectiveness of treatments that target motor behaviour. The article provides an example of how task-specific training protocols that influence motor learning and neuroplasticity could be implemented into a shoulder rehabilitation protocol (discussed further in 4.2 Skilled Training (intense repetition and task-specific)). These authors are not alone in recognising the potential that rehabilitation targeting neuroplastic changes could have on recovery from musculoskeletal injury. In their research into brain neuroplasticity in ACL injury, Kapreli et al. (2009) commented that,

“If differences in brain activation could be justified between injured and non-injured individuals, the rehabilitation strategies might have to be revised, provided that certain therapeutic components have influence in facilitating brain plastic changes that lead to beneficial functional outcomes.”
The authors proceed to state that rather than only optimising peripheral neuromuscular function, rehabilitation strategies should focus on central nervous system re-education as well. Given the adaptive abilities of the central nervous system, therapists should identify goals and provide tools to allow the central nervous system to find a solution (Kapreli et al., 2009). Recognition of the need to address neuroplasticity in musculoskeletal injury and rehabilitation has become increasingly prevalent (Boudreau et al., 2010; Gabler et al., 2013; Kapreli et al., 2009; Moseley and Flor, 2012; Pelletier et al., 2015a; b; Schabrun and Chipchase, 2012; Shi et al., 2012; Snodgrass et al., 2014; van Vliet and Heneghan, 2006). As Snodgrass et al. (2014) point out, whilst therapists are working with a non-lesioned brain, “the neurological basis of neuroplasticity and potential for motor learning is the same as for the person with brain damage such as stroke.”

This section reviews some of the existing and emerging approaches to musculoskeletal rehabilitation that address or account for underlying central neuroplastic adaptations.

4.1. Strength maintenance in immobilised limb

As discussed in 3. Neuroplasticity, time and functional recovery, cross-education through unilateral strength training can promote the maintenance (or even increase) in muscle size and strength of the homologous muscle of the contralateral, immobilised limb. These findings in healthy adults have also been investigated in an injured population. Magnus et al. (2013) investigated the effects of cross-education through unilateral strength training on muscle strength, range of motion and function during recovery from a contralateral distal radius fracture (Magnus et al., 2013). Women aged older than 50 years were randomised to a training group (standard rehabilitation plus strength training) or a control group (standard
rehabilitation) within two weeks after injury. Those in the training group were provided with a progressively increasing strength training program for the non-injured limb to be conducted at home three times per week, for a total of 26 weeks. The control and training groups were given standard range of motion exercises while in the cast. When the cast was removed, the both groups were given exercises to improve range of motion, stretching exercises and strengthening exercises (ten – twelve times per day) to the injured limb. After twelve weeks both groups were encouraged to continue these exercises with no limitations on activity levels. The average cast duration was 40.4 (± 5.2) days. Assessment of strength in the injured arm took place at weeks nine, twelve and 26 after injury. The study found that strength training in the non-injured arm was associated with improved strength and range of motion at twelve weeks post-fracture in the injured arm compared to the control group. There was no significant difference at nine weeks or 26 weeks. The study was not able to safely measure maximum strength at six weeks (time of cast removal) so no data is available on strength immediately after removal from immobilisation. It is unclear why there was no difference in strength at nine weeks, although the authors theorise that participants may have had too much pain to comfortably perform a maximal handgrip test. Nonetheless, the study shows that unilateral strength training of an uninjured limb can improve rehabilitation and recovery of an injured limb through cross-education.

These findings have important clinical implications and may be applicable in other settings. Rehabilitation targeting the uninjured limb could result in the maintenance of strength in an injured limb and/or expedite rehabilitation once the injured limb can be mobilised again.
4.2. Skilled training (intense repetition and task-specific)

In the stroke population, repetitive and task-specific practice has been shown to have the greatest effect on neuroplasticity, as neuroplasticity is experience and practice dependent (Richards et al., 2008; Snodgrass et al., 2014). As mentioned, the neurological basis for neuroplasticity is the same in the non-lesioned brain as in the lesioned brain. Therefore, repetitive task-specific training has the greatest potential for treating movement disorders and pain associated with musculoskeletal conditions (Snodgrass et al., 2014).

In their Manual Therapy article, “Motor control and the management of musculoskeletal dysfunction”, van Vliet and Heneghan (2006) discuss how task-specific movements such as “reach to grasp” involves two mechanisms, both feedback and feedforward. Feedback occurs through vision and proprioception, while feedforward (anticipatory control) mechanisms act to maintain stability, adjust to changes and anticipate reactive forces. Task-specific motor training has been shown to be beneficial in neurologically impaired patients (van Vliet and Heneghan, 2006). The authors suggest that this may occur as performing a simple task such as isolated wrist extension does not engage all the appropriate feedback and feedforward mechanisms of motor control that would be involved in wrist extension within a reaching to grasp task. The authors state that for optimal skill acquisition task-specific training is likely beneficial in the rehabilitation of those with musculoskeletal dysfunction and an intact central nervous system (van Vliet and Heneghan, 2006). With this in mind, Snodgrass et al. (2014) outline an example of a musculoskeletal rehabilitation regime for shoulder pain that applies the principles of experience dependent neuroplasticity. Components include part (individual movements) and whole (whole shoulder movements) practice, repetitive practice, task-specific training (i.e. reaching for overhead objects) and focus of attention.
There is evidence to show that in skilled movement training, motor-skill training coupled with strength training does not promote cortical neuroplastic change more than motor-skill training alone (Remple et al., 2001). This is likely because targeting specific movements requires greater skill, attention and precision than strength training in which all muscles are contracted (Boudreau et al., 2010). A study by Jull et al. (2009) investigated the effects of a low load cranio-cervical flexion (C-CF) exercise or neck flexor strengthening exercise for six weeks on the temporal and spatial characteristics of deep cervical flexor muscle (DCF) activation in those with chronic neck pain. C-CF exercises targeted the deep cervical flexor muscles and involved task and movement specific exercises, whereas strength training involved a non-specific progressive resistance program of the neck in which no muscles were specifically targeted. EMG activity was recorded from the DCF, sternocleidomastoid and anterior scalene muscles pre- and post- intervention. C-CF training increased DCF EMG amplitude (known to be impaired in those with neck pain) whereas no change in DCF EMG amplitude occurred following strength training. The study suggests that exercises that target specific deficits with task-specific techniques can achieve better rehabilitation outcomes than those using non-specific strength training. Likewise, more complex tasks that demand more cognitive effort can increase the extent of cortical neuroplasticity relative to simple tasks (Boudreau et al., 2010; Pascual-Leone et al., 1995; Sadato et al., 1996). Tsao et al. (2010) demonstrated that skilled motor training in participants with recurrent lower back pain was not only associated with improved functional outcomes, but also demonstrated a shift in motor cortical representation (towards that of a healthy population, as assessed with TMS) compared to participants who performed an unskilled walking exercise. The study demonstrates that skilled motor training can reverse reorganisation in the motor cortex of people with recurrent pain. Together these studies show that repetitive task-specific training
is a useful rehabilitation intervention that can target beneficial adaptive neuroplastic processes and reverse maladaptive neuroplastic reorganisation.

4.3. Mental imagery and action observation

Mental imagery is also being shown to have potential uses in musculoskeletal rehabilitation (Mulder, 2007; Snodgrass et al., 2014). In the previously discussed Pascual-Leone et al. (1995) study (discussed in 2.1 Use-dependent neuroplasticity) in which participants had to learn a five-finger piano exercise, the authors also found that mental practise generated the same cortical changes as physical practise. An extra group of participants had the same daily cortical mapping as the first two groups, however instead of physically practising this group was asked to visualise their fingers performing the exercise and hearing the sound. After 5 days, the cortical maps were very similar to the group who physically practised. This finding suggests that mental practise may be sufficient to cause cortical neuroplastic change; a finding that may have important implications in the rehabilitation of patients in whom movement is difficult. A systematic review by Slimani et al. (2016) found that mental imagery in patients with ACL injuries or immobilisation (e.g. due to wrist fracture) was effective at reducing strength loss and recovering motor function. Short-term immobilisation can lead to a decrease in corticospinal excitability (discussed in 2.2 Disuse-dependent neuroplasticity). Bassolino et al. (2014) demonstrated that action observation (observing hand actions performed by other individuals) during immobilisation prevented the decrease in corticospinal excitability (as measured by TMS) otherwise seen in the control group. Interestingly, imagining the action with the immobilised hand did not prevent the decrease in corticospinal excitability secondary to immobilisation. Mental imagery and action observation have the potential to encourage neuroplastic adaption or prevent maladaptive
change in patients who are immobilised due a musculoskeletal injury. When pain limits movement, mental imagery and action observation may be implemented used to induce beneficial neuroplastic change and prevent maladaptive changes (Snodgrass et al., 2014). The use of mental imagery in chronic pain is briefly discussed in 4.6 Consideration of pain in rehabilitation.

4.4. Focus of attention

Attention has been shown to increase the release of modulatory neurotransmitters and therefore enhance activity-dependent neuroplasticity (Moucha and Kilgard, 2006). In stroke rehabilitation patients often use an external focus of attention such as knocking an object off a table. In contrast, musculoskeletal rehabilitation often utilises an internal focus of attention, such as focusing on posture or pain (Snodgrass et al., 2014). Evidence from the stroke population suggests that an external focus of attention is more effective at improving motor performance (van Vliet and Wulf, 2006), and therefore this technique may provide more benefit than internal focus in musculoskeletal rehabilitation (Snodgrass et al., 2014). Laufer et al. (2007) investigated the effect of attentional focus (internal vs external) and dynamic balance training on ankle sprain recovery. Forty participants with a grade one or two ankle sprain within the last four months underwent baseline stability assessments and were randomly allocated to either an internal focus of attention or an external focus of attention instruction while balancing. After three training sessions stability was reassessed. Stability increased in both groups after training, however those participants with an external focus of attention (“keep your balance by stabilising the platform”) had greater improvement than the internal focus of attention (“keep your balance by stabilising your body”) group. Reviews of attentional focus in musculoskeletal rehabilitation support these findings, showing that an
external attention of focus can lead to significant improvements in motor performance compared to an internal focus of attention (Hunt et al., 2017; Sturmberg et al., 2013).

4.5. Augmented feedback

As discussed in 2.3.2 Joint and ligament injury, knee injury or surgery can result in arthrogenic muscle inhibition, resulting in persistent quadriceps weakness despite no ongoing structural damage. An example of electromyographic biofeedback (EMG-BF) has been used in conjunction with exercise to increase quadriceps activation and strength (Gabler et al., 2013). EMG-BF is able to measure electrical activity in the muscle and provide the patient with visual or auditory feedback regarding the magnitude of muscular activation. EMG-BF can provide the attention component for enhancing activity-dependent neuroplasticity and thereby increase quadriceps activation and strength, likely due to, “improving motor unit recruitment and optimising firing rates through cortically generated mechanisms” (Gabler et al., 2013). A systematic review by Sturmberg et al. (2013) into attentional focus of feedback in musculoskeletal dysfunction found no evidence of improvement in motor performance, function or pain with EMG-BF, however this was limited to two studies with small sample sizes. A recent study by Pietrosimone et al. (2015a) found increases in corticospinal excitability and increased maximal voluntary isometric contraction in the quadriceps with EMG-BF compared to no biofeedback in healthy individuals. The evidence for the use of EMG-BF in musculoskeletal rehabilitation remains mixed, although it does appear to be effective at improving quadriceps strength and overall knee function post-operatively (Gatewood et al., 2017; Giggins et al., 2013; Wasielewski et al., 2011). Verbal augmented feedback (external verbal instructions or cues) can be used to provide an external focus of attention, and has been shown to be effective for improving lower limb biomechanics and
postural control in the rehabilitation of lower extremity musculoskeletal dysfunction (Storberget et al., 2017).

### 4.6. Consideration of pain in rehabilitation

An awareness of the impact of neuroplasticity in musculoskeletal injury and rehabilitation also requires an awareness of factors that may influence or impact this. Acute pain has been shown to affect neuroplasticity (as discussed in 2.3.4 Musculoskeletal pain). Boudreau et al. (2007) investigated the impact of pain on rapid neuroplastic changes associated with learning a novel task. TMS was applied to the M1 both immediately before and after 15 minutes of training in a tongue protrusion task, with MEPs measured from the tongue musculature (and FDI muscle as a control). Those who had a vehicle cream (i.e. no algesic properties) applied to their tongue prior to training demonstrated an enhanced MEP stimulus-response curve and a reduced MEP threshold. However, those who had the capsaicin cream applied prior to training did not demonstrate these changes. Likewise, performance scores were higher in those who had the vehicle cream applied than in those who had capsaicin cream applied. This experiment demonstrates that acute pain can interfere with the rapid neuroplastic changes that would otherwise occur with a tongue training task. However, these findings may be secondary to performance modification due to pain, and if movement quality is maintained the training-induced changes may not have been influenced by pain (Hodges, 2011). Pain may therefore modulate motor cortex neuroplasticity and impair the ability to learn new tasks, or at the very least impair the ability to perform quality movement, thereby impairing learning. Therefore, adequate analgesia should be given to patients who are rehabilitating from an injury (Boudreau et al., 2010). Furthermore, there is some limited evidence within animal studies to suggest that motor-training can prevent central neuroplastic changes that
may otherwise occur in response to experimental pain (Boudreau et al., 2010; Hook et al., 2008). Whilst further research into this area is required, early motor training in an acute injury may reduce the risk of unfavourable neuroplastic changes that may otherwise occur as a result of the painful stimuli (Boudreau et al., 2010).

While a review of the chronic pain rehabilitation literature is beyond the scope of this article, it is worth briefly discussing rehabilitation protocols emerging within chronic pain rehabilitation as many of the therapeutic approaches implemented have been shown to normalise cortical changes that are associated with chronic pain (Moseley and Flor, 2012). Moseley and Flor (2012) outlined therapeutic strategies that could be implemented to target the underlying neuroplasticity in chronic pain conditions. Treatments were grouped as cognitive-behavioural, sensory and motor strategies. The cognitive-behavioural approach aims to reduce feelings of helplessness and uncontrollability while also establishing a sense of control over the pain and implement behaviours that limit the impact of pain on the patient’s quality of life. Within this approach, education alone has been found to positively impact on pain-related knowledge, catastrophizing and participation in subsequent cognitive-behavioural rehabilitation. For example, Moseley (2004) found that chronic lower back pain patients had improved physical performance (as measured by change in straight leg raise and forward bending) after a one-to-one education session in which there was a change in pain cognition. The research suggests that education should play a key role in changing pain attitudes and beliefs in the management of chronic pain conditions. Strategies to normalise sensory representations (in the cortex) requires patients to discriminate between different stimuli at the area of injury (e.g. stump in amputees with phantom limb pain). This based on the theory that sensory input that is salient or functionally important is more likely to induce changes in cortical representation. This approach has been shown to be effective in reducing
pain and disability (Moseley, 2004). Strategies to normalise motor representations aim at correcting cortical body maps to remove an incongruence between motor commands and sensory feedback. This strategy includes techniques such as graded motor imagery, which includes left/right limb judgement, imagined movements and mirror therapy, which has been shown to be effective in chronic regional pain syndrome and phantom limb pain (Moseley, 2004). There is also evidence to suggest that a reduction in pain can reverse cortical changes that occurred in response to chronic pain, as demonstrated by Seminowicz et al. (2011). Decreased cortical thickness and excessive activity during cognitive tasks was normalised in chronic lower back pain patients who had reduced pain secondary to zygapophyseal joint block or spinal surgery.

The chronic pain literature suggests that therapeutic strategies that address underlying neuroplastic processes can be effective in rehabilitation and reduction of pain in musculoskeletal conditions.

4.7. Therapeutic use of non-invasive brain stimulation techniques

The literature reviewed above shows (1) that the mature human cortex is capable of neuroplastic changes, (2) these changes occur following a musculoskeletal injury, and (3) injury-induced neuroplasticity is associated with functional recovery. Therefore, there is much interest in techniques that can induce neuroplasticity in the human cortex. Non-invasive brain stimulation (NIBS) techniques, such as TMS and transcranial direct current stimulation (tDCS) are able to modulate brain neuroplasticity and potentially prime the brain for rehabilitation (Hulme et al., 2013; Müller-Dahlhaus and Ziemann, 2015; Vallence and
Ridding, 2014). Repetitive transcranial magnetic stimulation (rTMS) applied over a specific time period and at a specific frequency is able to induce long-term potentiation-like changes in the brain, similar to those seen in learning (Buetefisch et al., 2015; Goldsworthy et al., 2012; Huang et al., 2005). rTMS has been utilised as priming tool for rehabilitation in the stroke population. While a review by Hao et al. (2013) did not find evidence to support the routine use of rTMS for the treatment of stroke there has been evidence since then that suggests that rTMS can improve motor outcomes in stroke patients and may play a role in neurorehabilitation strategies (Bashir et al., 2016). Theta-burst stimulation (a form of rTMS) appears to be an effective priming tool to modulate M1 neuroplasticity in younger adults but is ineffective in older adults (Opie et al., 2017; Schabrun and Chipchase, 2012). rTMS has been coupled with repeated passive-movement associative stimulation to modulate corticospinal excitability which potentially has therapeutic applications (Edwards et al., 2014). In chronic pain, rTMS has been shown to be effective at reducing pain scores (Goudra et al., 2017), and there may be a role for implementing rTMS in acute pain conditions to prevent the transition to chronic pain (Jodoin et al., 2017). There is recent evidence for enhanced cortical and motor function after tDCS, with some studies showing increases in strength after a single session of tDCS in healthy adults (Hendy and Kidgell, 2013). Peripheral electrical stimulation (PES) has also been shown to be an effective technique for modulating neuroplasticity (Schabrun and Chipchase, 2012). PES can be a useful tool to prime the corticomotor pathway alone, but can also be used in conjunction with other treatments (i.e. combined tDCS/PES has been shown to reduce pain and sensitisation as well as normalising motor cortical organisation and improve sensory function in individuals with chronic recurrent lower back pain) (Schabrun and Chipchase, 2012; Schabrun et al., 2014). A detailed review of the role of NIBS in modulating neuroplasticity and impacting functional outcomes is beyond the scope of this article, but by enhancing connectivity in the brain of the
patient with an acute musculoskeletal injury it may be possible to achieve greater motor learning and improved clinical outcomes than with traditional therapies alone (Bolognini et al., 2009; Schabrun and Chipchase, 2012).

5. Conclusion

Central nervous system neuroplasticity is a fundamental component of learning and memory formation. The central nervous system should be thought of as fluid in its ability to adapt; responding to ever-changing stimuli and demands. Animal and human models have demonstrated that both the motor and somatosensory cortex is capable of reorganisation in response to experience, learning and injury, and that the amount of cortex and strength of neural connections dedicated to certain areas are modifiable. Rapid changes in cortical neuroplasticity likely represent the motor cortex’s ability to reversibly increase its influence on the motor pool, whereas long-term practice results in the formation of new connections. Repetitive, meaningful behaviour is capable of causing lasting changes within the brain, as is mental imagery and action observation. Motor skill training that takes advantage of these neurophysiological changes will cause rapid cortical neuroplastic changes that will continue to evolve with ongoing training.

Evidence of substantial neuroplastic changes in musculoskeletal injuries is emerging. Chronic and acute pain influences neuroplasticity and can lead to a variety of central adaptations that may persist beyond any peripheral structural injury. Joint injuries can deprive the brain of important sensory information, to the point that an ACL injury could be considered a deafferentation injury. Altered sensory input causes bilateral changes in neurological
activation that can persist beyond the acute phase of the injury and predispose individuals to further injury due to altered biomechanics. Joint immobilisation can modulate cortical excitability and impair motor learning and memory consolidation. While awareness of the impact that a musculoskeletal injury can have on the central nervous system is increasing there is still a tendency to manage musculoskeletal injuries with traditional therapies that only treat local structural pathology.

Awareness is increasing of the need to consider neurophysiological changes in the management of musculoskeletal trauma. By not addressing central changes rehabilitation outcomes may be delayed. Lessons from the stroke population illustrate the extent of functional recovery is correlated with the extent of neuroplastic change and that therapies that are repetitive, task-specific and meaningful optimise recovery. Targeted rehabilitation techniques are available and should be utilised, addressing both peripheral structural changes and central adaptations. There are emerging techniques including mental imagery, cross education, focus of attention, augmented feedback and education that have promising results in rehabilitation of musculoskeletal injuries by accounting for neurophysiological changes. Managing pain adequately may prevent the detrimental impact it can otherwise have on neuroplasticity, and early musculoskeletal training may prevent pain-related maladaptive neuroplastic changes. Furthermore, NIBS techniques (such as rTMS and tDCS) may play a promising role in priming the brains of patients for rehabilitation, increasing connectivity and potentially improving therapeutic outcomes.

Currently our knowledge of acute neuroplastic change in acute musculoskeletal injuries is limited. Evidence is emerging in orthopaedic injuries, especially joints, but is lacking in other
acute injuries such as burns. As the evidence shows, cortical maps can change and adapt quickly. While harnessing these changes in rehabilitation is important, it is important to recognise that maladaptive changes may be occurring early as well. Changes in cortical maps may have occurred before any rehabilitative effort even begins. However, to date the capacity for change, the amount of change and the motor training required to reverse these acute changes in many conditions is largely unknown. If we can quantify the capacity for neuroplastic change in musculoskeletal injuries and the extent to which this impacts functional outcomes we will develop a greater understanding of the neurological mechanisms involved (Snodgrass et al., 2014). Research that quantifies the neuroplastic capacity for change (e.g. changes in corticospinal excitability and ICI) in the injured patient, as well as the amount and type of rehabilitation needed for improved functional outcomes in motor performance, could provide guidelines for each condition that may be tailored to the individual (van Vliet and Heneghan, 2006).
6. Bibliography


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7. Figure captions

Fig. 1. Neuroplasticity and chronic musculoskeletal disorders

Initial peripheral (e.g. musculoskeletal) injury causes altered sensory input, which includes enhanced nociceptive/neuropathic stimuli and altered cutaneous and proprioceptive input. This altered input can alter sensorimotor organisation and processes within the central nervous system (CNS), which in turn impacts a patient’s perception, pain and motor control processes (Pelletier et al., 2015b).
CHAPTER THREE – Decreased Neuroplasticity in Older Burn Injury Survivors Compared to Non-Injured Older Adults

Foreword

Chapter Two has outlined that neuroplastic adaptations occur in musculoskeletal injuries and impact the rehabilitation and recovery from these injuries. Targeted interventions can aim at harnessing beneficial neuroplastic adaptations or reversing maladaptive neuroplasticity. Older adults are especially at risk, given they have a decreased capacity for neuroplasticity relative to younger adults. NIBS techniques may be able to modulate neuroplasticity to prime the brain for rehabilitative intervention, thereby maximising motor learning and improving functional outcomes. However, in order to implement these techniques an understanding of the neuroplastic adaptations that occur in each specific injury is required. Currently, there is no known data available on the neuroplastic adaptations that occur acutely (within the first twelve weeks) post-burn injury in older adults.

Chapter Three presents the manuscript, “Decreased neuroplasticity in older burn injury survivors compared to non-injured older adults”. This manuscript is currently under review in Clinical Neurophysiology. This manuscript investigated the relationship between acute burn injury, rTMS-induced neuroplastic responses (at six and twelve weeks post-injury) and functional and quality of life outcomes at twelve weeks post-injury in older adults compared to non-injured, age-matched participants. Six and twelve weeks post-injury were chosen for investigation as burn injury outpatients follow up in the State Adult Burn Unit at Fiona Stanley Hospital at this time, and therefore minimal further travel is required to also attend the TMS laboratory at Murdoch University.
The manuscript is presented in the form that was submitted for publication, including an Abstract, Keywords, Highlights and a Bibliography. All references from this manuscript are presented in the Bibliography at the end of the manuscript, not in the Bibliography at the end of the thesis (although they may be in the final Bibliography as well). Figure captions are also presented after the Bibliography, as they were also required to be presented separate to the figures for publication. Note that the figure numbers in this manuscript do not continue from previous figures in this thesis, instead the first figure presented in the manuscript is presented as Figure 1.

Appendices are not referred to in the manuscript as these were not submitted for publication but are provided in this thesis. The attached appendices are applicable to both this manuscript and the manuscript in Chapter Four.

- Appendix A outlines the inclusion and exclusion criteria for burns survivors for participation in the research conducted. Non-injured control participants were subject to the same criteria, except they were to have no history of a burn injury requiring medical treatment.
- Appendix B provides the Transcranial Magnetic Stimulation Adult Safety Screen form. This is adapted from the guidelines by Rossi et al. (2009). All participants completed this form and were excluded from the study if any contraindications to TMS were identified.
- Appendix C provides further information regarding each of the Functional and Quality of Life measures utilised in the research.
• Appendix D outlines the reasons that four of the Burns Group participants were lost to follow up and therefore did not complete the second experimental session.

• Appendix E is a table that presents injury details for each of the Burns Group participants, including those lost to follow up.

• Appendix F is a list of drugs that form strong or simply relative hazards for the use of TMS. This list was adapted from Rossi et al. (2009).

• Appendix G provides the mean scores (and standard deviation) for the Burns Group and Control Group in each of the SF-36 Domains.
Decreased neuroplasticity in older burn injury survivors compared to non-injured older adults.

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Abstract

Objective: To investigate repetitive transcranial magnetic stimulation (rTMS)-induced neuroplastic changes in the primary motor cortex (M1) post-burn injury in adults aged over 45, and investigate whether capacity for neuroplasticity is associated with time and functional recovery from burn injury.

Methods: Burns Group and Control Group participants underwent two experimental sessions, six and twelve weeks post-injury. Transcranial magnetic stimulation (TMS) assessed motor-evoked potentials (MEPs) from the M1, which were obtained pre- and post-spaced continuous theta-burst stimulation (cTBS). Functional/Quality of Life measures were obtained in the experimental session twelve weeks post-injury.

Results: Control Group showed decreased MEP amplitude 15 – 30 minutes (but not 0 – 5 minutes) after spaced cTBS in both sessions. No evidence of significant MEP amplitude change after spaced cTBS in the Burns Group. Burns participants showed a significant positive relationship between general health scores and MEP amplitude change post-spaced cTBS at twelve weeks post-injury.

Conclusions: Non-injured older adults demonstrated delayed decrease in MEP amplitude after spaced cTBS, reproducible at a group level across two sessions, that was not demonstrated in burn injured participants.

Significance: Results suggest rTMS-induced neuroplastic response is reduced in individuals with a minor burn injury, and may suggest a role of neuroplasticity in recovery from burn injury.
Keywords: neuroplasticity, burn, ageing, injury, repetitive TMS, motor cortex

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Highlights:

- Burn injury participants showed no significant rTMS-induced neuroplastic response at six or twelve weeks post-injury.
- Non-injured older adults showed delayed but not immediate rTMS-induced neuroplastic brain response that is reproducible across sessions.
- Minor burn injury might be associated with reduced rTMS-induced neuroplastic brain responses.
1. Introduction

Burn injuries cause significant pain and trauma. Rehabilitating from a burn injury is often a slow and challenging process, with physical dysfunction and quality of life often impacted long after the initial injury (Klein et al., 2011; Renneberg et al., 2014; Wasiak et al., 2014). With an ageing population, the proportion of elderly burns survivors is increasing, and with improving standards of care the mortality rate of elderly burns victims is decreasing (Edgar et al., 2013). However, age has a significant negative impact on burns victims’ morbidity and mortality: older survivors often have prolonged wound healing times, require more intense rehabilitation and have longer hospital stays (Abu-Sittah et al., 2016; Duke et al., 2012; Edgar et al., 2013; Pham et al., 2009; Rani and Schwacha, 2012). Older burns survivors also experience decreased mobility, loss of independence, limited function, pain, depressed mood and poor nutrition (Abu-Sittah et al., 2016). Research from the State Adult Burn Unit (Western Australia) shows that deterioration in physical function post-burn injury is greater in older adults than younger adults, with this deterioration apparent from approximately 45-50 years of age (Edgar et al., 2013).

Strong evidence suggests that structural and functional neuroplasticity underlies the ability to acquire, consolidate and retain motor skills (Dayan and Cohen, 2011). Neuroplasticity is also thought to underlie functional recovery following brain injury, such as stroke (Di Lazzaro et al., 2010; Hamdy et al., 1998; Takeuchi and Izumi, 2012), as well as following musculoskeletal injury (Boudreau et al., 2010; Pelletier et al., 2015b). Neuroplastic changes have been observed following a variety of musculoskeletal injuries in both acute and chronic stages, including tendons (Heales et al., 2014; Rio et al., 2016), muscles (On et al., 2004), ligaments and joints (Berth et al., 2009; Berth et al., 2010; Grooms et al., 2015a; Grooms et
al., 2015b; Héroux and Tremblay, 2006; Kapreli and Athanasopoulos, 2006; Kapreli et al., 2009; Shi et al., 2012; Te et al., 2017; Ward et al., 2015), and in chronic/recurrent lower back pain (Elgueta-Cancino et al., 2018; Flor et al., 1997; Goossens et al., 2018; Schabrun et al., 2017; Strutton et al., 2005; Tsao et al., 2008; 2010). Such evidence of neuroplastic changes following musculoskeletal injury has led to rehabilitation strategies targeting maladaptive neuroplastic changes induced by injury, rather than focusing solely on the injured tissue (Boudreau et al., 2010; Pelletier et al., 2015a; b; Snodgrass et al., 2014). Rehabilitation strategies that induce beneficial neuroplasticity will likely improve functional rehabilitation outcomes (Boudreau et al., 2010; Snodgrass et al., 2014). However, there is evidence to suggest that the capacity for neuroplasticity declines with age (Berghuis et al., 2017; Fathi et al., 2010; Freitas et al., 2011; Müller-Dahlhaus et al., 2008; Sawaki et al., 2003; Seidler et al., 2010); it is plausible that the poorer rehabilitation outcomes evident in older burns survivors than younger burns survivors (Edgar et al., 2013) is due to a reduced capacity for neuroplasticity.

Non-invasive brain stimulation (NIBS) can be used to both measure and induce neuroplasticity in the human primary motor cortex (M1) (Dayan et al., 2013; Hallett, 2007; Ridding and Rothwell, 2007; Vallence and Ridding, 2014). Transcranial magnetic stimulation (TMS) is a commonly used form of NIBS. In single-pulse TMS, a brief, high current electrical pulse is delivered through a handheld coil placed over the scalp, which induces a magnetic field that passes through the scalp and skull with little attenuation. The magnetic field induces current flow in the underlying brain tissue, and if the stimulation is sufficiently intense, will result in depolarisation of the neurons (Barker et al., 1985; Hallett, 2007). A single, suprathreshold pulse delivered to the M1 will depolarise neurons in the underlying brain tissue and elicit a motor-evoked potential (MEP) in the muscle controlled by the
cortical representation over which the pulse was delivered. The amplitude of the MEP provides a measure of corticospinal excitability; a change in the amplitude of the MEP provides a marker of neuroplasticity (Dayan et al., 2013; Di Lazzaro et al., 2008; Hallett, 2007; Rothwell et al., 1999; Vallence and Ridding, 2014).

TMS can be used to induce neuroplasticity when applied in trains of pulses, known as repetitive TMS (rTMS). When trains of pulses are applied at a particular frequency and of a particular intensity, changes in cortical excitability that outlast the period of stimulation can be induced, which is indicative of neuroplasticity (Hallett, 2007). Varying the frequency and temporal pattern of rTMS can induce bidirectional changes in cortical excitability (Hallett, 2007). A commonly used rTMS protocol is continuous theta-burst stimulation (cTBS), which comprises high frequency bursts of stimuli delivered at 50 Hz, every 200 ms (i.e. 5 Hz) for 40 seconds (Hallett, 2007; Huang et al., 2005). This paradigm can decrease excitability, which is thought to be a marker of long-term depression (LTD)-like neuroplastic brain response (Hallett, 2007). Responses to TMS protocols, including cTBS, have been shown to have large inter-individual variation (Hamada et al., 2013). One protocol that has shown to be effective in reducing inter-individual variability is ‘spaced cTBS’; specifically, two applications of cTBS spaced by a 10-minute interval. Goldsworthy et al. (2012) demonstrated that spaced cTBS led to a greater, longer-lasting, and more reliable suppression of MEP amplitude compared to a single application of cTBS (Goldsworthy et al., 2015). This suggests that spaced cTBS is an effective protocol to examine LTD-like neuroplastic responses in the human motor cortex.
To date there are no known studies examining neuroplasticity in individuals with acute burn injuries. If there are demonstrable differences in neuroplastic brain responses in burns survivors and non-injured controls, then targeted rehabilitation strategies that address these changes coupled with NIBS techniques may have the potential to modulate neuroplasticity to facilitate better rehabilitation outcomes (Schabrun and Chipchase, 2012). Given the deterioration in physical function post-burn injury in older adults, we investigated the relationship between acute burn injury, neuroplastic responses and functional outcomes/quality of life in those aged 45+ years. We hypothesised that burns survivors would show a smaller change in MEP amplitude following spaced cTBS than non-injured, age-matched control participants. It was also hypothesised that burns survivors who showed the greater change in MEP amplitude following spaced cTBS would have the highest scores on the Functional and Quality of Life measures.

2. Methods

2.1. Participants

Sixteen burn injury participants (ten males, $M = 56.8 \pm 7.9$ years, range = 45 – 71 years) and thirteen control participants (six males, $M = 62.4 \pm 10.3$ years, range = 47 – 83 years) took part in the study. Due to limitations in time and participant availability participants are not matched one-to-one for age and gender. Burn injury survivors were recruited through the State Adult Burn Unit (henceforth referred to as the ‘Burns Group’). The inclusion criteria for burn injury survivors were: aged 45 years or older at the time of recruitment; total body surface area (TBSA) of burn injury less than 20%; burn injury occurred less than six weeks prior to recruitment. A TBSA of 20% was chosen as individuals with larger burn injuries
often have prolonged hospital stays and consequently may skew neurophysiological and functional data. This also reduces confounding due to the Systemic Inflammatory Response Syndrome which is evident in burns greater than \(~20\%\) TBSA (Greenhalgh et al., 2007; Nunez Lopez et al., 2017). The exclusion criteria for burn injury survivors were: conditions that may confound the measurement of recovery or hinder rehabilitation beyond the burn injury such as neurological incidents (e.g. stroke) and reported musculoskeletal injury or surgery within the last three months; severe or recent heart disease; sleep deprivation (self-assessed); any contraindication to TMS (Rossi et al., 2011; Rossi et al., 2009). Burns Group participants underwent normal treatment for their burn injury, through the State Adult Burn Unit Inpatient and/or Outpatient Services, which included an extensive medical history and physical examination, and a functional assessment at twelve weeks post-burn injury.

Non-injured volunteers were recruited from the public to form the ‘Control Group’. The inclusion criteria for these participants were: aged 45 years or older at time of recruitment; no history of a burn injury that required medical treatment. The same exclusion criteria as for the Burns Group applied.

The protocol was performed in accordance with the Declaration of Helsinki and was approved by the Murdoch University Human Research Ethics Committee (MU HREC Reference: 2016-166), East Metropolitan Health Service (EMHS HREC Reference: 16-012), South Metropolitan Health Service (SIRO HREC Reference: 16-012) and University of Western Australia (UWA HREO RA/4/1/8354). Governance approval was obtained from East and South Metropolitan Health Services. All subjects gave written informed consent.
prior to testing and were screened for conditions that would contraindicate TMS (Rossi et al., 2011; Rossi et al., 2009).

2.2. Experimental procedures

2.2.1. Transcranial magnetic stimulation

All participants attended two experimental sessions at the TMS laboratory at Murdoch University, six weeks apart. In both sessions participants were seated in a comfortable chair with their right hand resting on a soft pillow on their lap. The first dorsal interosseous (FDI) muscle on the right hand was palpated (participants asked to gently abduct the index finger against resistance for palpation of the muscle), and the overlying skin cleaned with an alcohol-based solution. The FDI muscle was chosen due to its accessibility for both surface EMG and stimulation within the M1. The anticipated small sample size and variability in burn location did not facilitate targeted measurement of muscles specifically in/near the location of the burn injury in each individual. Surface electromyography (EMG) was recorded from two Ag-AgCl electrodes (with water-based lubricant applied) taped into position; one electrode was placed over the belly of the muscle and one electrode was placed over the tendon insertion. A grounding electrode was attached to the skin over the distal ulna at the wrist. The EMG signal was amplified (x1000) and band-pass filtered (20 Hz – 1 kHz) using a CED 1902 signal conditioner (Cambridge Electronic Design Co. Ltd, Cambridge, UK). The signal was then digitized at 2 kHz using a CED 1401 analog-to-digital converter (Cambridge Electronic Design Co. Ltd, Cambridge, UK) and was then stored on a computer to allow for later off-line analysis. TMS pulses (monophasic) were delivered using a figure-of-eight coil connected to a Magstim BiStim 2002 stimulator (The Magstim Company Limited, Whitland, Wales, UK). The coil was held tangentially to the scalp at an angle of 45
degrees to the sagittal plane with the handle pointed posteriorly to induce a posterior-anterior current flow.

The site for optimal stimulation and the resting motor threshold (RMT) were determined for the right FDI with left M1 stimulation. To determine the optimal stimulation site, suprathreshold pulses were delivered at a number of sites to identify the site from which FDI MEPs were evoked consistently. The optimal site was marked on a tight-fitting material swimming cap to ensure reliable placement of the coil throughout the experimental session.

RMT was defined as the minimum stimulus intensity (as a percentage of the maximum stimulator output) that produced a MEP of at least 50 μV in at least three out of six trials in which the FDI was completely relaxed (Groppa et al., 2012; Opie et al., 2017; Rogasch et al., 2013; Rossini et al., 1999).

2.2.2. Repetitive TMS

The current study used a rTMS protocol known as cTBS. cTBS was applied using a Magstim Rapid stimulator (Magstim) connected to an air-cooled figure-of-eight coil (biphasic pulses). The optimal site for stimulation and RMT was determined using the air-cooled coil and the Magstim Rapid prior to commencing cTBS. cTBS comprised a total of 600 pulses, applied in bursts of three pulses delivered at 50 Hz, and repeated at a frequency of 5 Hz (Huang et al., 2005). cTBS intensity was set at 70% RMT (determined using the Magstim Rapid). In the current study, ‘spaced cTBS’ was applied: two trains of cTBS were delivered ten minutes apart. This protocol was demonstrated by Goldsworthy et al. (2012) to be effective at inducing reliable and long-lasting (at least two hours) suppression of MEP amplitude.
Three blocks of TMS were delivered before (baseline), and at several time points after the spaced cTBS protocol (see Figure 1). There was a total of 14 single-pulses per block; the test stimulus intensity was set to 120% RMT.

(It is important to note here that TMS was delivered in blocks of 14 single-pulse and 14 paired-pulse trials (order randomised; the interval between trials was 5 seconds (±20% jitter)). The paired-pulse trials were delivered to measure short-interval intracortical inhibition (SICI): the SICI results are reported in Whife et al. (Unpublished data).)

![Fig. 1: Experimental protocol for each TMS session. Abbreviations: RMT, resting motor threshold; MEPs, motor-evoked potentials; cTBS, continuous theta-burst stimulation; Post-cTBS\text{\textsubscript{EARLY}}, averaged early blocks; Post-cTBS\text{\textsubscript{LATE}}, averaged late blocks.](image)

2.3. Functional and Quality of Life measures

Functional and Quality of Life measures were obtained from all participants at the second TMS session (median days after injury for Burns Group = 88, range = 79 – 103). In addition,
all Burns Group participants completed both the Short-Form Health Survey version 2 (SF-36) and the Burn Specific Health Scale – Brief (BSHS-B) Quality of Life assessments. The SF-36 is a self-completed questionnaire that is an indicator of overall health status across eight domains (Ware and Sherbourne, 1992); the BSHS-B is a self-completed questionnaire that is an abbreviated outcome scale, designed specifically for burns survivors, used to evaluate burn-specific aspects of health status across nine domains or three broad categorisations (Kildal et al., 2001; Willebrand and Kildal, 2008). Burns Group participants who had sustained a burn injury to their upper limb (n = 8) were also asked to complete the QuickDASH (Disabilities of the Arm, Shoulder and Hand) Outcome Measure (QuickDASH). The QuickDASH is a self-completed questionnaire that uses eleven items to measure physical function and symptoms in people with any or multiple musculoskeletal disorders of the upper limb (Wu et al., 2007). Burns Group participants who had sustained a burn injury to their lower limb (n = 6) were also asked to complete the Timed Up and Go (TUG) test and the Lower Limb Function Index-10 (LLFI). The TUG test involves participants starting in a seated position in a chair, standing, walking three metres quickly to a line identified on the floor, and then returning to the seated position (Herman et al., 2011; Shumway-Cook et al., 2000). The LLFI is a self-assessment questionnaire that assesses functional status in individuals with lower limb conditions (Gabel et al., 2012). All of these assessments have been validated for use in a burn injury population (Edgar et al., 2010; Finlay et al., 2010; Finlay et al., 2014b; Gittings et al., 2016; Ryland et al., 2016; Wu et al., 2007). Some Burns Group participants had both upper and lower limb burn injuries (n = 2), and therefore completed both upper and lower limb functional measures.

All Control Group participants completed the Short-Form Health Survey version 2 (SF-36) and the Timed Up and Go (TUG) test. (Only the TUG test and SF-36 were assessable across
both groups as the BSHS-B, QuickDASH and LLFI are either burn or injury specific and not easily completed by a Control Group without injury. As noted above, TUG measures were only available from those Burns Group participants with a lower limb burn injury.)

2.4. Data analysis

Four of the sixteen participants in the Burns Group were lost to follow-up after the first TMS session (two participants were unavailable to attend the second experimental session; another participant had commenced a new medication that contraindicated the use of TMS; in one participant EMG recordings were unable to be interpreted). These four participants did not complete the Functional and Quality of Life assessments (completed at the second TMS session). Their data from the first TMS session at six weeks are included in the analyses comparing Session 1 between groups but are not included in analyses that compares Session 1 to Session 2 or Session 1 outcomes to Functional and Quality of Life measures (because Functional and Quality of Life measures were obtained in Session 2).

EMG activity was analysed by visual inspection of the offline recordings. Any trial with muscle activity in the 250 ms preceding the onset of the MEP was excluded from analysis. The peak-to-peak MEP amplitude (mV) was obtained from the 40 ms of EMG activity beginning 15 ms after the test stimulus.
2.4.1. Baseline MEP data

To test for differences in RMT and baseline MEP amplitude between groups and across experimental sessions, two-way mixed analysis of variance (ANOVAs) were performed, with within-subject factor of SESSION (Session 1, Session 2) and between-subjects factor of GROUP (Burns, Control). Separate ANOVAs were performed on RMT and baseline MEP data.

2.4.2. Spaced cTBS-induced change in MEP amplitude

To test for differences in MEP amplitude in the three baseline blocks, repeated measures analysis of variance (RM-ANOVA) with within-subject factors of BLOCK were performed on the raw MEP amplitudes. Separate ANOVAs were performed for each session and each group. No significant differences were found between baseline blocks (Burns Group Session 1: $F_{1,15} = 2.38$, $p = .14$, $\eta^2_p = .14$, Session 2: $F_{1,11} = 1.01$, $p = .34$, $\eta^2_p = .08$, and Control Group Session 1: $F_{1,12} = 0.37$, $p = .55$, $\eta^2_p = .03$, Session 2: $F_{1,12} = 1.51$, $p = .24$, $\eta^2_p = .11$); therefore, the three baseline blocks were averaged.

To test for differences between MEP amplitudes obtained at 0 and 5 minutes post-spaced cTBS (post-spaced cTBS is referred to simply as post-cTBS from here onwards), paired-samples t-tests were performed on the raw MEP amplitudes. Separate t-tests were performed for each session and each group. No significant differences were found between 0 minutes post-cTBS and 5 minutes post-cTBS time points (all $t < 0.18$, all $p > .19$); therefore, these two post-cTBS blocks were averaged, and analysed as ‘post-cTBSearly’. To test for differences between MEP amplitudes obtained at 15 and 30 minutes post-cTBS, paired-
samples t-tests were performed on the raw MEP amplitudes. Separate t-tests were performed for each session and each group. No significant differences were found between 15 minutes post-cTBS and 30 minutes post-cTBS time points (all $t < 0.81$, all $p > .43$); therefore, these two post-cTBS blocks were also averaged, and analysed as ‘post-cTBS\textsubscript{LATE}’.

To test for differences in MEP amplitude following spaced cTBS, two-way RM-ANOVAs (with polynomial contrasts) were performed with within-subject factors of SESSION and TIME (baseline, post-cTBS\textsubscript{EARLY}, post-cTBS\textsubscript{LATE}). Separate RM-ANOVAs were performed on MEP data from Burns Group participants and Control Group participants. Greenhouse-Geisser corrections were used for analyses in which the assumption of sphericity was violated (Mauchly’s test of sphericity).

To test for differences in MEP amplitude changes following spaced cTBS between the Burns Group and the Control Group, two-way mixed RM-ANOVAs were performed, with within-subject factor of TIME (baseline, post-cTBS\textsubscript{EARLY}, post-cTBS\textsubscript{LATE}) and between-subjects factor of GROUP (Burns, Control). Separate RM-ANOVAs were performed on MEP data from Session 1 and Session 2.

To examine reliability of spaced cTBS-induced neuroplastic responses across sessions, correlations were performed between post-cTBS\textsubscript{EARLY} and post-cTBS\textsubscript{LATE} for Session 1 and 2.
Conditional on significant main effects of interactions, post-hoc analyses were performed. Statistical significance was accepted at \( \alpha < 0.05 \). Multiple comparisons were not corrected for as this study was exploratory. Data are presented as mean \( \pm \) standard deviation, except in the figures where the standard error of the mean (SEM) is presented.

### 2.4.3. Functional and Quality of Life measures

Functional and Quality of Life measures were compared between the Burns Group and Control Group using independent t-tests. The relationship between Functional and Quality of Life domain scores and spaced cTBS-induced change in MEP amplitude was assessed using correlational analysis.

### 3. Results

#### 3.1. Participant characteristics

There was no significant difference in age or gender between groups (Age \( p = .11 \), Gender \( p = .40 \)). The average TBSA of burn size in the Burns Group was 2.14\%, with burn size ranging from 0.12\% to 7.25\%. All burn injuries in this study had a TBSA of less than 15\% and are therefore considered to be minor burn injuries (Finlay et al., 2014a). The location of burn injury included nine upper limb burns (two individuals sustained burns to both upper limbs but are only counted once), nine lower limb burns (buttocks included; four individuals sustained burns to both lower limbs but are only counted once), three thorax burns (chest, abdomen, pelvis (including genitals but excluding buttocks) and back) and three head and neck burns. Only one participant sustained a burn injury over the area targeted by TMS (right
hand, first dorsal interosseous muscle). Interestingly, this patient was unable to complete the second TMS session as EMG values were unable to be reliably recorded from this area. This may have been secondary to the changes (maturation) in scarring over this area (this was one of the four participants in the Burns Group who was lost to follow-up). Six of the Burns Group participants required surgical management of their injuries, ten did not. The median days between sessions for the Burns Group was 44 (range = 32 – 55); the median days between sessions for the Control Group was 50 (range = 39 – 56).

3.2. Baseline corticospinal excitability

There was no significant difference in RMT or baseline MEP amplitude across sessions or between groups. The mean RMT (determined using a monophasic pulse waveform) in the Burns Group was 53.1% (±6.9) and 51.8% (±6.1) of maximal stimulator output (MSO) for Session 1 and Session 2. The mean RMT in the Control Group was 52.8% (±8.0) and 53.6% (±9.3) MSO for Sessions 1 and Session 2. A two-way RM-ANOVA showed no main effect of SESSION \( (F_{1,23} = 0.52, p = .48, \eta^2_p = .02) \), no main effect of GROUP \( (F_{1,23} = 0.30, p = .59, \eta^2_p = .01) \), and no SESSION*GROUP interaction \( (F_{1,23} = 0.05, p = .83, \eta^2_p < .01) \). The RM-ANOVA performed on the Baseline MEP amplitude data showed no main effect of SESSION \( (F_{1,23} = 0.77, p = .39, \eta^2_p = .03) \), no main effect of GROUP \( (F_{1,23} = 0.10, p = .75, \eta^2_p < .01) \), and no SESSION*GROUP interaction \( (F_{1,23} = 1.04, p = .32, \eta^2_p = .04) \).

3.3. Effect of spaced cTBS on MEP amplitude

Figure 2 shows MEP amplitude at baseline and post-cTBS for the Burns Group and Control Group for the two experimental sessions. The Burns Group showed no systematic change in
MEP amplitude following spaced cTBS in either Session 1 or Session 2. The two-way within-subject ANOVA showed no main effect of TIME \((F_{1,11} = 0.04, p = .84, \eta^2_p < .01)\), no main effect of SESSION \((F_{1,11} = 0.20, p = .89, \eta^2_p < .01)\), and no SESSION*TIME interaction \((F_{1,11} = 1.07, p = .32, \eta^2_p = .09)\).

Within the Control Group, MEP amplitude decreased from baseline to the post-cTBS\textsubscript{LATE} time point in both Session 1 and Session 2 (see Figure 2). The two-way within-subject ANOVA showed a main effect of TIME \((F_{1,12} = 10.90, p < .01, \eta^2_p = .48)\), but no main effect of SESSION \((F_{1,12} = 1.49, p = .25, \eta^2_p = .11)\) and no SESSION*TIME interaction \((F_{1,12} = 0.37, p = .56, \eta^2_p = .03)\). To further investigate the main effect of TIME, post-hoc one-way within-subject ANOVAs were performed on MEP amplitudes, with separate ANOVAs for Session 1 and Session 2. Both ANOVAs showed a main effect of TIME \((both \ F_{1,12} > 5.36, \ both \ p < .04, \ both \ \eta^2_p > .30)\). Paired sample t-tests showed a significant difference in MEP amplitude from Baseline to post-cTBS\textsubscript{LATE} in both sessions \((both \ t > 2.31, \ both \ p < .04)\), but no significant difference between Baseline and post-cTBS\textsubscript{EARLY} \((both \ t < 0.91, \ both \ p > .38)\) or between post-cTBS\textsubscript{EARLY} and post-cTBS\textsubscript{LATE} \((both \ t < 1.57, \ both \ p > .14)\).
Fig. 2: MEP amplitudes (+/- SEM) at baseline (BL), post-cTBS\textsubscript{EARLY} (EARLY), and post-cTBS\textsubscript{LATE} (LATE) measurements in Session 1 (A) and Session 2 (B). Normalised MEP data for Burns and Control Groups in Session 1 (C) and Session 2 (D) at post-cTBS\textsubscript{LATE}, with values less than 1.0 reflecting a decrease from baseline MEP amplitude (expected response), and values greater than 1.0 reflecting an increase in MEP amplitude.

* = statistically significant change from BL to Late measurements in the Control Group.

Note: Large SEM in Session 1 Control Group is due to one control participant who elicited very large MEPs (TS of 120% RMT); sensitivity analyses were performed excluding this individual and outcomes of analyses were unchanged.
3.3.1. **Comparison of Burns Group and Control Group**

At Session 1 (six weeks post-injury for Burns Group participants) there was no systematic change in MEP amplitude following spaced cTBS in the Burns Group, but a decrease in MEP amplitude from baseline to the post-cTBSLATE time point in the Control Group. The mixed ANOVA showed a significant TIME*GROUP interaction ($F_{1,27} = 8.16, p < .01, \eta^2_p = .23$), but no main effect of TIME ($F_{1,27} = 2.27, p = .14, \eta^2_p = .08$) or GROUP ($F_{1,27} = 0.55, p = .47, \eta^2_p = .02$). A post-hoc one-way ANOVA of the Burns Group MEP amplitude data from Session 1 showed no effect of time ($F_{1,15} = 1.60, p = .23, \eta^2_p = .01$). Analyses reported above showed that there was a significant difference between baseline and post-cTBSLATE MEP amplitudes for the Control Group in Session 1. At Session 2 (twelve weeks post-injury for Burns Group participants) there was no significant TIME*GROUP interaction ($F_{1,23} = 2.01, p = .17, \eta^2_p = .08$) and no main effect of TIME ($F_{1,23} = 3.49, p = .08, \eta^2_p = .13$) or GROUP ($F_{1,23} = 0.21, p = .65, \eta^2_p = .01$).

3.3.2. **Intra-individual neuroplastic response associations between Session 1 and Session 2**

Figure 3 shows scatterplots of post-cTBS\textsubscript{EARI\textsubscript{L}} and post-cTBS\textsubscript{LATE} normalised MEP data in Session 1 and Session 2 for the Burns Group and Control Group participants. Correlational analyses of Burns Group post-cTBS\textsubscript{EARI\textsubscript{L}} normalised MEP data showed no significant relationship between Session 1 and Session 2 ($r = .22, p = .50, 95\% \text{ confidence interval } [CI] = -.41, .70$). Correlational analyses of Burns Group post-cTBS\textsubscript{LATE} normalised MEP data showed a significant relationship between spaced cTBS-induced neuroplastic responses in Session 1 and Session 2 ($r = .75, p < .01, 95\% \text{ CI } = .30, .92$), demonstrating a consistency in response to post-cTBS\textsubscript{LATE} across sessions; individuals who showed a late MEP depression...
following spaced cTBS in Session 1 also showed a late MEP depression following spaced cTBS in Session 2 (Fig. 3, panel B). Three of the twelve burns participants showed the expected MEP suppression following spaced cTBS in both sessions, at both time points (see lower left quadrants, Fig. 3 panels A and B). Correlational analyses of Control Group normalised MEP data showed no significant relationship between Session 1 and Session 2 for either post-cTBSearly ($r = .21, p = .49, 95\% CI = -.38, .68$). or post-cTBSlate ($r = .51, p = .07, 95\% CI = -.05, .83$). Seven of the thirteen control participants showed the expected MEP suppression following spaced cTBS in both sessions at the post-cTBSlate time point (see lower left quadrant, Fig. 3 panel D).
3.4. Functional and Quality of Life measures

Functional and Quality of Life measures were recorded at Session 2 (approximately twelve weeks post-injury for the Burns Group) prior to the TMS protocol. There was a significant difference in the TUG times between the Burns Group and Control Group ($p = .02$), with a Burns Group mean TUG time of 7.95 seconds ($\pm 1.38$), compared to 6.46 seconds ($\pm 1.13$) for the Control Group. There was no significant difference between groups in SF-36 outcomes.

* = statistically significant relationship between spaced cTBS-induced neuroplasticity in Session 1 and Session 2
3.5. Associations between neuroplastic responses and Functional and Quality of Life measures

Given there was no main effect of TIME for the Burns Group, MEP data post-cTBS was quantified as average MEP change across all time points post-cTBS; ‘post-cTBS\textsubscript{AVE}’. In Session 1 there were no evidence of significant relationships between the change in MEP amplitude post-cTBS\textsubscript{AVE} and any Functional or Quality of Life measures. In Session 2 there was a significant relationship between the SF-36 Domain General Health and post-cTBS\textsubscript{AVE} \((r = .62, p = .03, 95\% \text{ CI} = .07, .88)\), as shown in Figure 4. There were no significant findings on correlational analysis between average MEP amplitude change post-cTBS and Burn Specific Health Scale – Brief, Lower Limb Functional Index or QuickDASH measures or TUG times in the Burns Group.

![Graph showing MEP change and SF-36 General Health](image)

**Fig. 4**: Average MEP change post-cTBS (post-cTBS\textsubscript{AVE}) and SF-36 Domain General Health in Session 1 and 2 in the Burns Group.

* = statistically significant relationship.
4. Discussion

Here we examined rTMS-induced neuroplasticity in the M1 of burns survivors at six and twelve weeks post-injury and in non-injured age-matched controls. Although burns survivors had minor burns (with the largest burn being 7.25% TBSA) they showed reduced rTMS-induced neuroplasticity, as indicated by reduced changes in MEP amplitude post-cTBS, compared to control participants at six weeks post-injury. There was no significant difference in neuroplasticity markers between burns survivors and control participants at twelve weeks post-injury. These findings suggest that motor cortical neuroplastic responses might be influenced following even minor burn injuries, which could have important implications for rehabilitation following burn injury.

4.1. Spaced cTBS-induced changes in MEP amplitude in Burns and Control Groups

In the current study, there was no evidence of significant changes in MEP amplitude following spaced cTBS in burns survivors at either six or twelve weeks post-injury. In contrast, in control participants, MEP amplitude was significantly decreased 15 – 30 minutes post-cTBS in both experimental sessions. cTBS is thought to induce MEP depression via LTD-like mechanisms (Goldsworthy et al., 2012; Huang et al., 2005): the change in MEP amplitude following cTBS can outlast the period of stimulation, is dependent on the temporal pattern of stimulation, and is N-methyl-D-aspartate (NMDA) receptor dependent (Berardelli et al., 1998; Chen et al., 1997; Hamada et al., 2013; Huang et al., 2007; Maeda et al., 2000). However, recent literature examining rTMS-induced neuroplasticity shows high inter- and intra-subject variability (Hamada et al. 2012). Here we used the spaced cTBS protocol, which involves the application of two trains of cTBS spaced by ten minutes; spaced cTBS has been shown to induce longer lasting and more reliable MEP depression than a single application of
cTBS (Goldsworthy et al., 2015; Goldsworthy et al., 2012). Therefore, the current findings suggest reduced spaced cTBS-induced neuroplasticity in burns survivors compared to controls, which might reflect a reduced LTD-like neuroplasticity following burn injury. The reduced LTD-like neuroplasticity reflects a global reduction rather than a burn-location specific change, as all measurements were taken from the right FDI muscle.

At six weeks post-injury, there was a significant difference between the change in MEP amplitude following spaced cTBS in the two groups; the controls but not the burns survivors showed a significant decrease in MEP amplitude following spaced cTBS, suggesting a reduced capacity for LTD-like neuroplastic responses in burns survivors at six weeks post-injury. At twelve weeks post-injury, there was no significant difference in the change in MEP amplitude in response to spaced cTBS between the two groups, suggesting that the reduced LTD-like neuroplastic response evident in burns survivors at six weeks post-injury is normalising at twelve weeks post-injury. Given the role of neuroplasticity in motor learning and the importance of motor function for recovery from injury, a decreased capacity for a neuroplastic response could substantially influence functional outcomes following burn injury (Boudreau et al., 2010; Dayan and Cohen, 2011; Pascual-Leone et al., 2005).

4.2. Delayed spaced cTBS-induced neuroplastic response in non-injured older adults

In the current study, control participants showed significant MEP amplitude suppression following spaced cTBS at 15 – 30 minutes but not 0 – 5 minutes. This finding was replicated at the group level, with the significant MEP depression at 15 – 30 minutes post-cTBS observed in two experimental sessions separated by six weeks. Although we didn’t have a
young adult group in the current study, previous work has shown a significant MEP amplitude suppression from 0 – 120 minutes post-cTBS in young healthy adults (Goldsworthy et al., 2012). Therefore, the current results showing a significant MEP suppression from 15 – 30 minutes post-cTBS in older healthy adults suggests a delayed neuroplastic response to spaced cTBS in older adults. To our knowledge, this is the first study to examine neuroplasticity following spaced cTBS in older adults, however, recent research examining neuroplasticity following other forms of non-invasive brain stimulation, including anodal transcranial direct current stimulation, have shown a delayed change in MEP amplitude in older compared to younger adults (Fujiyama et al., 2014), consistent with the current results. Sidhu et al. (2017) demonstrated that older subjects had a greater response to spaced paired associative stimulation (PAS) priming with an inter-PAS interval of thirty minutes compared to ten minutes. Puri et al. (2016) have demonstrated a trend towards delayed MEP change in older adults using the facilitatory protocol intermittent TBS (iTBS). It is important to note that the primary comparison in the current study was the neuroplastic response induced by spaced cTBS in burns survivors and non-injured older adults, therefore, we did not include a young healthy control sample or a sham control condition. It is necessary for future studies to replicate the current findings and incorporate additional conditions to show that older adults exhibit a delayed neuroplastic response to spaced cTBS compared to younger adults and compared to sham stimulation. Nonetheless, the current results suggest that the capacity for neuroplasticity might not be reduced with age, but that neuroplastic induction follows a different time-course in younger and older adults; future research should examine neuroplasticity following spaced cTBS in a sample of younger and older adults to characterise the time-course of this neuroplastic response.
4.3. *Intra-individual variability to spaced cTBS*

Although the Control Group showed a significant MEP suppression in both Session 1 and Session 2 at the group level, scatterplots and correlational analysis of normalised MEP data in Session 1 and Session 2 showed no significant correlation at both post-cTBS\textsubscript{EARLY} and post-cTBS\textsubscript{LATE} between Sessions. At the post-cTBS\textsubscript{LATE} time point 54\% of participants demonstrated the expected MEP suppression across both sessions. This suggests that although there is a reliable and reproducible effect of spaced cTBS at a group level there is still considerable variation in participants at an individual level. The literature shows that variability in neurophysiological responses to NIBS is high and that causes are multifactorial (Hamada et al., 2013; Hordacre et al., 2017; Ridding and Ziemann, 2010): factors that may influence NIBS-induced neuroplasticity include priming (recent synaptic activity), prior voluntary motor activity, parallel voluntary motor activity, aerobic exercise, age, attention, gender, medication, genetics and time of day. Further, inter-individual physiological differences in the cortical network that is activated by NIBS can also influence variability (Hamada et al., 2013; Hordacre et al., 2017). There is evidence to suggest that a greater proportion of the variability in response to cTBS is due to inter-individual variability compared to intra-individual variability (Vallence et al., 2015). However, there is currently no evidence regarding intra-individual variability in response to spaced cTBS and therefore it is not known whether the variability in this study is representative of a wider healthy, older population.
4.4. Associations between spaced cTBS-induced neuroplastic response and Functional and Quality of Life measures

There was a significant association between SF-36 Domain General Health and average change in MEP amplitude following spaced cTBS in burns survivors at twelve weeks post-injury (but not six weeks post-injury). Overall, burns survivors who showed a typical response to spaced cTBS (i.e. MEP suppression) scored higher on the General Health Domain than those with an atypical response (MEP facilitation) to spaced cTBS. The General Health Domain of the SF-36 (version 2) predominantly measures perception of health in general (i.e. “In general, would you say your health is: Excellent, Very Good, Good, Fair, Poor”), and the questions are not time-specific. Although speculative, the association between neuroplasticity and general health scores may reflect that individuals who showed the expected neuroplastic response may be able to adapt to the injury and improve their perceived general health-related outcomes faster than those with a reduced capacity for neuroplasticity. As the follow up in this study was not extended beyond three months it is not clear whether or not this relationship would persist or resolves as burns survivors continue to recover. This was a pilot study with a small sample size, therefore these findings need to be replicated in future studies with larger samples. There was variability between sessions, with no clear relationship evident in analysis of General Health Domain outcomes and post-cTBSave in Session 1. This may be secondary to Functional and Quality of Life measures only being recorded at twelve weeks post-injury, and therefore no Functional or Quality of Life data is available at six weeks (Session 1).
4.4.1. Participants and Functional and Quality of Life measures

There was no significant difference in SF-36 scores between the Burns Group and the Control Group in this study. As these were relatively minor burns, twelve weeks of State Adult Burn Unit treatment and rehabilitation may be sufficient for Burns Group participants to return to baseline (i.e. compared to Control Group) in the SF-36 Quality of Life assessment (SF-36 has been shown to be more sensitive to change after burn injury than the BSHS-B after approximately one month (Edgar et al., 2010)). However, despite the relatively minor burns, TUG times were longer in the Burns Group than in the Control Group at twelve weeks. The TUG has been shown to provide useful clinical information regarding functional recovery up to six months after a lower limb burn injury (Finlay et al., 2010). A longer follow up period was beyond the scope of the current study; it is unclear whether the burns survivors had plateaued in their functional recovery by twelve weeks or whether the TUG time would have improved beyond this point. While no baseline TUG data is available for the Burns Group, the fact that their TUG time is longer than the Control Group despite no difference in Quality of Life (SF-36) may suggest that TUG is a more sensitive measure of recovery in lower limb burns. TUG has been shown to be a sensitive and specific measure for identifying older adults that are at risk of falls (Shumway-Cook et al., 2000).

This study has investigated the effect of spaced cTBS on LTD as this had been shown to be the most reliable protocol for inducing neuroplastic change (Goldsworthy et al., 2012). However, since data collection began recent evidence suggests that spaced intermittent theta burst stimulation (iTBS) can induce LTP-like neuroplasticity, however there remains significant variability between individuals (Tse et al., 2018) and spaced iTBS does not appear to have a significant effect in older adults (Opie et al., 2017). Despite this, future research
should look to test both LTP and LTD-like plasticity in burns survivors. It is reasonable to speculate that both LTP and LTD-like plasticity would be impaired following burn injury as both are affected by changes in afferent input and both play important roles in motor learning and function (Buonomano and Merzenich, 1998; Massey and Bashir, 2007; Rioult-Pedotti et al., 2000; Rioult-Pedotti et al., 1998; Sanes and Donoghue, 2000; Ziemann et al., 2004).

Four of the Burns Group participants did not complete the study and therefore there is no data regarding Functional and Quality of Life measures or neuroplastic changes at twelve weeks post-injury for these participants. Furthermore, Functional and Quality of Life measures were only measured at twelve weeks. Future studies should aim to record Functional and Quality of Life data at multiple time points, as changes in this data may correlate with changes in neuroplastic markers after burn injury. Measurements of pain were limited to a score out of ten prior to Session 1 (with participants to be excluded if pain or overnight itch scores were greater than five out ten due to the effect on sleep). It is possible that the initial decrease in LTD-related neuroplasticity at Session 1 in the Burns Group, which then normalises relative to the Control Group by Session 2, reflects the resolution of pain over twelve weeks. Further research should account for the ongoing effect of pain on neuroplasticity. Within the Burns Group there was some variation in rehabilitation input. Those participants who were admitted to the State Adult Burn Unit would have received more intensive rehabilitation early in their burn injury recovery than those who did not require inpatient admission. Future research may need to account for differences in rehabilitation within this cohort, and perhaps stratify these participants into separate groups to better measure the impact of neuroplasticity, rehabilitation and functional recovery.
5. **Conclusion**

The findings of this study suggested that a minor burn injury can affect rTMS-induced neuroplastic brain responses up to six weeks after injury in burns survivors aged over 45 years, relative to controls. In burns survivors, when comparing Functional and Quality and Life outcomes, those with smaller rTMS-induced neuroplasticity (smaller decrease in MEP amplitude post-cTBS) also had poorer General Health Domain scores. The results suggest that neuroplastic changes occur acutely after small burn injuries and may play a role in delaying recovery.
6. **Bibliography**


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7. **Figure captions**

**Fig. 1:** Experimental protocol for each TMS session. Abbreviations: RMT, resting motor threshold; MEPs, motor-evoked potentials; cTBS, continuous theta-burst stimulation; Post-cTBS\textsubscript{EARLY}, averaged early blocks; Post-cTBS\textsubscript{LATE}, averaged late blocks.

**Fig. 2:** MEP amplitudes (+/- SEM) at baseline (BL), post-cTBS\textsubscript{EARLY} (EARLY), and post-cTBS\textsubscript{LATE} (LATE) measurements in Session 1 (A) and Session 2 (B). Normalised MEP data for Burns and Control Groups in Session 1 (C) and Session 2 (D) at post-cTBS\textsubscript{LATE}, with values less than 1.0 reflecting a decrease from baseline MEP amplitude (expected response), and values greater than 1.0 reflecting an increase in MEP amplitude.

* = statistically significant change from BL to Late measurements in the Control Group.

Note: Large SEM in Session 1 Control Group is due to one control participant who elicited very large MEPs (TS of 120% RMT); sensitivity analyses were performed excluding this individual and outcomes of analyses were unchanged.

**Fig. 3:** Intra-individual neuroplastic response variation in normalised MEP values across Session 1 and Session 2 for Burns Group post-cTBS\textsubscript{EARLY} (A) and post-cTBS\textsubscript{LATE} (B) and Control Group post-cTBS\textsubscript{EARLY} (C) and post-cTBS\textsubscript{LATE} (D).

* = statistically significant relationship between spaced cTBS-induced neuroplasticity in Session 1 and Session 2

**Fig. 4:** Average MEP change post-cTBS (post-cTBS\textsubscript{AVE}) and SF-36 Domain General Health in Session 1 and 2 in the Burns Group.
* = statistically significant relationship.
CHAPTER FOUR – No Difference in Short-interval Intracortical Inhibition in Older Burn Injury Survivors Compared to Non-Injured Older Adults

Foreword

Chapter Three presented the manuscript, “Decreased neuroplasticity in older burn injury survivors compared to non-injured older adults”, which showed that older adults with a minor burn injury demonstrated a decreased rTMS-induced neuroplastic response relative to non-injured participants. Short-interval intracortical inhibition (SICI) provides a measure of motor cortical inhibition, which is known to facilitate rapid change within the motor cortex and can be modified by injury. Chapter Three does not provide insight into whether the differences in rTMS-induced neuroplastic responses between older burns survivors and non-injured older adults after burn injury is mediated by changes in SICI. This was investigated in the manuscript presented in Chapter Four.

Chapter Four presents the manuscript, “No difference in short-interval intracortical inhibition in older burn injury survivors compared to non-injured older adults”. This manuscript is to be submitted for publication. This manuscript investigated the relationship between acute burn injury, SICI (at six and twelve weeks post-injury) and functional and quality of life outcomes at twelve weeks post-injury in older adults compared to non-injured, age-matched participants. The manuscript is presented in the form that is required when submitted for publication, including an Abstract, Keywords, Highlights and a Bibliography. All references from this manuscript are presented in the Bibliography at the end of the manuscript, not in the Bibliography at the end of the thesis (although they may be in the final Bibliography as well). Figure captions are presented after the Bibliography, as they are also required to be presented separate to the figures for publication. Note that figure numbers in
this manuscript do not continue from previous figures in this thesis, or in the previous manuscript (Chapter Three). The first figure presented in this manuscript will be presented as Figure 1.

The research in this manuscript was undertaken in the same population (and at the same time) as the research presented in Chapter Three. Therefore, much of the Methods section of this manuscript will be the same as the Methods section in the manuscript in Chapter Three. The main exception will be concerning the measurement of SICI and spaced cTBS-induced changes in SICI.

Appendices are not referred to in the manuscript as these are not to be submitted for publication but are provided in this thesis. The attached appendices are applicable to both this manuscript and the manuscript in Chapter Three, and these have been discussed in the Chapter Three Foreword.
No difference in short-interval intracortical inhibition in older burn injury survivors compared to non-injured older adults.

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Abstract

Objective: Investigate differences in short-interval intracortical inhibition (SICI) between older (age 45+ years) burns survivors and non-injured controls at baseline and the change in SICI following repetitive transcranial magnetic stimulation (rTMS).

Methods: Burns Group and Control Group participants underwent two experimental sessions, six and twelve weeks post-injury. Paired-pulse transcranial magnetic stimulation (TMS) was applied to the primary motor cortex (M1) to measure SICI pre- and post-spaced continuous theta-burst stimulation (cTBS). Functional/Quality of Life measures were obtained in the experimental session twelve weeks post-injury.

Results: No significant difference in baseline SICI was evident between the Burns Group or Control Group in either session or between sessions. There was no evidence of a correlation between baseline SICI and Functional/Quality of Life measures in the Burns Group.

Conclusions: No evidence that minor burn injury alters SICI at six or twelve weeks post-injury. No evidence that rTMS (spaced cTBS) induces changes in SICI in older burns survivors or older healthy adults.

Significance: Minor burn injury does not alter short-acting motor cortical inhibition. A reduction in the capacity for neuroplasticity following burn injury is unlikely to be mediated by changes in short-acting motor cortical inhibition.
Keywords: neuroplasticity, burn, short-interval intracortical inhibition, injury, repetitive TMS, motor cortex

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Highlights:

- There was no difference in baseline SICI between recently burn injured participants and controls.
- Neither burns survivors or controls demonstrated rTMS-induced changes in SICI at six or twelve weeks post-injury.
- Baseline SICI was not correlated with Functional or Quality of Life measures in the Burns Group.
1. Introduction

It is well established that the brain is capable of changing—a phenomenon known as neuroplasticity. Structural and functional neuroplasticity underlie the ability to acquire, consolidate and retain motor skills (Dayan and Cohen, 2011). Neuroplasticity has been observed in a variety of musculoskeletal injuries, and the capacity for neuroplastic change likely impacts the functional recovery from these injuries (Boudreau et al., 2010; Snodgrass et al., 2014). The mechanisms underlying neuroplasticity in the motor cortex are of great importance. Neuroplasticity of the primary motor cortex (M1) is thought to be mediated by unmasking of existing, but functionally silent, connections via disinhibition (Jacobs and Donoghue, 1991; Sanes and Donoghue, 1997; 2000). For example, transient deafferentation can lead to a rapid expansion of motor cortical maps coupled with reduced intracortical inhibition (Ziemann et al., 1998). The rapid change in inhibition facilitates the unmasking of otherwise functionally silent connections (Chen et al., 2002). Inhibition at the cortical level is mediated by GABA-ergic (GABA: gamma-aminobutyric acid) inhibitory interneurons (Jacobs and Donoghue, 1991; Kujirai et al., 1993). Ageing has been shown to lead to a decline in both resting-state and task-related GABA-ergic activity in the M1, with declines in resting-state inhibition leading to declines in motor performance (Heise et al., 2013; Levin et al., 2014). Rehabilitation and recovery from a burn injury is a challenging process, especially in the older population. Age is known to have a significant negative impact on burn victims’ morbidity and mortality (Abu-Sittah et al., 2016; Duke et al., 2012; Edgar et al., 2013; Pham et al., 2009; Rani and Schwacha, 2012). It is plausible that functional recovery from a burn injury is impacted negatively by an age-related decline in motor cortex inhibition through the GABA-ergic system.
Transcranial magnetic stimulation (TMS), a form of non-invasive brain stimulation (NIBS), is a useful tool to investigate the activity of cortical GABA-ergic inhibitory interneurons. Paired-pulse TMS can be used to measure the excitability of several different inhibitory and excitatory processes within the M1. One major inhibitory process that has been identified is short-interval intracortical inhibition (SICI) (Kujirai et al., 1993). When a subthreshold conditioning stimulus (CS) precedes a suprathreshold test stimulus (TS) by 1 – 5 ms, the amplitude of the motor-evoked potential (MEP) elicited by the TS is suppressed because of the activation of SICI circuits (Di Lazzaro et al., 1998; Hallett, 2000; 2007; Kujirai et al., 1993; Nakamura et al., 1997; Reis et al., 2008). Pharmacological studies provide strong evidence that SICI is mediated by GABA\(_A\) receptor activity (Ilic et al., 2002; Ziemann et al., 1996).

Repetitive TMS (rTMS) can modify the excitability of the motor cortex. Continuous theta burst stimulation (cTBS) has been demonstrated to be an effective protocol that can induce long-term depression-like (LTD) changes in excitatory pathways and has been shown to reduce MEP amplitude and reduce SICI (Chung et al., 2016; Huang et al., 2007; Huang et al., 2005). Goldsworthy et al. (2012) have demonstrated that two applications of cTBS spaced by a ten-minute inter-train interval is more effective at inducing a long lasting (at least two hours) reduction in MEP amplitude than a single application of cTBS. We have shown reduced spaced cTBS-induced neuroplastic response in burns survivors (six weeks post-injury) compared to non-injured control participants (Whife et al., Under review). It is plausible that the reduced spaced cTBS-induced neuroplastic response in burns survivors is due to reduced SICI at baseline, or a reduction in the change in SICI following the application of spaced cTBS. This study aims to investigate whether SICI is modulated by an acute burn injury in older adults compared to a non-injured, age-matched control group, and
whether differences in baseline SICI are associated with functional outcome measurements within an older burn injury population. It was hypothesised that burns survivors would have less SICI than non-injured, age-matched controls.

2. Methods

2.1. Participants

Sixteen burn injury participants (ten males, $M = 56.8 \pm 7.9$ years, range 45 – 71 years) and thirteen control participants (six males, $M = 62.4 \pm 10.3$ years, range 47 – 83 years) took part in the study. Burn injury survivors were recruited through the State Adult Burn Unit (henceforth referred to as the ‘Burns Group’). The inclusion criteria for burn injury survivors were: aged 45 years or older at the time of recruitment; total body surface area (TBSA) of burn injury less than 20%; burn injury occurred less than six weeks prior to recruitment. The exclusion criteria for burn injury survivors were: conditions that may confound the measurement of recovery or hinder rehabilitation beyond the burn injury such as neurological incidents (e.g. stroke) and reported musculoskeletal skeletal injury or surgery within the last three months; severe or recent heart disease; sleep deprivation (self-assessed); any contraindication to TMS (Rossi et al., 2011; Rossi et al., 2009). Burns Group participants underwent normal treatment for their burn injury, through the State Adult Burn Unit Inpatient and/or Outpatient Services, which included an extensive medical history and physical examination, and a functional assessment at twelve weeks post-burn injury.

Non-injured volunteers were recruited from the public to form the ‘Control Group’. The inclusion criteria for these participants were: aged 45 years or older at time of recruitment; no
history of a burn injury that required medical treatment. The same exclusion criteria as for the Burns Group applied.

The protocol was performed in accordance with the Declaration of Helsinki and was approved by the Murdoch University Human Research Ethics Committee (MU HREC Reference: 2016-166), East Metropolitan Health Service (EMHS HREC Reference: 16-012), South Metropolitan Health Service (SIRO HREC Reference: 16-012) and University of Western Australia (UWA HREO RA/4/1/8354). Governance approval was obtained from East and South Metropolitan Health Services. All subjects gave written informed consent prior to testing and were screened for conditions that would contraindicate TMS (Rossi et al., 2011; Rossi et al., 2009).

2.2. Experimental procedures

2.2.1. Transcranial magnetic stimulation

All participants attended two experimental sessions at the TMS laboratory at Murdoch University, six weeks apart. In both sessions participants were seated in a comfortable chair, with their right hand resting on a soft pillow on their lap. The first dorsal interosseous (FDI) muscle on the right hand was palpated (participants asked to gently abduct the index finger against resistance for palpation of the muscle), and the overlying skin cleaned with an alcohol-based solution. Surface electromyography (EMG) was recorded from two Ag-AgCl electrodes (with water-based lubricant applied) taped into position; one electrode was placed over the belly of the muscle and one electrode was placed over the tendon insertion. A grounding electrode was attached to the skin over the distal ulna at the wrist. The EMG signal
was amplified (x1000) and band-pass filtered (20 Hz – 1 kHz) using a CED 1902 signal conditioner (Cambridge Electronic Design Co. Ltd, Cambridge, UK). The signal was then digitized at 2 kHz using a CED 1401 analog-to-digital converter (Cambridge Electronic Design Co. Ltd, Cambridge, UK) and was then stored on a computer to allow for later off-line analysis. TMS pulses (monophasic) were delivered using a figure-of-eight coil connected to a Magstim BiStim 200\(^2\) stimulator (The Magstim Company Limited, Whitland, Wales, UK). The coil was held tangentially to the scalp at an angle of 45 degrees to the sagittal plane with the handle pointed posteriorly to induce a posterior-anterior current flow.

The site for optimal stimulation and the resting motor threshold (RMT) were determined for the right FDI with left M1 stimulation. To determine the optimal stimulation site, suprathreshold pulses were delivered at a number of sites to identify the site from which FDI MEPs were evoked consistently. The optimal site was marked on a tight-fitting material swimming cap to ensure reliable placement of the coil throughout the experimental session. RMT was defined as the minimum stimulus intensity (as a percentage of the maximum stimulator output) that produced a MEP of at least 50 \(\mu V\) in at least three out of six trials in which the FDI was completely relaxed (Groppa et al., 2012; Opie et al., 2017; Rogasch et al., 2013; Rossini et al., 1999).

2.2.2. Repetitive TMS

The current study used a rTMS protocol known as cTBS. cTBS was applied using a Magstim Rapid stimulator (Magstim) connected to an air-cooled figure-of-eight coil (biphasic pulses). The optimal site for stimulation and RMT was determined using the air-cooled coil and the
Magstim Rapid prior to commencing cTBS. cTBS comprised a total of 600 pulses, applied in bursts of three pulses delivered at 50 Hz, and repeated at a frequency of 5 Hz (Huang et al., 2005). cTBS intensity was set at 70% RMT (determined using the Magstim Rapid). In the current study, ‘spaced cTBS’ was applied: two trains of cTBS were delivered ten minutes apart. This protocol was demonstrated by Goldsworthy et al. (2012) to be effective at inducing reliable and long-lasting (at least two hours) suppression of MEP amplitude.

Three blocks of paired-pulse TMS were delivered before (baseline), and at several time points after the spaced cTBS protocol (see Figure 1). There was a total of 14 single- and 14 paired-pulses per block (order randomised; the interval between trials was 5 seconds (±20% jitter)). For single-pulse trials, test stimulus intensity was set to 120% of RMT. For paired-pulse trials the conditioning stimulus intensity was set to 80% of RMT, the test stimulus intensity was set to 120% RMT, and the inter-stimulus interval (ISI) was 2 milliseconds (ms). An ISI of 2 ms was chosen due to the limited effect of short-interval intracortical facilitation (SICF) at this ISI (Peurala et al., 2008).

![Fig. 1: Experimental protocol for each TMS session. Abbreviations: RMT, resting motor threshold; SICI, short-interval intracortical inhibition; cTBS, continuous theta-burst stimulation; Post-cTBS\textsubscript{EARLY}, averaged early blocks; Post-cTBS\textsubscript{LATE}, averaged late blocks.](image-url)
2.3. Functional and Quality of Life measures

Functional and Quality of Life measures were obtained from all participants at the second TMS session (median days after injury for Burns Group = 88, range = 79 – 103). In addition, all Burns Group participants completed both the Short-Form Health Survey version 2 (SF-36) and the Burn Specific Health Scale – Brief (BSHS-B) Quality of Life assessments. The SF-36 is a self-completed questionnaire that is an indicator of overall health status across eight domains (Ware and Sherbourne, 1992); the BSHS-B is a self-completed questionnaire that is an abbreviated outcome scale, designed specifically for burns survivors, which is used to evaluate burn-specific aspects of health status across nine domains or three broad categorisations (Kildal et al., 2001; Willebrand and Kildal, 2008). Burns Group participants who had sustained a burn injury to their upper limb (n = 8) were also asked to complete the QuickDASH (Disabilities of the Arm, Shoulder and Hand) Outcome Measure (QuickDASH). The QuickDASH is a self-completed questionnaire that uses eleven items to measure physical function and symptoms in people with any or multiple musculoskeletal disorders of the upper limb (Wu et al., 2007). Burns Group participants who had sustained a burn injury to their lower limb (n = 6) were also asked to complete the Timed Up and Go (TUG) test and the Lower Limb Function Index-10 (LLFI). The TUG test involves participants starting in a seated position in a chair, standing, walking three metres quickly to a line identified on the floor, and then returning to the seated position (Herman et al., 2011; Shumway-Cook et al., 2000). The LLFI is a self-assessment questionnaire that assesses functional status in individuals with lower limb conditions (Gabel et al., 2012). All of these assessments have been validated for use in a burn injury population (Edgar et al., 2010; Finlay et al., 2010; Finlay et al., 2014b; Gittings et al., 2016; Ryland et al., 2016; Wu et al., 2007). Some Burns Group participants had both upper and lower limb burn injuries (n = 2), and therefore completed both upper and lower limb functional measures.
All Control Group participants completed the Short-Form Health Survey version 2 (SF-36) and the Timed Up and Go (TUG) test. (Only the TUG test and SF-36 were assessable across both groups as the BSHS-B, QuickDASH and LLFI are either burn or injury specific and not easily completed by a Control Group without injury. As noted above, TUG measures were only available from those Burns Group participants with a lower limb burn injury.)

2.4. Data analysis

Four of the sixteen participants in the Burns Group were lost to follow-up after the first TMS session (two participants were unavailable to attend the second experimental session; another participant had commenced a new medication that contraindicated the use of TMS; in one participant EMG recordings were unable to be interpreted). These four participants did not complete the Functional and Quality of Life assessments (completed at the second TMS session). Their data from the first TMS session at six weeks are included in the analyses comparing Session 1 between groups, but are not included in analyses that compares Session 1 to Session 2 or Session 1 outcomes to Functional and Quality of Life measures (because Functional and Quality of Life measures were obtained in Session 2).

EMG activity was analysed by visual inspection of the offline recordings. Any trial with muscle activity in the 250 ms preceding the onset of the MEP was excluded from analysis. The peak-to-peak MEP amplitude (mV) was obtained from the 40 ms of EMG activity beginning 15 ms after the test stimulus.
2.4.1. Baseline SICI data

To quantify SICI, the mean MEP amplitude from paired-pulse trials was expressed as the ratio of the mean MEP amplitude from single-pulse trials. To test for differences in RMT and baseline SICI between groups and across experimental sessions, two-way mixed analysis of variance (ANOVs) were performed, with within-subject factor of SESSION (Session 1, Session 2) and between-subjects factor of GROUP (Burns, Control). Separate ANOVAs were performed on RMT and baseline SICI data.

2.4.2. Spaced cTBS-induced change in SICI

To test for differences in SICI in the three baseline blocks, repeated measures analysis of variance (RM-ANOVA) with within-subject factors of BLOCK were performed on the raw SICI data. Separate ANOVAs were performed for each session and each group. No significant differences were found between baseline blocks (Burns Group Session 1: $F_{1,15} = 2.32$, $p = .15$, $\eta_p^2 = .13$, Session 2: $F_{1,11} = 2.83$, $p = .12$, $\eta_p^2 = .21$, and Control Group Session 1: $F_{1,12} = 0.02$, $p = .88$, $\eta_p^2 < .01$, Session 2: $F_{1,12} = 0.13$, $p = .72$, $\eta_p^2 = .01$); therefore, the three baseline blocks were averaged.

To test for differences in SICI at 0 and 5 minutes post-cTBS, paired-samples t-tests were performed on the raw SICI data. Separate t-tests were performed for each session and each group. No significant differences were found between 0 minutes post-cTBS and 5 minutes post-cTBS time points (all $t < 1.19$, all $p > .25$); therefore, these two post-cTBS blocks were averaged, and analysed as ‘post-cTBSearly’. To test for differences in SICI at 15 and 30 minutes post-cTBS, paired-samples t-tests were performed on the raw SICI data. Separate t-
tests were performed for each session and each group. No significant differences were found between 15 minutes post-cTBS and 30 minutes post-cTBS time points (all $t < 0.98$, all $p > .18$); therefore, these two post-cTBS blocks were also averaged, and analysed as ‘post-
cTBS\textsubscript{LATE}’.

To test for differences in SICI following spaced cTBS, two-way RM-ANOVAs (with polynomial contrasts) were performed with within-subject factors of SESSION and TIME (baseline, post-cTBS\textsubscript{EARLY}, post-cTBS\textsubscript{LATE}). Separate RM-ANOVAs were performed on SICI data from Burns Group participants and Control Group participants. Greenhouse-Geisser corrections were used for analyses in which the assumption of sphericity was violated (Mauchly’s test of sphericity).

To test for differences in SICI following spaced cTBS between the Burns Group and the Control Group, two-way mixed RM-ANOVAs were performed, with within-subject factor of TIME (baseline, post-cTBS\textsubscript{EARLY}, post-cTBS\textsubscript{LATE}) and between-subjects factor of GROUP (Burns, Control).

Conditional on significant main effects of interactions, post-hoc analyses were performed. Statistical significance was accepted at $\alpha < 0.05$. Data are presented as mean ± standard deviation, except in the figures where the standard error of the mean (SEM) is presented.
2.4.3. **Functional and Quality of Life measures**

The relationship between Functional and Quality of Life domain scores and baseline SICI was assessed using correlational analysis.

3. **Results**

3.1. **Participant characteristics**

There was no significant difference in age or gender between groups (Age $p = .11$, Gender $p = .40$). The average TBSA of burn size in the Burns Group was 2.14%, with burn size ranging from 0.12% to 7.25%. All burn injuries in this study had a TBSA of less than 15% and are therefore considered to be minor burn injuries (Finlay et al., 2014a). The location of burn injury included nine upper limb burns (two individuals sustained burns to both upper limbs but are only counted once), nine lower limb burns (buttocks included; four individuals sustained burns to both lower limbs but are only counted once), three thorax burns (chest, abdomen, pelvis (including genitals but excluding buttocks) and back) and three head and neck burns. Only one participant sustained a burn injury over the area targeted by TMS (right hand, first dorsal interosseous muscle). Six of the Burns Group participants required surgical management of their injuries, ten did not. The median days between sessions for the Burns Group was 44 (range = 32 – 55); the median days between sessions for the Control Group was 50 (range = 39 – 56).
3.2. Baseline SICI

There was no difference in baseline SICI across session or between groups. The RM-ANOVA performed on the baseline SICI data showed no main effect of SESSION \((F_{1,23} = 1.67, p = .21, \eta^2 = .07)\), no main effect of GROUP \((F_{1,23} = 0.32, p = .58, \eta^2 = .01)\), and no SESSION*GROUP interaction \((F_{1,23} = 0.06, p = .81, \eta^2 < .01)\).

3.3. Effect of spaced cTBS on SICI

Within the Burns Group there was no effect of spaced cTBS on SICI ratios. The RM-ANOVA showed no main effect of TIME \((F_{1,11} = 1.65, p = .23, \eta^2 = .13)\), no main effect of SESSION \((F_{1,11} = 1.01, p = .34, \eta^2 = .08)\) and no SESSION*TIME interaction \((F_{1,11} = 0.53, p = .48, \eta^2 = .05)\). Within the Control Group there was no effect of spaced cTBS on SICI ratios. The RM-ANOVA showed no main effect of TIME \((F_{1,12} = 1.34, p = .27, \eta^2 = .10)\), no main effect of SESSION \((F_{1,12} = 1.09, p = .32, \eta^2 = .08)\) and no SESSION*TIME interaction \((F_{1,12} = 0.01, p = .93, \eta^2 < .01)\). The amplitude of the test stimulus was not matched over time in this study. Average single pulse MEP amplitude and average SICI for each time point in Session 1 and Session 2 is presented in Table 1.
<table>
<thead>
<tr>
<th></th>
<th>Session 1</th>
<th>Mean BL</th>
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<tr>
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<tr>
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<td>Control</td>
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<tr>
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<td>Burns</td>
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<tr>
<td>MEP</td>
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</table>

**Table 1:** Average single-pulse MEP amplitude and SICI at baseline (BL) and at time points post-cTBS for Session 1 and 2.

3.4. **Baseline SICI and Functional and Quality of Life measures**

In this study, there was no change in SICI following spaced cTBS (in either the Burns Group or Control Group) and therefore associations between spaced cTBS-induced SICI and Functional and Quality of Life measures have not been investigated. Within the Burns Group there was no significant relationship between baseline SICI and any Functional or Quality of Life measures. The Control Group however did show significant relationships between baseline SICI and TUG times in Session 1 \( r = .64, p = .02, 95\% \) confidence interval \( [CI] = .14, .88 \), and between baseline SICI and TUG times \( r = .59, p = .04, 95\% \) CI = .05, .85) and SF-36 Domain Physical scores \( r = .58, p = .04, 95\% \) CI = .04, .86) in Session 2. Scatterplots for baseline SICI and TUG times across both sessions in the Control Group is presented in Figure 2, while Figure 3 shows the scatterplots for baseline SICI and SF-36 Domain Physical in the Burns Group and Control Group in Session 2.
**Fig. 2:** Baseline SICI and TUG times across Session 1 and 2 in the Control Group.

* = statistically significant relationship.

**Fig. 3:** Baseline SICI and SF-36 Domain Physical scores in the Burns Group and Control Group in Session 2.

* = statistically significant relationship.
4. **Discussion**

In this study, we have used paired-pulse TMS and rTMS to examine SICI in older burns survivors at six and twelve weeks post-burn injury and in non-injured age-matched controls. The results of the current study show that there is no evidence of a difference in SICI at baseline or change in SICI following spaced cTBS in burns survivors and non-injured control participants. Previous research (Whife et al., Under review) showed a reduced capacity for spaced cTBS-induced neuroplastic response in burns survivors six weeks post-injury; the current results suggest that the reduced capacity for spaced cTBS-induced neuroplastic response is not due to altered SICI.

### 4.1. No difference in baseline SICI between Burns and Control Groups

In this study, there was no significant difference in baseline SICI between burns survivors and non-injured control participants; this absence of a difference in baseline SICI between burns survivors and control participants was evident at six and twelve weeks post-injury. Given that SICI is mediated by GABA\(_A\) receptor activity (Di Lazzaro et al., 2000; Ilic et al., 2002; Ziemann et al., 2006), the current findings suggest that a minor burn injury does not lead to a change in GABA\(_A\) mediated inhibition in the M1 between six and twelve weeks post-injury. Therefore, there was no evidence from this research that the globally (i.e. regardless of burn injury location) reduced capacity for spaced cTBS-induced neuroplastic response in older burns survivors detected in Whife et al. (Under review) is mediated by global changes in SICI. At first glance, the absence of a difference in SICI between burns survivors and non-injured controls is somewhat surprising given that there is a reduction in SICI following peripheral injury such as amputation and short-term deafferentation using ischemic nerve block (Chen et al., 2002; Chen et al., 1998). However, there are two plausible
explanations for the lack of difference in SICI in the current sample of burns survivors and non-injured control participants.

First, SICI in the current study was measured from a hand muscle (right FDI), but only one of the burns survivors had a burn to their right hand, and one had a burn to the volar surface of all fingers bilaterally. Experiments studying mechanisms of cortical neuroplasticity secondary to deafferentation measured inhibition acting on the muscle immediately proximal to deafferentation (Chen et al., 2002). Similarly, measures of inhibition in amputees were obtained from muscles just proximal to the amputation site (Chen et al., 2002; Chen et al., 1998). Furthermore, in amputees, a significant decrease in SICI in the amputated limb has been shown compared to the intact limb, but SICI in the intact limb is not significantly different to non-injured subjects (Chen et al., 1998). Therefore, it is reasonable to speculate that a decrease in SICI in burns survivors relative to non-injured control participants may be observed if SICI acting on muscles immediately proximal to the area of burn injury is measured. It would be valuable for future research to examine SICI in upper limb burn injury survivors, comparing SICI in the burn injured and non-injured upper limb.

Second, whilst the results of the current study did not provide any evidence of a difference in GABA\textsubscript{A} mediated inhibition between burns survivors and non-injured control participants, it is possible that other types of inhibition are altered following minor burn injury. Indeed, a recent study examined differences in the cortical silent period (period of inactivity in the EMG following a suprathreshold TMS pulse to a voluntarily contracted muscle) between burns survivors and non-injured controls (Garside et al., 2018). The cortical silent period is likely mediated by GABA-ergic inhibition, although it is likely that both GABA\textsubscript{A} and
GABAB receptor activity is implicated (McDonnell et al., 2006; Paulus et al., 2008; Stetkarova and Kofler, 2013; Tremblay et al., 2013). Garside et al. (2018) demonstrated a significantly shorter cortical silent period in the burned arm of burns survivors with an upper limb injury compared to non-injured controls in those who were injured less than two years ago, those with partial thickness burns, those with upper limb burns only and those with burns less than 10% TBSA. As in this study, EMG recordings were taken from the FDI muscle in all participants in the Garside et al. (2018) study, regardless of burn location. The research suggests that there is a reduction in intracortical inhibition in burns survivors compared to non-injured controls, although this may not be mediated by GABA_A receptor activity. Given the possible role of intracortical inhibition mediated by GABA_B receptor activity future research should examine long-interval intracortical inhibition (LICI). An inter-stimulus interval of 100 – 200 ms between conditioning and test stimuli can reduce MEP amplitude secondary to LICI, with pharmacological evidence showing that LICI is mediated by GABA_B receptor activity (McDonnell et al., 2006; Müller-Dahlhaus et al., 2008; Sanger et al., 2001; Werhahn et al., 1999).

4.2. No change in SICI following spaced cTBS

Both burns survivors and non-injured control participants demonstrated no change in SICI following spaced cTBS. This may show that spaced cTBS does not modulate the excitability of SICI circuits, and therefore suggests that GABA_A mediated inhibition does not underlie the change in neuroplastic responses following spaced cTBS. To our knowledge there is no other research examining the effect of spaced cTBS on SICI. Changes in SICI following a single application of cTBS are mixed; a recent systematic review and meta-analysis on the use of theta-burst stimulation in the motor cortex found that cTBS induced a decrease in SICI
at the early time point (within five minutes), but no significant decrease was seen at 20 – 30 minutes post application of cTBS (Chung et al., 2016). Taken together, the available evidence suggests that the role of GABA\(_A\) mediated intracortical inhibition in (single or spaced) cTBS-induced neuroplasticity is not well understood.

4.3. Associations between baseline SICI and Functional and Quality of Life measures

No significant relationship was demonstrated between baseline SICI ratios and Functional or Quality of Life measures within the Burns Group. Given there was no difference in baseline SICI between burns survivors and non-injured controls it might be expected that there are no associations between baseline SICI and Functional and Quality of Life measures in burns survivors. Further, the Functional and Quality of Life measures used may not be sufficiently sensitive to detect associations between SICI and motor function. SICI measurements were taken from a hand muscle, whereas Functional and Quality of Life measures were either limb specific or very generalised (i.e. overall wellbeing). Associations may be identified if more specific measures of motor function were measured, or if SICI was obtained from the area of injury specifically.

In the Control Group, baseline SICI ratios were significantly associated with TUG times in Session 1 and Session 2: greater SICI (lower SICI ratio) was associated with slower performance on the TUG. This finding is surprising for two reasons. First, SICI was measured in a resting hand muscle and the TUG primarily involves lower-limb function. Whilst there is some evidence in the literature that SICI is associated with motor control these studies show associations between SICI recorded from a muscle that is engaged in the motor
Indeed, SICI is typically measured from a muscle involved in task performance and measures of SICI are obtained both at rest and during task performance (Berghuis et al., 2016; Berghuis et al., 2017; Duque et al., 2017; Duque and Ivry, 2009; Opie et al., 2015). Second, the direction of the association between SICI and TUG performance is such that greater SICI is associated with poorer performance. Evidence from the literature suggests that greater SICI is associated with better motor performance in visuomotor tasks in younger adults (Berghuis et al., 2017) and with improved reaction times (Fujiyama et al., 2012) and relative motor skill acquisition (Berghuis et al., 2016) in older adults. Therefore, while the current results provide some evidence to suggest an association between SICI measured in a hand muscle and performance on the TUG, further research is necessary to first replicate this association, and second determine whether SICI measured in a lower limb muscle is also associated with performance on the TUG. Likewise, in the second session greater SICI was associated with lower SF-36 Domain Physical scores in the Control Group. Although also a surprising finding (for the reasons outlined in this paragraph), the Physical Domain score is a very generalised measure of physical function (Ware and Sherbourne, 1992) and SICI measurements from a hand muscle at rest might not accurately reflect the role of intracortical inhibition in generalised physical function.

4.4. Limitations

This was a pilot project with a small sample size and therefore these findings need to be replicated in larger studies. All burn injuries in this study were minor and some burn injuries were very small (i.e. less than 1% TBSA). This research needs to be replicated in a population with larger burn injuries, as results may differ in a population with a more significant injury. SICI was measured from the right FDI muscle regardless of burn injury
location. Future research should examine potential changes in SICI following acute burn injury by examining SICI in body parts adjacent to the burn injury. No data regarding changes in SICI were available before six weeks post-injury, this would be a valuable addition in future research. In this study SICI was only measured with a single conditioning stimulus intensity and a single inter-stimulus interval (ISI). SICI is dependent on the intensities of the conditioning stimulus. Variation of the conditioning stimulus intensity results in a U-shaped variation in SICI magnitude. The descending arm of this U-shape reflects increasing inhibition with increasing stimulus intensity, with the ascending arm reflecting decreasing inhibition with continuing increases in stimulus intensity (Peurala et al., 2008). Obtaining the full SICI curve as a function of conditioning stimulus intensity provides a measure of both the sensitivity of circuits that mediate SICI (that is, the descending limb) and the magnitude of SICI (that is, maximal inhibition). In the current study, SICI was measured at a single conditioning stimulus intensity that likely corresponds to maximal inhibition; it would be valuable to measure SICI as a function of CS intensity in burns survivors and non-injured controls to determine whether the sensitivity of SICI circuits is altered following a burn injury. Furthermore, measures of SICI are influenced by short-interval intracortical facilitation (SICF). SICF provides a measure of intracortical facilitatory processes. SICF can be measured when the preceding stimulus (set to produce a 1 mV MEP alone) precedes a second stimulus (set at 90% of RMT). The extent of SICF induced is dependent upon the ISI between stimuli, with research showing that ISIs of 1.5 ms, 2.5 – 3.1 ms and 4.5 ms elicit significantly larger MEP amplitudes than a control MEP elicited by the preceding stimulus alone (Peurala et al., 2008). Although SICI measured at an ISI of 3 ms is likely to be influenced more by SICF than SICI measured at an ISI of 2 ms, SICF would still have some influence on SICI measured at an ISI of 2 ms. Burn injury may alter the balance between motor cortical inhibition and excitation. Therefore, future research should examine
SICI at different conditioning stimulus intensities and ISIs, taking into account the possible effect of SICF. The amplitude of the test stimulus was not matched over time in this study; future research should consider this.

5. *Conclusion*

The results of the current study show no evidence of a difference in SICI between burn injury survivors and non-injured control participants. In addition, there was no evidence of a change in SICI following spaced cTBS, suggesting that GABA_A mediated inhibition was not underlying spaced cTBS-induced neuroplastic responses.
6. Bibliography


7. **Figure captions**

**Fig. 1:** Experimental protocol for each TMS session. Abbreviations: RMT, resting motor threshold; SICI, short-interval intracortical inhibition; cTBS, continuous theta-burst stimulation; Post-cTBS\text{\textsubscript{EARLY}}, averaged early blocks; Post-cTBS\text{\textsubscript{LATE}}, averaged late blocks.

**Fig. 2:** Baseline SICI and TUG times across Session 1 and 2 in the Control Group.

* = statistically significant relationship.

**Fig. 3:** Baseline SICI and SF-36 Domain Physical scores in the Burns Group and Control Group in Session 2.

* = statistically significant relationship.

**Table 1:** Average single-pulse MEP amplitude and SICI at baseline (BL) and at time points post-cTBS for Session 1 and 2.
CHAPTER FIVE – Summation of Discussion

In this thesis, I have demonstrated that injury to the musculoskeletal system, such as burn injury, can plausibly induce central nervous system neuroplastic change. The capacity of an individual to evoke a neuroplastic response secondary to musculoskeletal injury may impact rehabilitation due to the importance of LTP and LTD processes in motor learning. Targeting neuroplasticity in rehabilitation may improve functional outcomes. Within older adults, burn injury appears to decrease neuroplastic responses at six weeks after injury relative to non-injured controls (as shown in Chapter Three), and it is unlikely that SICI plays a role in this altered neuroplastic capacity (as shown in Chapter Four). Non-injured older adults demonstrate delayed neuroplastic responses secondary to spaced cTBS (relative to that demonstrated by Goldsworthy et al. (2012) in younger adults) which is reproducible at a group level.

As outlined in the Chapter Two review article, musculoskeletal injury can affect afferent sensory information which can drive central neuroplastic changes. Burn injury can lead to damage to sensory nerve fibres, leading to increased nociception as well as altered cutaneous input (Coderre and Choinière, 2000) and may affect proprioceptive input depending on the location of the injury. There is evidence to suggest that both primary hyperalgesia (within the site of injury) and secondary hyperalgesia (to surrounding undamaged tissues) are mediated by both peripheral and central sensitisation (Coderre and Choinière, 2000). Central sensitisation occurs when inputs from the peripheral tissue are no longer required to maintain hyperalgesia. Prolonged pre-emptive anaesthetic nerve blocks (Pedersen et al., 1996) and local anaesthetic infiltration (Dahl et al., 1993) prior to burn injury prevents or delays the onset of hyperalgesia, with pre-emptive anaesthetic nerve blocks significantly reducing
primary and secondary hyperalgesia. These studies demonstrate that burn injury can lead to significant nociceptive input that may drive a persistent pain perceived state in which neuroplastic adaptation can occur.

In Chapter Three (investigating changes in markers of neuroplasticity following burn injury), results showed that spaced cTBS-induced neuroplastic change was reduced in older burns survivors compared to non-injured control participants. Activation and sensitisation of nociceptive fibres could play a role in the decrease in neuroplastic response following spaced cTBS seen at six weeks post-injury in burns survivors. Acute pain has been shown to decrease corticospinal excitability (Burns et al., 2016b), while sustained pain has been shown to increase corticospinal excitability after four days (Schabrun et al., 2016). A recent meta-analysis has found no difference in corticospinal excitability between those with chronic pain and controls (Parker et al., 2016). Acute pain states have been shown to increase intracortical inhibition while chronic pain states have demonstrated a reduction in intracortical inhibition (Burns et al., 2016a; Parker et al., 2016; Schabrun et al., 2016; Schabrun and Hodges, 2012). However, there is no evidence to suggest that corticospinal excitability and intracortical inhibition is increased, decreased or unchanged at six weeks after injury. Measuring the changes in corticospinal excitability and intracortical inhibition post-injury provide some insight in to how burn injury can result in neuroplastic changes over time. The research from this thesis suggests that neuroplastic adaptation is maximal in the first six weeks after minor burn injury in older adults, as differences between groups were not statistically significant by twelve weeks. To my knowledge this is one of only three studies to investigate motor cortical changes following burn injury (Garside et al., 2018; Portilla et al., 2013), and the only study to investigate neuroplastic motor cortex change in an acute burn injury population. In the current study, it is important not to confound burn injury participants with chronic pain.
suffers. Participants were enrolled in the study within the first six weeks post-injury. A detailed pain history was not taken in the course of this research, but participants were asked about their levels of overnight pain (between one and ten) in an attempt to assess their quality of sleep and were excluded if they had pain greater than five out of ten. Therefore, while pain may have some impact on the capacity for neuroplastic response, it is unlikely that prolonged pain alone is responsible for the difference seen between burn injury participants and non-injured controls. Instead, the causes are likely multi-factorial and may include alteration of cutaneous and proprioceptive sensation secondary to damage to sensory receptors, as well as other factors, such as inflammation.

Burn injury is known to cause sustained inflammation and a hypermetabolic state (Atiyeh et al., 2008; Jeschke et al., 2008; Jeschke et al., 2011; Stanojcic et al., 2018). Burn injury has also been shown to be correlated with an increase in nervous system morbidity (Vetrichevvel et al., 2016). Burn injury is known to cause systemic alterations in nerve fibre density and function (Anderson et al., 2010; Hamed et al., 2011; Morellini et al., 2012). Increased brain tissue levels of tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 have also been demonstrated post-burn injury (Ji et al., 2010). Inflammation is increasingly being recognised as an important factor in neurological and psychiatric diseases. Peripheral derived immune factors (such as lymphocytes and cytokines) are thought to participate in the modulation of a variety of neurological and psychiatric diseases by impacting neuroplasticity (Hayley, 2014). Many psychiatric and neurological diseases, which have been associated with changes in neuroplasticity and neuroinflammatory processes, have shown reductions in adult hippocampal neurogenesis, diminished cortical dendritic arbors, deficits in LTP and impaired synaptogenesis (Hayley, 2014). An over-production of pro-inflammatory cytokines may
damage neuronal structure and function, which leads to a decreased ability of the central nervous system to perceive, respond and adapt to external or internal stimuli (Calabrese et al., 2014). There is evidence that pro-inflammatory factors (such as TNF-α and IL-1β) provoke neuroplastic deficits and, in contrast, anti-inflammatory and neurotrophic cytokines enhance neuroplastic adaptation (Calabrese et al., 2014; Hayley, 2014; Hayley and Litteljohn, 2013; McEwen and Gianaros, 2011; Phillips et al., 2014). The neurotrophin brain-derived neurotrophic factor (BDNF) is a major regulator of synaptic transmission and neuroplasticity at synapses within the CNS (Allen and Dawbarn, 2006; Bramham and Messaoudi, 2005), and has been shown to be reduced by increased levels of pro-inflammatory cytokines (Calabrese et al., 2014). It is possible that burn injury induced inflammation contributed to the difference in neuroplastic responses between burns survivors and non-injured controls in this research. However, there was no measure of inflammatory markers (or BDNF) in this study. Exercise has been shown to be neuroprotective, positively correlated with plasma levels of BDNF and decreases inflammatory markers (Phillips et al., 2014). Future research into the relationship between burn injury and neuroplasticity would warrant measuring levels of inflammatory markers, BDNF and either measuring activity levels or implementing exercise as a treatment variable that may change measured outcomes.

The research presented in *Chapter Four* showed no differences in SICI at baseline between burns survivors and non-injured controls, and no change in SICI following spaced cTBS in either group. These results suggest that SICI does not mediate the differences in spaced cTBS-induced neuroplastic responses evident between the burns survivors and non-injured controls. The literature suggests that there are decreases in intracortical inhibition in the motor cortex after amputation and short-term deafferentation (Chen et al., 2002; Chen et al.,
Intracortical inhibition (mediated by GABA-ergic mechanisms) appears to play an important role in the rapidly occurring neuroplastic changes seen in amputation or deafferentation (Chen et al., 2002; Chen et al., 1998). A reduction in GABA-ergic inhibition may induce a permissive state within the cortex in which long-term changes can then occur (Chen et al., 1998). A difference in baseline SICI between groups may not be evident for a number of reasons (as outlined in Chapter Four), however it is also possible that a minor burn injury is not a sufficiently large injury (when compared to amputation or deafferentation) to induce a long-lasting decrease in SICI. However, minor burns have been shown to induce systemic responses (Duke et al., 2015b; O’Halloran et al., 2016; Stevenson et al., 2017; Vetrichievvel et al., 2016) and it is therefore not unreasonable that even a minor burn could induce changes in intracortical inhibition. Rapid changes (within hours) of SICI secondary to acute burn injury remains unknown but may reflect those seen in individuals with acute pain.

The research in Chapter Three demonstrates that older non-injured adults have a delayed (15 – 30 minutes) neuroplastic response to spaced cTBS. This was reproducible at a group level across two sessions. To my knowledge this is the only study investigating the effect of spaced cTBS in older adults. The evidence for the efficacy of spaced cTBS with an inter-train interval of ten minutes showed a decrease in corticospinal excitability from five minutes after the second application of cTBS, however that study was conducted in young adults (mean age: 24.2 ± 1.4 years) (Goldsworthy et al., 2012). The rational for using a ten minute inter-train interval was based on efficacy of this time in animal data (Abraham et al., 2002; Goldsworthy et al., 2012). Gamboa et al. (2011) have also investigated the effect of spaced cTBS, although the cTBS protocol differed from that used by Goldsworthy et al. (2012). Inter-train intervals of five and 20 minutes were utilized and showed no increased after-
effects compared to a single application of cTBS. The Gamboa et al. (2011) study was also conducted with a young cohort (mean age: 24.6 ± 1.7 years). Opie et al. (2017b) found that priming theta-burst stimulation was effective for modulating M1 plasticity in younger adults but was ineffective in older adults. A similar result was obtained when priming paired associative stimulation (PAS) was applied to the M1, with priming being effective for younger adults but ineffective in older adults (Opie et al., 2017a). Both these priming protocols implemented a ten-minute gap between the application of priming and test interventions. However, recent evidence suggests that older adults may respond similarly to younger adults to priming protocols if interval times are increased. When priming PAS protocols similar to those used by Opie et al. (2017a) were applied to older adults with either a ten minute inter-PAS interval (IPI) or a 30 minute IPI, the induction of LTP-like plasticity was significantly greater with the longer IPI of 30 minutes (Sidhu et al., 2017). The findings suggest that age-related changes in neuroplasticity occur in a time-dependent manner. It is therefore plausible that the application of spaced cTBS with an inter-train interval longer than ten minutes may induce neuroplastic changes similar to those seen in young adults with shorter inter-train intervals.

A decrease in neuroplastic response secondary to burn injury may impact functional recovery. The correlation between the SF-36 Domain General Health and normalised MEP change suggests that there are worse outcomes for those with less capacity for neuroplasticity. No other correlations were seen either in SF-36 or BSHS-B outcomes with TMS measurements. The SF-36 has been shown to be more sensitive to change after burn injury than the BSHS-B after approximately one month (Edgar et al., 2010), and may explain the lack of correlation of TMS measures with any BSHS-B measurements. The Burns Group had significantly slower TUG times than the Control Group which may be secondary to burn-
induced neuroplastic change, but this is speculation only. Future research investigating the association between decreased neuroplasticity post-burn injury and functional outcomes should look to include repeated functional measures, such as the SF-36 and TUG, coupled with repeated measurements of neuroplastic change. This would provide insight into how functional (and quality of life) outcomes evolve post-burn injury relative to neuroplastic adaptation, which may prove valuable when looking to implement meaningful rehabilitative therapy.

No correlation was seen between baseline SICI and Functional or Quality of Life measures in the burn injury population. Decreases in the ability to control cortical inhibition with advancing age may explain functional declines observed in healthy ageing, such as longer reaction times, impaired coordination and deterioration of fine motor functions (Levin et al., 2014). Measuring SICI after visuomotor tasks has demonstrated a decrease in inhibition within older adults that is not seen in younger adults (Berghuis et al., 2017). Specifically, within older adults, higher performance on a visuomotor task has been correlated with a greater ability to modulate SICI relative to lower performing older adults (Fujiyama et al., 2012). A younger control group was not available in this study, and therefore it is unclear whether the relationship between baseline SICI and burn injury evolves with age. There is some evidence that age-related changes in brain structure and function can be reversed and skills revived by training aimed at inducing neuroplasticity, and anodal transcranial direct current stimulation (tDCS) has been shown to improve the efficiency of inhibitory control (Levin et al., 2014).
The sample size in these studies is small, and these results will need to be replicated in larger studies. However, should future studies support the findings of a decreased neuroplastic response post-burn injury, strategies to promote or normalise neuroplasticity can be implemented. This could include rehabilitation strategies outlined in Chapter Two, such as intense repetition and task-specific movements, visualisation of movements and graded motor imagery. Implementing these techniques that rely on neuroplasticity may prove difficult, however, in a population that has been shown to have a decreased neuroplastic response both secondary to age and also due to recent burn injury. Given the decline in neuroplasticity and impaired motor skill acquisition in older adults there is an increasing focus on whether NIBS techniques can be used to ‘prime’ the brain. By modulating synaptic activity prior to further intervention, the cortex can primed to be more receptive, thus facilitating a greater neuroplastic response (Müller-Dahlhaus and Ziemann, 2015). Inducing functionally meaningful neuroplasticity may help improve motor skill acquisition and improve motor function in older adults where neuroplasticity is otherwise decreased. However, the current evidence is mixed as to whether priming is effective in older adults (Fujiyama et al., 2017; Opie et al., 2017b; Sidhu et al., 2017; Zimerman et al., 2013). Zimerman et al. (2013) demonstrated substantial improvements in the acquisition of complex motor skills in older adults when tDCS was applied to the motor cortex concurrently with training. Fujiyama et al. (2017) showed that skill acquisition in older adults was improved when preconditioning tDCS was applied prior to subsequent tDCS concurrent with skilled training. In contrast, priming theta-burst stimulation (Opie et al., 2017b) and PAS (Opie et al., 2017a) has been found to be ineffective for modulating M1 plasticity in older adults, but was effective in younger adults. As discussed, this may be secondary to the time-dependent nature of neuroplasticity in older adults. If priming protocols can be shown to induce neuroplasticity in
older adults, they may prove a useful tool in maximising rehabilitation outcomes in the older burn injury population.
CHAPTER SIX – Clinical Recommendations and Conclusions

Burn injury in older adults appears to reduce rTMS-induced corticospinal excitability (as a measure of neuroplasticity) at six weeks post-injury, with some evidence that the reduced capacity for neuroplastic response is related to Functional and Quality of Life measures. Identifying altered neuroplasticity secondary to burn injury in older adults may provide an opportunity for intervention. This may include targeted rehabilitation techniques aimed at maximising neuroplastic potential, as well as NIBS techniques to induce neuroplastic adaptation, thereby priming the brain for intervention and facilitating improved rehabilitation outcomes. Improving recovery in a population that is known to have declines in physical functioning and quality of life post-injury may have significant benefits not only at an individual level, but also financially with decreased length of stay in hospital and increasing early discharge to home rather than into a care provider.

Further research is required, both to replicate these findings and investigate underlying causes or associated factors that were not within the scope of this research. Future research should consider measuring Functional and Quality of Life measures at multiple time points post-injury, as the research in this thesis only provided one measure at twelve weeks post-injury; the relationship between neuroplastic changes and Functional or Quality of Life measures prior to this is unknown. In addition, measuring markers of neuroplasticity at earlier time points post-injury may provide further insight into how acute burn injury affects neuroplastic responses at critical times in recovery and rehabilitation. Additional research should look to include burns survivors with larger TBSA burn injuries to investigate whether the findings in this research extrapolate to those with a more significant burn injury. A reliable measure of pain post-injury would be a valuable addition to further research in this area. As discussed,
pain potentially impacts neuroplasticity and this research was lacking data in this area. Given the role of inflammation in burn injury and neurological disease, a measure of inflammatory markers post-injury could also provide a useful insight into how neuroplasticity is impacted by inflammation in burn injury. BDNF is affected by inflammation and is involved in synaptic neuroplasticity, and therefore future research may include measures of BDNF (which can now be reliably measured in human serum (Polacchini et al., 2015)). The effect of exercise as a rehabilitation intervention and the relationship to neuroplasticity in burns survivors also warrants further research.

The delayed rTMS-induced neuroplastic responses in older adults warrants further investigation. To my knowledge this is the first time this has been demonstrated using a spaced cTBS protocol. Further research into whether increasing inter-train intervals modulates rTMS-induced neuroplastic responses in older adults is also necessary. The understanding that older adults have a decreased capacity for neuroplasticity may be challenged if they display similar rTMS-induced neuroplastic responses to younger adults at a longer inter-train interval. This would instead suggest that neuroplastic response in older adults is not necessarily decreased, but time-dependent.

This thesis shows that acute burn injury in older adults induces a change in neuroplastic responses that may impair functional recovery. Better understanding the mechanisms underlying this change and further characterising how this evolves over time may facilitate the implementation of rehabilitation strategies that improve outcomes, such as physical dysfunction, in older adults.
BIBLIOGRAPHY


### Inclusion Criteria

Participants were aged 45 years or older at the time of recruitment.

Total body surface area of burn injury was less than 20%.

Burn injury occurred less than six weeks prior to recruitment.

### Exclusion Criteria

Inability to understand written English and, or unable to provide consent.

Pregnancy

Conditions that may confound the measurement of recovery or hinder rehabilitation beyond the burn injury such as neurological incidents (e.g. stroke) and reported musculoskeletal skeletal injury or surgery within the last three months. Participants will not be excluded if age related or chronic conditions are reported.

Conditions that may impact the safety of rTMS use, including:

- History of epilepsy (treated or untreated)
- Vascular, traumatic, tumoural, infectious, or metabolic lesion of the brain, even without history of seizure, and without anticonvulsant medication
o Administration of drugs that potentially lower seizure threshold (see Appendix F for list of medications), without concomitant administration of anticonvulsant drugs which potentially protect against seizure occurrence

o Sleep deprivation*, alcoholism

o Implanted brain electrodes (cortical or deep-brain electrodes)

o Severe or recent heart disease

*Sleep deprivation can occur in survivors with a burn injury. Should survivors report pain >5/10 or overnight itch >5/10 on being recruited into the study their recruitment will be postponed to assess whether symptoms resolve. If symptoms continue to impact the survivors’ sleep up to the time of the first TMS session (six weeks) then the survivor will be excluded from the study.
Appendix B - Transcranial Magnetic Stimulation† (TMS) Adult Safety Screen

<table>
<thead>
<tr>
<th>Name:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date:</td>
</tr>
<tr>
<td>Age:</td>
</tr>
</tbody>
</table>

Please answer the following:

Do you have epilepsy or have you ever had a convulsion or a seizure?

☐ Yes  ☐ No

Have you ever had a fainting spell or syncope*? If yes, please describe in which occasions on the following page.

☐ Yes  ☐ No

Have you ever had severe (i.e., followed by loss of consciousness) head trauma?

☐ Yes  ☐ No

Do you have any hearing problems or ringing in your ears?

☐ Yes  ☐ No

Are you pregnant or is there a chance you might be?

☐ Yes  ☐ No

Do you have cochlear implants?

☐ Yes  ☐ No

Do you have an implanted neurostimulator? (e.g., DBS, epidural/subdural, VNS)

☐ Yes  ☐ No

Do you have a cardiac pacemaker or intracardiac lines or metal in your body?

☐ Yes  ☐ No

Do you have a medication infusion device?

☐ Yes  ☐ No

Are you taking any medications? (Please list)

☐ Yes  ☐ No

Have you had a surgical procedure to your spinal cord?

☐ Yes  ☐ No

Did you ever undergo TMS in the past?

☐ Yes  ☐ No

Did you ever undergo MRI in the past?

☐ Yes  ☐ No

Subject signature:

Experimenter name:  Signature:
* Syncope refers to a temporary loss of consciousness, described as “fainting” or “passing out”. It's usually related to temporary insufficient blood flow to the brain.

If you answered yes to any of the above, please provide details.

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† For use with single-pulse TMS, paired-pulse TMS, or repetitive TMS.

adapted from Rossi et al., 2009.
Appendix C - Functional and Quality of Life measures

Short-Form Health Survey version 2 (SF-36)

The SF-36 is a self-completed questionnaire that is an indicator of overall health status. The survey has been shown to have good reliability and validity (Edgar et al., 2010) and covers the following sections:

- Vitality (energy/fatigue; “Vitality”)
- Physical functioning (“Physical”)
- Bodily pain (“Pain”)
- General health perceptions (“General Health”)
- Physical role functioning (role limitations because of physical health problems; “Role Physical”)
- Emotional role functioning (role limitations because of emotional problems; “Role Emotional”)
- Social functioning (“Social”)
- Mental health (psychological distress and psychological wellbeing; “Mental Health”)

Burn Specific Health Survey – Brief (BSHS-B)

The BSHS-B (self-completed questionnaire) is an abbreviated outcome scale designed specifically for burns survivors used to evaluate burn-specific aspects of health status. The BSHS-B has been shown to be a valid burn specific measure of health status and forms a useful test battery when used in conjunction with tests such as the TUG (Finlay et al., 2010; Finlay et al., 2014).
The QuickDASH Outcome Measure

The QuickDASH Outcome Measure is a shortened form of the DASH (Disabilities of the Arm, Shoulder and Hand) Outcome Measure. It is a self-completed questionnaire that uses eleven items to measure physical function and symptoms in people with any or multiple musculoskeletal disorders of the upper limb. The QuickDASH Outcome Measure is already utilised by the State Adult Burn Unit in Western Australia for survivors with upper limb injuries, and prior research by investigators in this study has confirmed, “the validity, repeatability and responsiveness of the QuickDASH outcome measure in patients with upper limb burns. It supports the use of the QuickDASH in this population to help assess change in functional level” (Wu et al., 2007).

Timed Up and Go (TUG)

The TUG is a simple, quick and reliable clinical performance-based measure of lower limb function, mobility and falls risk (Herman et al., 2011; Shumway-Cook et al., 2000). Research by some of the investigators in this study has shown that the TUG is a reliable and valid test in the burns population for measuring recovery from a lower limb burn injury (Finlay et al., 2010). Participants are asked to sit back in a chair with a line identified on the ground three metres away. When the investigator says “Go”, the participant is to stand up from the chair, walk quickly at a safe pace to the line on the floor, turn around and walk back to the chair and sit down again (Shumway-Cook et al., 2000). The time taken from the word “Go” to the participant sitting down again is recorded.
**Lower Limb Functional Index (LLFI)**

The LLFI is a self-assessment questionnaire that assesses functional status in individuals with lower limb conditions. It has been shown to be superior to previously used scales and is used routinely by the State Adult Burn Unit in Western Australia (Gabel et al., 2012; Gittings et al., 2016; Ryland et al., 2016).
Appendix D – Participants lost to follow up

- Participant 2: unable to contact for second TMS session and Functional and Quality of Life measures at twelve weeks post-injury.
- Participant 5: right hand burn including burn injury to dorsum of hand over FDI muscle. Unable to read EMG recordings in second session, possibly due to scar maturation.
- Participant 10: new medication commenced between sessions. The new medication commenced was a contraindication to rTMS, therefore the second session was cancelled.
- Participant 15: not available for second TMS session and Functional and Quality of Life measures at twelve weeks post-injury.
## Appendix E – Burns participant injury information

<table>
<thead>
<tr>
<th>Participant</th>
<th>Burn Location</th>
<th>TBSA</th>
<th>Burn Depth</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Upper Limb</td>
<td>2%</td>
<td>All Superficial/Partial thickness</td>
<td>Non-surgical</td>
</tr>
<tr>
<td></td>
<td>Thorax</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2*</td>
<td>Bilateral Lower Limb</td>
<td>2%</td>
<td>All Superficial/Partial thickness</td>
<td>Surgical</td>
</tr>
<tr>
<td></td>
<td>Thorax</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Bilateral Lower Limb</td>
<td>1.3%</td>
<td>0.6% Superficial Thickness</td>
<td>Surgical</td>
</tr>
<tr>
<td></td>
<td>Head/neck</td>
<td></td>
<td>0.7% Full Thickness</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Bilateral Lower Limb</td>
<td>2.75%</td>
<td>0.25% Superficial</td>
<td>Non-surgical</td>
</tr>
<tr>
<td></td>
<td>Upper Limb</td>
<td></td>
<td>2.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Superficial/Partial Thickness</td>
<td></td>
</tr>
<tr>
<td>5*</td>
<td>Bilateral Upper Limb</td>
<td>7.25%</td>
<td>3.25%</td>
<td>Surgical</td>
</tr>
<tr>
<td></td>
<td>Upper Limb</td>
<td></td>
<td>Superficial/Partial Thickness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Head/neck</td>
<td></td>
<td>4% Deep/Partial Thickness</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Bilateral Lower Limb</td>
<td>2.75%</td>
<td>0.25% Superficial Thickness</td>
<td>Surgical</td>
</tr>
<tr>
<td></td>
<td>Location</td>
<td>2.5%</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>--------------------------------</td>
<td>--------------------</td>
<td>------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Superficial/Partial Thickness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Upper Limb Head/neck</td>
<td>0.35% All Superficial Thickness</td>
<td></td>
<td>Non-surgical</td>
</tr>
<tr>
<td>8</td>
<td>Bilateral Upper Limb</td>
<td>0.2% All Superficial/Partial Thickness</td>
<td></td>
<td>Non-surgical</td>
</tr>
<tr>
<td>9</td>
<td>Thorax</td>
<td>3% 1% Superficial Thickness</td>
<td>2% Superficial/Partial Thickness</td>
<td>Non-surgical</td>
</tr>
<tr>
<td>10*</td>
<td>Lower Limb</td>
<td>2.2% 0.2% Superficial/Partial Thickness</td>
<td>2% Deep/Partial Thickness</td>
<td>Non-surgical</td>
</tr>
<tr>
<td>11</td>
<td>Lower Limb</td>
<td>7% All Deep/Partial Thickness</td>
<td></td>
<td>Surgical</td>
</tr>
<tr>
<td>12</td>
<td>Lower Limb</td>
<td>1.01% 1% Superficial Thickness</td>
<td>0.01%</td>
<td>Non-surgical</td>
</tr>
<tr>
<td>Participant</td>
<td>Body Part</td>
<td>Superficial/Partial Thickness</td>
<td>Deep/Partial Thickness</td>
<td>Treatment</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------</td>
<td>-------------------------------</td>
<td>------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>13</td>
<td>Upper Limb</td>
<td>0.12%</td>
<td>0.11%</td>
<td>Non-surgical</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.01% Deep/Partial Thickness</td>
<td>Non-surgical</td>
</tr>
<tr>
<td>14</td>
<td>Upper Limb</td>
<td>0.6%</td>
<td>All Superficial/Partial Thickness</td>
<td>Non-surgical</td>
</tr>
<tr>
<td>15*</td>
<td>Lower limb</td>
<td>1%</td>
<td>All Deep/Partial Thickness</td>
<td>Surgical</td>
</tr>
<tr>
<td>16</td>
<td>Upper Limb</td>
<td>0.5%</td>
<td>All Superficial/Partial Thickness</td>
<td>Non-surgical</td>
</tr>
</tbody>
</table>

*next to participant number indicates that participant was lost to follow up. TBSA = Total body surface area.

Note: All surgery was performed prior to Session 1, no surgical intervention occurred between Session 1 and Session 2.
Appendix F – Drugs that form strong or simply relative hazards for the use of rTMS

Intake of one or a combination of the following drugs forms a strong potential hazard for application of rTMS due to their significant seizure threshold lowering potential:

- Imipramine
- Amitriptyline
- Doxepine
- Nortriptyline
- Maprotiline
- Chlorpromazine
- Clozapine
- Foscarnet
- Ganciclovir
- Ritonavir
- Amphetamines
- Cocaine
- (MDMA, ecstasy)
- Phencyclidine (PCP, angel’s dust)
- Ketamine
- gamma-hydroxybutyrate (GHB)
- Alcohol
- Theophylline

Intake of one or a combination of the following drugs forms a relative hazard for application of rTMS due to their significant seizure threshold lowering potential:

- Mianserin
- Fluoxetine
- Fluvoxamine
- Paroxetine
- Sertraline
- Citalopram
- Reboxetine
- Venlafaxine
- Duloxetine
- Bupropion
- Mirtazapine
- Fluphenazine
- Pimozide
- Haloperidol
- Olanzapine
- Quetiapine
• Aripiprazole  
• Ziprasidone  
• Risperidone  
• Chloroquine  
• Mefloquine  
• Imipenem  
• Penicillin  
• Ampicillin  
• Cephalosporins  
• Metronidazole  
• Isoniazid  
• Levofloxacin  
• Cyclosporine  
• Chlorambucil  
• Vincristine  
• Methotrexate  
• Cytosine arabinoside  
• BCNU  
• Lithium  
• Anticholinergics  
• Antihistamines  
• Sympathomimetics

Withdrawal from one of the following drugs forms a strong relative hazard for application of rTMS due to the resulting significant seizure threshold lowering potential:

• Alcohol  
• Barbiturates  
• Benzodiazepines  
• Meprobamate  
• Chloral hydrate.
### Appendix G – SF-36 outcome scores for Burns Group and Control Group

<table>
<thead>
<tr>
<th>SF-36 DOMAIN</th>
<th>BURNS GROUP</th>
<th>CONTROL GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VITALITY</strong></td>
<td>$M = 73.44$</td>
<td>$M = 71.15$</td>
</tr>
<tr>
<td></td>
<td>$SD = 16.45$</td>
<td>$SD = 14.33$</td>
</tr>
<tr>
<td><strong>PHYSICAL</strong></td>
<td>$M = 91.25$</td>
<td>$M = 95.00$</td>
</tr>
<tr>
<td></td>
<td>$SD = 16.53$</td>
<td>$SD = 5.40$</td>
</tr>
<tr>
<td><strong>PAIN</strong></td>
<td>$M = 90.08$</td>
<td>$M = 86.46$</td>
</tr>
<tr>
<td></td>
<td>$SD = 16.99$</td>
<td>$SD = 14.79$</td>
</tr>
<tr>
<td><strong>GENERAL HEALTH</strong></td>
<td>$M = 86.75$</td>
<td>$M = 79.23$</td>
</tr>
<tr>
<td></td>
<td>$SD = 12.93$</td>
<td>$SD = 12.22$</td>
</tr>
<tr>
<td><strong>ROLE PHYSICAL</strong></td>
<td>$M = 93.75$</td>
<td>$M = 92.79$</td>
</tr>
<tr>
<td></td>
<td>$SD = 8.00$</td>
<td>$SD = 9.15$</td>
</tr>
<tr>
<td><strong>ROLE EMOTIONAL</strong></td>
<td>$M = 90.97$</td>
<td>$M = 96.15$</td>
</tr>
<tr>
<td></td>
<td>$SD = 13.97$</td>
<td>$SD = 8.06$</td>
</tr>
<tr>
<td><strong>SOCIAL</strong></td>
<td>$M = 95.83$</td>
<td>$M = 85.58$</td>
</tr>
<tr>
<td></td>
<td>$SD = 8.14$</td>
<td>$SD = 20.95$</td>
</tr>
<tr>
<td><strong>MENTAL HEALTH</strong></td>
<td>$M = 85.83$</td>
<td>$M = 86.15$</td>
</tr>
<tr>
<td></td>
<td>$SD = 10.62$</td>
<td>$SD = 9.61$</td>
</tr>
</tbody>
</table>

$M =$ mean; $SD =$ standard deviation