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An exploration of homeostatic plasticity in musculoskeletal pain

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I dedicate this thesis to my mother, and sister.

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

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Tribikram Thapa (Rana)

Publications, abstracts, and presentations from this thesis

Published journal articles

Thapa, Tribikram., & Schabrun, M, S. (2018). Test-retest reliability of homeostatic plasticity induced and assessed using non-invasive brain stimulation in the human primary motor cortex. *Neural Plasticity.* 2018 (9). doi: 10.1155/2018/6207508.

Thapa, T., Graven-Nielsen, T., Chipchase, L. S., & Schabrun, S.M. (2018). Disruption of cortical synaptic homeostasis in individuals with chronic low back pain. *Clinical Neurophysiology*. 129(5), 1090-1096. doi: 10.1016/j.clinph.2018.01.060.

Conference abstracts

Thapa, T., Chipchase, L., Graven-Nielsen, T., & Schabrun, S. (2016) Homeostatic plasticity in the primary motor cortex of patients with non-specific chronic low back pain. *Front. Hum. Neurosci. Conference Abstract: ASP2016 - The 26th Annual Meeting of the Australasian Society for Psychophysiology, Adelaide, Australia*. doi: 10.3389/conf.fnhum.2016.221.00028, 12th - 14th of December 2016.

Conference presentations

Thapa, Tribikram., Ridding, Michael., Graven-Nielsen, Thomas., & Schabrun, Siobhan. (2018). Aberrant plasticity in musculoskeletal pain: a failure of homeostatic control? *The 28th Annual Meeting of the Australasian Society for Psychophysiology, Geelong, Victoria Australia., 19th – 21st of November 2018.*

Thapa, Tribikram., Ridding, Michael., Graven-Nielsen, Thomas., & Schabrun, Siobhan. (2018). The influence of sustained nociception on homeostatic plasticity in humans. *IASP 2018. The 17th World Congress of Pain, Boston, United States of America, 12th – 16th of September 2018*.

Thapa, Tribikram., Graven-Nielsen, Thomas., Chipchase, Lucinda., & Schabrun, Siobhan. (2017). Homeostatic plasticity is impaired in the primary somatosensory cortex of people with chronic low back pain. *SPRING 2017. Sydney Pain Researchers: The Next Generation, Sydney, Australia, 20th November 2017*.

Thapa, T., Chipchase, L., Graven-Nielsen, T., & Schabrun, S. (2016). Disruption of homeostatic plasticity in the primary motor cortex of individuals with chronic low back pain. *The Australian Pain Society Conference – 37th Annual Scientific Meeting, Adelaide Convention Centre, Adelaide, Australia. 9th – 12th of April 2017*.

Thapa, T., Chipchase, L., Graven-Nielsen, T., & Schabrun, S. (2016) Homeostatic plasticity in the primary motor cortex of patients with non-specific chronic low back pain. *Front. Hum. Neurosci. Conference Abstract: ASP2016 - The 26th Annual Meeting of the Australasian Society for Psychophysiology, Adelaide, Australia. doi: 10.3389/conf.fnhum.2016.221.00028, 12th – 14th of December 2016*.

Grants and awards from this thesis

2018 Nominated for the Young Investigator Awards Clinical Neurophysiology (€5,000).

2018 IASP Financial Aid Working Group Award to attend the 17th World Congress on Pain ($1300 USD).

2017 Winner of the Best Laboratory Training video (BRAiN-u, Western Sydney University) for demonstration on use of pressure algometer, transcranial direct current stimulation, and transcutaneous electrical nerve stimulation.

2017 Best Free Paper Award at the 37th Annual Scientific Meeting, Australian Pain Society ($2000 AUD).

2017 PhD Student Travel Grant to the 37th Annual Scientific Meeting, Australian Pain Society ($500 AUD).

2016 Commendable mention for oral presentation at the Australasian Society of Psychophysiology conference.

2016 PhD Student Travel Grant to the Australasian Society of Psychophysiology conference ($250 AUD).

Other related publications, and abstracts

Published journal articles

Schabrun, M. S., Burns, E., **Thapa, T.**, & Hodges, W.P. The response of the primary motor cortex to neuromodulation is altered in chronic low back pain: a preliminary study (2017). *Pain Medicine*. doi: 10.1093/pm/pnx168.

Schabrun, M. S., Palsson, S. T., **Thapa, T.**, & Graven-Nielsen, T. Movement does not promote recovery of motor output following acute experimental pain (2017). *Pain Medicine*. doi: 10.1093/pm/pnx099.

David A Seminowicz., **Tribikram Thapa.**, Andrew J Furman., Simon J Summers., Rocco Cavaleri., Jack S Fogarty., Genevieve Z Steiner, & Siobhan M Schabrun (2017). Slow peak alpha frequency and corticomotor depression linked to high pain susceptibility in the transition to sustained pain. bioRxiv. doi: 10.1101/278598

Roccol Cavaleri., **Tribikram Thapa.**, Paula R Beckenkamp, & Lucy S Chipchase (2018). The influence of kinesiology tape colour on performance and corticomotor activity in healthy adults: a randomised crossover controlled trial. *BMC Sports Science, Medicine and Rehabilitation*. doi: 10.1186/s13102-015-0106-4

Conference presentations

Renee Timmers, Jennifer MacRitchie, Siobhan Schabrun, **Tribikram Thapa**, Manuel Varlet., & Peter Keller. (2016). The influence of audio-visual information and motor simulation on synchronization with a prerecorded co-performer. *European Society for the Cognitive Sciences of Music (ESCOM 2017), University of Ghent, Belgium, 31/7 – 4/8, 2017.*

Author's note

This thesis has been prepared in the format of thesis as a series of papers. Study one (chapter two), and study two (chapter three) have been published in Neural Plasticity, and Clinical Neurophysiology, while study three (chapter four) is currently under review in the Journal of Pain. The content of each publication and manuscript under review has been preserved. However, minor editorial changes have been made to maintain a standard formatting throughout this document. References are presented alphabetically as a continuous list towards the end of the document to avoid repetition and to improve readability. A copy of the publications in its original format is provided in appendix A (study one; chapter two), appendix B (study two; chapter three), and appendix C (editorial to study two).

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Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
aMT	Active Motor Threshold
APB	Abductor Pollicis Brevis
APV	(2R)-amino-5-phosphonopentanoate
CI	Confidence Interval
cLBP	Chronic Low Back Pain
CNS	Central Nervous System
CRPS	Complex Regional Pain Syndrome
cTBS	Continuous Theta Burst Stimulation
DPC	Dorsal Premotor Cortex
ECRB	Extensor Carpi Radialis Brevis
FDI	First Dorsal Interosseous
GABA	Gamma-aminobutyric Acid
HFS	High Frequency Stimulation
Hz	Hertz
iTBS	Intermittent Theta Burst Stimulation
LBP	Low Back Pain
LTD	Long Term Depression
LTP	Long Term Potentiation

M1	Primary Motor Cortex
mA	Milliampere
m. ECRB	Extensor Carpii Radialis Brevis Muscle
MEP	Motor Evoked Potential
mEPSPs	Miniature Excitatory Postsynaptic Potentials
Mg^{2+}	Magnesium
MN-SSEP	Median Nerve Somatosensory Evoked Potential
msec	Milliseconds
mV	Millivolts
NaCl	Sodium Chloride
NFR	Nociception Flexion Reflex
NGF	Nerve Growth Factor
NIBS	Non-invasive Brain Stimulation
NMDA	*N*-methyl-$_D$-aspartate
NMJ	Neuromuscular Junction
NRS	Numerical Rating Scale
OA	Osteoarthritis
PAS	Paired Associative Stimulation
pHFS	Peripheral High Frequency Stimulation
PPT	Pressure Pain Threshold
PRTEEQ	Patient Rated Tennis Elbow Questionnaire

QPS	Quadri-pulse Transcranial Magnetic Stimulation
rMT	Resting Motor Threshold
rTMS	Repetitive Transcranial Magnetic Stimulation
S1	Primary Somatosensory Cortex
S_{1mV}	Peak-to-peak MEP amplitude of 1 mV
SD	Standard Deviation
SEM	Standard Error of Mean
SEP	Somatosensory Evoked Potential
SMA	Supplementary Motor Area
TBS	Theta Burst Stimulation
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation
TNFα	Tumor Necrosis Factor α
TTX	Tetrodotoxin
V1	Primary Visual Cortex
VEP	Visual Evoked Potential

Abstract

The brain has a remarkable capacity to reorganise itself through life. When changes occur at a cellular level between neurons, this is known as synaptic plasticity. Synaptic plasticity has been proposed to be a key mechanism underpinning the learning and memory formation that occurs following afferent input (i.e., incoming stimuli from movement and sensation). However, synaptic plasticity in the human brain follows a positive loop cycle where incoming stimuli can lead to excessive synaptic strengthening (long-term potentiation; LTP) or weakening (long-term depression; LTD). To prevent overexpression of LTP or LTD, regulatory mechanisms termed '*homeostatic plasticity*' promote stability during synaptic plasticity.

A large body of evidence suggests that short- or long-term changes to synaptic plasticity takes place following afferent input. Similarly, evidence also suggests synaptic plasticity is altered in individuals experiencing incoming stimuli that are painful. However, no study has examined homeostatic plasticity during pain. Published studies that have examined homeostatic plasticity in individuals with pathology have been conducted in neurological conditions such as writer's cramp, and chronic migraine. These studies provide preliminary evidence that impaired homeostatic plasticity is associated with altered synaptic plasticity with patients displaying abnormally high primary motor cortex (M1) excitability, altered cortical organisation, increased pain perception, and sensorimotor dysfunction. As altered synaptic plasticity and similar clinical features have been observed in individuals with

chronic musculoskeletal pain, it is possible that homeostatic plasticity is impaired during pain. Thus, the broad goal of this thesis was to explore the effect of pain, using a clinical chronic musculoskeletal pain population and an experimental pain model, on homeostatic plasticity in the M1.

To address this broad goal, three primary research studies were conducted. In study one, test-retest reliability of M1 homeostatic regulation, induced and assessed using non-invasive brain stimulation, was examined in ten, right-handed, healthy volunteers at intervals of 48 hours, 7 days, and 2 weeks. To induce M1 homeostatic plasticity, a double anodal transcranial direct current stimulation (tDCS) protocol was applied. This involved two blocks of excitatory anodal tDCS applied in succession for 7-minutes and 5-minutes with a 3-minute rest period in between (double tDCS protocol). Following the double tDCS protocol, moderate-to-good test-retest reliability (ICC= 0.43 to 0.67) was observed for M1 homeostatic plasticity when induced and assessed at intervals of 48 hours, 7 days, and 2 weeks. Thus, study one provides the first data that, by using the double tDCS protocol, M1 homeostatic plasticity can be induced and assessed reliably over time in healthy individuals. Further, these data provide the foundation for assessment of M1 homeostatic plasticity using repeated measures, and longitudinal study designs in humans.

Using the double tDCS protocol described above, study two investigated homeostatic plasticity in the M1 of 50 individuals with non-specific chronic low back pain (cLBP), and

25 healthy, pain-free, controls. In individuals with non-specific cLBP, the first block of 7-minutes anodal tDCS increased M1 excitability consistent with the response observed in healthy, pain-free, controls ($P<0.003$). However, in contrast to healthy, pain-free controls, the application of a second block of 5-minutes anodal tDCS failed to reduce M1 excitability in individuals with non-specific cLBP ($P\geq0.1$). Results from study two provide evidence of impaired M1 homeostatic plasticity in individuals with non-specific cLBP. These data may explain previous observations of altered synaptic plasticity and pain persistence in individuals with non-specific cLBP.

In study three, the double tDCS protocol described in study one was used to investigate homeostatic plasticity as experimentally induced muscle pain developed, peaked, and resolved. Twenty-one, right-handed, healthy individuals were enrolled in the study for twenty-one days. Muscle pain was induced using nerve-growth factor (NGF) injected into the right extensor carpi radialis brevis muscle on three occasions: day 0, 2, and 4. Homeostatic plasticity was assessed in the M1 on days 0, 2, 4, 6, and 14, with an online diary that included measures of pain intensity, muscle soreness, disability, and sleep quality administered on alternative days from day 1 to 21. A normal M1 homeostatic response was observed on day 0 ($P<0.001$), and day 14 ($P<0.001$). However, the progressive development of experimentally induced muscle pain altered M1 homeostatic plasticity in otherwise healthy individuals on day 2 ($P=0.07$), day 4 ($P=0.7$), and day 6 ($P=0.5$). These

data provide the first information on M1 homeostatic plasticity as pain develops, peaks, and resolves.

These three studies provide original and novel data on M1 homeostatic plasticity during pain. First, M1 homeostatic plasticity was established as reliable across two weeks in healthy individuals. Second, study two, and study three suggest homeostatic plasticity is impaired during pain as participants failed to regulate excessive increases in M1 excitability during painful stimuli. The findings from study two is based on a cross sectional study design in a clinical chronic musculoskeletal pain population, while study three used an experimental pain model. When viewed together, the work presented in this thesis deepens our understanding of M1 homeostatic plasticity in response to pain and suggests that homeostatic plasticity may be responsible for altered synaptic plasticity that has been observed in a number of chronic musculoskeletal pain conditions. However, longitudinal studies in clinical pain populations are needed to extend the work presented in this thesis to fully understand the role of homeostatic plasticity during pain. If homeostatic plasticity is demonstrated to underpin the development of pain, therapies that seek to target altered synaptic plasticity may need to target homeostatic, rather than synaptic plasticity mechanisms to improve treatment effectiveness, and patient responsiveness to therapy.

Thesis overview

Neuroplasticity, broadly understood as 'the way the brain changes itself', underpins learning, and memory formation. Change in neuronal connections take place in response to new situations, changes in the environment, and alterations to afferent input (i.e., incoming stimuli from movement or sensation) (Bryan et al., 2003; Joseph, 2013; Kolb & Gibb, 2011). Originally, the adult human brain was thought to be immutable (Cajal, 1928; Hubel & Wiesel, 1962, 1970). However, the discovery that the brain can change and adapt throughout life has led to a surge of investigations on neuroplasticity in a variety of clinical conditions, and as a potential target for the treatment and prevention of pathology (Boudreau et al., 2010; Flor, 2002; Flor, 2003; Merzenich et al., 1984; Stahnisch & Nitsch, 2002).

A large body of research has focussed on mechanisms that underpin change in the human brain (Citri & Malenka, 2008; Joseph, 2013). This body of work has identified a number of functional and structural mechanisms, including the expression of long-term potentiation (LTP)- and long-term depression (LTD)-like changes in synaptic plasticity, thought to underpin brain changeability. However, synaptic plasticity follows a positive feedback loop, where it is possible to have too much LTP or LTD formation (Joseph, 2013; Turrigiano, 1999). As a result, in addition to mechanisms that promote change, the human brain is governed by mechanisms that promote stability (Turrigiano & Nelson, 2000, 2004). These mechanisms, termed *homeostatic plasticity*, prevent overexpression of LTP

and LTD-like plasticity, and ensure that a balance between change and stability is maintained within neural networks at all times (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). Thus, homeostatic control is essential to ensure healthy brain activity. However, to date, investigation of this mechanism in healthy populations and those with clinical conditions is limited.

Previously published studies exploring homeostatic plasticity in clinical populations have been conducted only in individuals with writer's cramp, and chronic migraine (Karabanov et al., 2015). Evidence from these studies suggest an association between impaired homeostatic control and clinical symptoms of pain and sensorimotor dysfunction (Antal et al., 2008b; Cosentino et al., 2014b; Kang et al., 2011; Quartarone & Pisani, 2011). As similar clinical symptoms including pain, and sensorimotor dysfunction are observed in individuals with chronic musculoskeletal pain (Apkarian et al., 2009; Apkarian et al., 2011), it is possible that homeostatic plasticity may also be impaired in these individuals. Therefore, the broad goal of this thesis was to explore the effect of pain on homeostatic plasticity in the human primary motor cortex (M1).

To answer the overarching goal, three primary research studies were conducted. Study one aimed to determine whether methods used to induce and assess homeostatic plasticity in the M1 were reliable over time in human subjects. Study two examined homeostatic plasticity in the M1 of individuals with non-specific chronic low back pain. Study three

examined the effect of progressively developing muscle pain (induced using repeated injection of nerve growth factor [NGF]) on M1 homeostatic plasticity in otherwise healthy individuals.

The specific aims and hypotheses for each study were as follows:

Study one

Aim: To determine test-retest reliability of M1 homeostatic plasticity in healthy individuals when induced and assessed using two successive blocks of excitatory non-invasive brain stimulation at intervals of 48 hours, 7 days, and 2 weeks.

Hypothesis: Homeostatic plasticity will be reliably induced and assessed in the M1 of healthy individuals at intervals of 48 hours, 7 days, and 2 weeks.

Study two

Aim: To investigate homeostatic plasticity in the M1 of individuals with non-specific chronic low back pain, and healthy, pain-free, controls using two successive blocks of anodal transcranial direct current stimulation applied for 7-minute and 5-minutes respectively.

Hypothesis: Individuals with non-specific chronic low back pain will fail to display a reversal of excitation towards inhibition following two successive blocks of anodal transcranial direct current stimulation consistent with impairment in homeostatic control. Normal homeostatic plasticity will be observed in healthy, pain-free, controls following two successive blocks of anodal transcranial direct current stimulation.

Study three

Aim: To investigate the influence of progressively developing muscle pain on homeostatic plasticity in the human M1 elicited using two successive blocks of anodal transcranial direct current stimulation.

Hypothesis: Normal homeostatic response observed following two blocks of anodal transcranial direct current stimulation will be reduced after four days of sustained muscle pain.

Peer reviewed journal publications arising from the studies conducted in this thesis are as follows:

Study 1: Thapa, Tribikram., & Schabrun, M, S. (2018). Test-retest reliability of homeostatic plasticity induced and assesed using non-invasive brain stimulation in the human primary motor cortex. *Neural Plasticity*, 2018 (9). [Impact factor: 3.161]

Study 2: Thapa, T., Graven-Nielsen, T., Chipchase, L. S., & Schabrun, S. M. (2018). Disruption of cortical synaptic homeostasis in individuals with chronic low back pain. *Clinical Neurophysiology*, 129(5), 1090-1096. [Impact factor: 3.614]

Study 3: Thapa, T., Ridding, C. M., Graven-Nielsen, T., & Schabrun, S. M. (2018). Aberrant plasticity in musculoskeletal pain: a failure of homeostatic control? *The Journal of Pain (Under review).* [Impact factor: 4.859]

The three studies are presented in numerical order in chapters two to four. Each chapter is presented as it has been published or submitted for review. A critical review of the literature specific to chapters two to four is provided in the introduction and discussion sections of each chapter. Prior to this, chapter one provides an in-depth critical review of pertinent literature on synaptic plasticity, and the mechanisms that support change and stability within neural networks. Following this, available literature on homeostatic plasticity, and the methods for assessing homeostatic regulation in humans are discussed. This is followed by a review of literature that examined homeostatic regulation in clinical populations. Chapter five includes a narrative synthesis of the findings of all three studies, their contribution to the field of homeostatic plasticity during pain and future directions, their clinical implications, and limitations.

Chapter 1: Introduction and literature review

This chapter provides an overview of synaptic plasticity mechanisms thought to underpin brain changes and adaptability to afferent input. First, homeostatic plasticity is introduced as a mechanism that regulates synaptic plasticity. Then current methodologies used to induce and assess homeostatic regulation are discussed. This is followed by a review of studies investigating the role of homeostatic plasticity in pathology. The chapter ends with a rationale for each study included in this thesis.

1.1 Neuroplasticity

Neuroplasticity has been defined as the ability of the brain to change and adapt to afferent input (i.e., incoming stimuli arising from movement, sensation, and nociception) (Bryan et al., 2003; Joseph, 2013; Kaas, 2001; Kolb & Gibb, 2011). Initially thought to take place only during the postnatal developmental period, and believed to be hard-wired and immutable in the adult brain (Cajal, 1928; Hubel & Wiesel, 1962, 1970), neuroplasticity is now understood to be a process of neural change and adaptability throughout life (Merzenich et al., 1984; Ottersen & Helm, 2002; Stahnisch & Nitsch, 2002). Fundamental to neuroplasticity are the functional units of the nervous system known as '*neurons*' (Joseph, 2013; Mayford et al., 2012).

Neurons are nerve cells that relay sensory or motor information through electrical signals known as action potentials (Cuevas, 2007; Mosier, 2010; Stuart et al., 1997). An action potential is generated through voltage gated channels that are activated following a triggering event (e.g., afferent input arising from touch or heat) (Feher, 2017; McCormick, 2014). Here, an influx of sodium ions through voltage gated sodium channels produce an action potential characterised by increased positive charge within a neuron. As communication between neurons is central to neuroplasticity, an action potential generated in one neuron is conveyed to another through junctions known as a '*synapse*'.

Synapses are gaps between neurons that allow information to move from one nerve cell to another (Mayford et al., 2012; Südhof & Malenka, 2008). Information relayed between neurons through synapses are central to neuroplasticity, and the brain's ability to adapt and change in response to afferent input (Cohen & Greenberg, 2008; Green & Bavelier, 2008). When the brain adapts, new synapses are formed, or old synapses strengthened, weakened or eliminated (Bi & Poo, 1998; Bliss & Collingridge, 1993; Bliss & Lomo, 1973; Carew & Sahley, 1986). This process determines the magnitude of neuroplastic change and is known as synaptic plasticity at a cellular level (Mayford et al., 2012; Pascual-Leone et al., 2005).

1.2 Synaptic plasticity

Synaptic plasticity is the modification of information transmission between neurons (Feldman, 2009; Joseph, 2013; Pascual-Leone et al., 2005), and is central to incorporating transient learning experiences to permanent memory traces in the human brain (Fernandes & Carvalho, 2016; Hebb, 1949; Pascual-Leone et al., 2005). Mechanisms underlying synaptic plasticity for learning and memory formation have been divided into two categories: '*short-term synaptic plasticity*', and '*long-term synaptic plasticity*' (Carasatorre & Ramirez-Amaya, 2013; Fröhlich, 2016; Pascual-Leone et al., 2005).

Short-term synaptic plasticity is a rapid process that involves immediate change to synaptic strength on a sub-second timescale (Zucker & Regehr, 2002). This process temporarily upregulates or downregulates synaptic plasticity which leads to short-term learning and memory formation (Citri & Malenka, 2008). In contrast, long-term synaptic plasticity lasts from hours to days or even years following afferent input (Daoudal & Debanne, 2003). Long-term synaptic plasticity may lead to permanent changes in brain function, and primarily involves structural changes to neurons that contribute to information storage and memory formation (Alberini, 2009; Grutzendler et al., 2002). The following sections discuss short- and long-term synaptic plasticity during learning and memory formation.

1.2.1 Short-term synaptic plasticity

Manipulation of incoming stimuli has been shown to drive mechanisms of short-term synaptic plasticity (Jacobs & Donoghue, 1991; Zucker & Regehr, 2002). These mechanisms include unmasking and activation of silent synapses (Atwood & Wojtowicz, 1999; Ziemann et al., 2001), and activity-dependent synaptic plasticity (Bliss & Lomo, 1973; Bliss & Cooke, 2011).

Silent synapses are excitatory glutamatergic synapses identified as those with post-synaptic *N*-methyl-$_{D}$-aspartate (NMDA) receptors, but no α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Atwood & Wojtowicz, 1999; Isaac et al.,

1995; Liao et al., 1995). Due to the absence of AMPA receptors, silent synapses fail to mediate synaptic transmission between pre- and post-synaptic neurons. However, a common mechanism proposed to activate silent synapses is the insertion of AMPA receptors in the post-synaptic neuron (Gomperts et al., 1998; Liao et al., 1999; Lu et al., 2001). This insertion is argued to release the magnesium channel block on NMDA receptors to allow calcium influx (Contractor & Heinemann, 2002; Lu et al., 2001; Sweatt, 2010). An influx of calcium ions switches a silent synapse to an active synapse, and allows action potentials (or information) to flow from one neuron to another (Kerchner & Nicoll, 2008; Lüscher & Frerking, 2001).

Alternatively, a widely accepted mechanism of short-term synaptic plasticity is activity driven changes to synaptic strength between neurons (Bliss & Lomo, 1973; Bliss & Cooke, 2011; Lynch et al., 1977; Mulkey & Malenka, 1992). Initially demonstrated in the first region of *Cornu Ammonis* (CA1) in animal hippocampal slices, high- and-low frequency repetitive stimulation has been shown to increase and decrease the excitability of post-synaptic neurons such that synaptic strength is either enhanced (termed '*long-term potentiation; LTP*') or reduced (termed '*long-term depression; LTD*') (Bliss & Lomo, 1973; Lomo, 1966; Lynch et al., 1977; Madison et al., 1991; Massey & Bashir, 2007; Mulkey & Malenka, 1992; Nicoll, 2017).

Following early observations, a large body of research have demonstrated activity driven changes to short-term synaptic plasticity (i.e., LTP and LTD induction) in a number of brain regions (Malenka & Bear, 2004; Martin et al., 2000; Rioult-Pedotti et al., 2000; Sacchetti et al., 2001; Sacktor, 2008). These studies indicate the activation of NMDA type glutamate receptors is central to activity-dependent short-term synaptic plasticity, and is known to involve several requirements for LTP – LTD induction (Bütefisch et al., 2000; Hell, 2014; Lisman et al., 2012; Madison et al., 1991). For instance, several pre-synaptic neurons need to 'fire' together to activate post-synaptic neurons to remove the magnesium channel block and allow calcium influx (Bear & Malenka, 1994; Catterall et al., 2013; Hebb, 1949). A fast and large influx of calcium ions enhances synaptic strength, and triggers LTP formation, while a slow and less pronounced influx of calcium ions decreases synaptic strength, and triggers LTD formation (Catterall et al., 2013; Hell, 2014; Lisman et al., 2012). Additionally, concurrent activation of pre- and post-synaptic neurons is necessary for LTP and LTD induction (Bi & Poo, 2001; Debanne et al., 1998). This requirement is in line with Hebb's postulate or Hebbian plasticity where "when an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased" (Hebb, 1949. p. 62).

The unmasking and activation of silent synapses, and activity-dependent LTP and LTD formation suggests modulation of synaptic strength between neurons is central to short-term synaptic plasticity (Feldman, 2009; Zucker & Regehr, 2002). However, evidence also exists for long lasting changes in synaptic efficacy (termed 'long-term synaptic plasticity') between pre- and post-synaptic neurons (Daoudal & Debanne, 2003). For example, Engert & Bonhoeffer (1999b) demonstrated long-lasting increases in excitability in the CA1 neurons of the hippocampus following prolonged electrical stimulation (Engert & Bonhoeffer, 1999b). These long-lasting changes to post-synaptic neuronal excitability make long-term synaptic plasticity a candidate mechanism for consolidation of learning and memory.

1.2.2 Long-term synaptic plasticity

The mechanisms involved in long-term synaptic plasticity lead to structural changes to neurons that may permanently alter brain function (Ottersen & Helm, 2002; Trachtenberg et al., 2002). These mechanisms include neurogenesis, synaptogenesis, and synaptic remodeling (Maren & Baudry, 1995; Yau et al., 2015; Zhao et al., 2013).

Neurogenesis leads to the formation of new neurons, and has been observed in the dentate gyrus of the rat hippocampus following afferent input arising from task performance or learning (Dupret et al., 2008; Shors et al., 2002; Zhao et al., 2008), while synaptogenesis,

that leads to formation of new synapses, has been demonstrated in the rat hippocampus following stimulation of the stratum radiatum of the same hippocampal slice (Watson et al., 2016). Increase in dendritic spine density and change in its morphology have also been observed in rats following spatial learning (Moser et al., 1994; Yuste & Bonhoeffer, 2001). However, whether the induction or maintenance of LTP and LTD is necessary for these morphological changes and consequently, long-term synaptic plasticity, remains unclear (Bosch & Hayashi, 2012; Yuste & Bonhoeffer, 2001). While some studies have demonstrated these morphological changes following LTP and LTD formation (Buchs & Muller, 1996; Engert & Bonhoeffer, 1999a; Trommald et al., 1996), others have not (Bosch & Hayashi, 2012; Sorra & Harris, 1998; Yuste & Bonhoeffer, 2001). Therefore, the role of LTP and LTD during morphological changes that underpin long-term synaptic plasticity is yet to be shown.

1.2.3 Regulation of synaptic plasticity

LTP and LTD formation is central to synaptic plasticity (Carasatorre & Ramirez-Amaya, 2013; Citri & Malenka, 2008; Martin et al., 2000; Mayford et al., 2012). In line with Hebb's seminal idea that neurons that fire together wire together, LTP and LTD formation occurs based on a positive-feedback mechanism (Brown et al., 2009; van Hemmen, 2001). This means that the induction of LTP drives further strengthening of a neural circuit (LTP expression), while the induction of LTD drives further weakening of a neural circuit (LTD

expression) (Chen & Maghsoodi, 2007). Left unchecked, this positive feedback loop can lead to excessive strengthening or excessive weakening of a neural circuit (Abbott & Nelson, 2000; Abraham & Bear, 1996; Turrigiano & Nelson, 2000).

To prevent excessive strengthening (LTP) or weakening (LTD) within a neural circuit, the CLO model, (an acronym derived from the authors' initials), was proposed by Cooper, Liberman, and Oja (Cooper et al., 1979). Here, anti-Hebbian rules were proposed in addition to Hebbian rules, such that synaptic strength was modified bidirectionally in a manner that promoted synaptic strengthening (LTP formation) or weakening (LTD formation). The authors indicated that the direction of synaptic modification was dependent upon where a neuron's post-synaptic response stood relative to a given threshold level (termed 'modification threshold,' θ_m). However, when the θ_m was set to a single value, the CLO model proved to be rigid and unadaptable to continuous LTP or LTD formation relative to incoming stimuli (Bienenstock et al., 1982; Cooper et al., 1979).

A few years later, Bienenstock, Cooper, and Munro modified the CLO model [now known as the Bienenstock-Cooper-Munro (BCM) model] by proposing a sliding modification threshold (θ_m) for bidirectional synaptic plasticity (Bienenstock et al., 1982). The BCM theory proposed that the θ_m dynamically adapts to the activation history of a neural circuit such that a history of low post-synaptic activity will lower the θ_m for future LTP induction

and increase the threshold for LTD. Conversely, a history of high post-synaptic activity will lower the θ_m for future LTD induction and increase the threshold for LTP (Abraham et al., 2001; Bienenstock et al., 1982; Turrigiano, 2012; Turrigiano, 1999, 2008; Turrigiano & Nelson, 2000). In other words, excessive strengthening of a neural circuit will bias neurons towards weakening (LTD-induction), while excessive weakening of a neural circuit will bias neurons towards strengthening (LTP-induction) (Carvalho et al., 2015; Karabanov & Siebner, 2012; Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015; Ni et al., 2014). In this way, homeostatic plasticity is argued to ensure stability is retained in neural circuits. A pictorial representation of homeostatic regulation consistent with the BCM theory is provided in Figure 1.1.

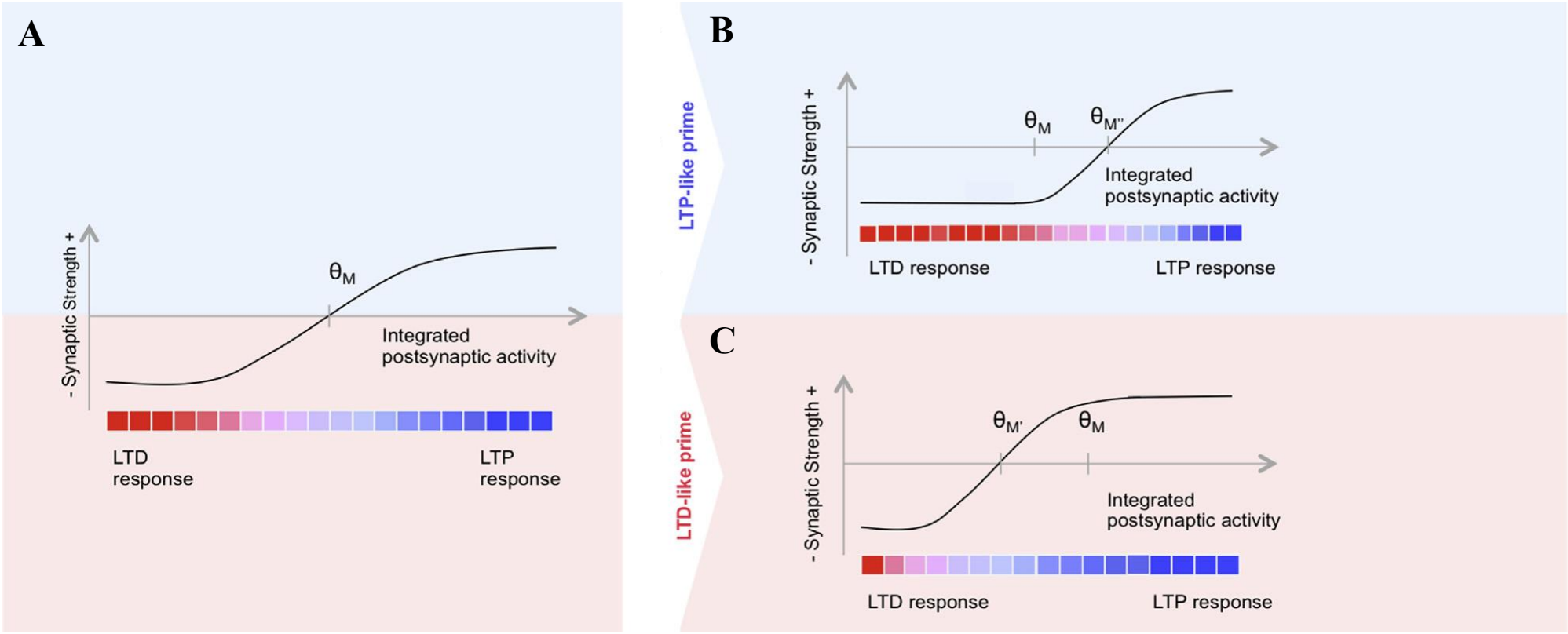

Figure 1.1: *Pictorial representation of homeostatic regulation. Picture A demonstrates the bidirectional shift of LTP – LTD induction at the crossover point (θ_m) without prior stimulus. Picture B and C demonstrates shift in the LTP – LTD induction crossover point (θ_m) as predicted by the BCM theory following LTP (B) or LTD (C) priming. The LTP – LTD crossover point slides to the right of the x-axis ($\theta_{m''}$) if preceded by increased levels of LTP (B). However, if preceded by increased levels of LTD (C), the θ_m slides to the left of the x-axis ($\theta_{m'}$).*

Reprinted from Brain Stimulation, Anke Karabanov, Ulf Ziemann, Masashi Hamada, Mark S. George, Angelo Quartarone, Joseph Classen, Marcello Massimini, John Rothwell, and Hartwig Roman Siebner. Consensus Paper: Probing homeostatic plasticity of human cortex with non-invasive transcranial brain stimulation, 8(3), 442-454, Copyright (2015) with permission from Elsevier.

1.2.4 Summary

The previous section reviewed short and long term synaptic plasticity and its role in learning and memory formation. While synaptic plasticity is best understood using Hebb's principle, a limitation in this principle is its positive feedback nature that leads to excessive LTP or LTD formation. To avoid overexpression of LTP or LTD, several studies suggest stability mechanisms, termed homeostatic plasticity, regulate and maintain synaptic homeostasis during neuroplastic change. Currently proposed mechanisms thought to underpin homeostatic regulation are critically analysed in the following sections.

1.3 Mechanisms of homeostatic plasticity

Following the discovery of homeostatic plasticity (Bienenstock et al., 1982; Cooper et al., 1979; Fox & Stryker, 2017), the BCM theory has now become an influential model to explain synaptic plasticity, and has guided experimental work in the last three decades (Cooper & Bear, 2012; Turrigiano & Nelson, 2000, 2004; Ziemann & Siebner, 2008). Although the original experiments were conducted in the visual cortex where binocular deprivation lowered the threshold for further LTP induction and normal vision restored this threshold (Bienenstock et al., 1982), evidence of a sliding modification threshold (θ_m) regulating synaptic modifications has since been demonstrated in numerous animal (Yee et al., 2017; Yger & Gilson, 2015) and human models (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). These findings establish homeostatic plasticity as a key

regulator of synaptic plasticity. In particular, global or local bidirectional modifications in synaptic strength between neurons have been suggested to explain homeostatic regulation within neural circuits (Turrigiano, 2008; Turrigiano & Nelson, 2000). The following sections discuss homeostatic mechanisms at a global and local level in further detail.

1.3.1. Global homeostatic plasticity

As synaptic plasticity is based upon a positive feedback process, potentiated synapses promote further LTP formation, while de-potentiated synapses promote further LTD formation (Abbott & Nelson, 2000; Abraham & Bear, 1996; Turrigiano & Nelson, 2000). One effective way of preventing runaway LTP – LTD formation is through compensatory increases or decreases in overall synaptic strength while simultaneously maintaining synaptic plasticity (i.e., LTP – LTD) dynamics (Ju et al., 2004; Lissin et al., 1998; O'Brien & Fischbach, 1986; Thiagarajan et al., 2002; Turrigiano, 2011, 2012). A pictorial representation of compensatory changes in overall synaptic strength representing global homeostatic plasticity is provided in Figure 1.2.

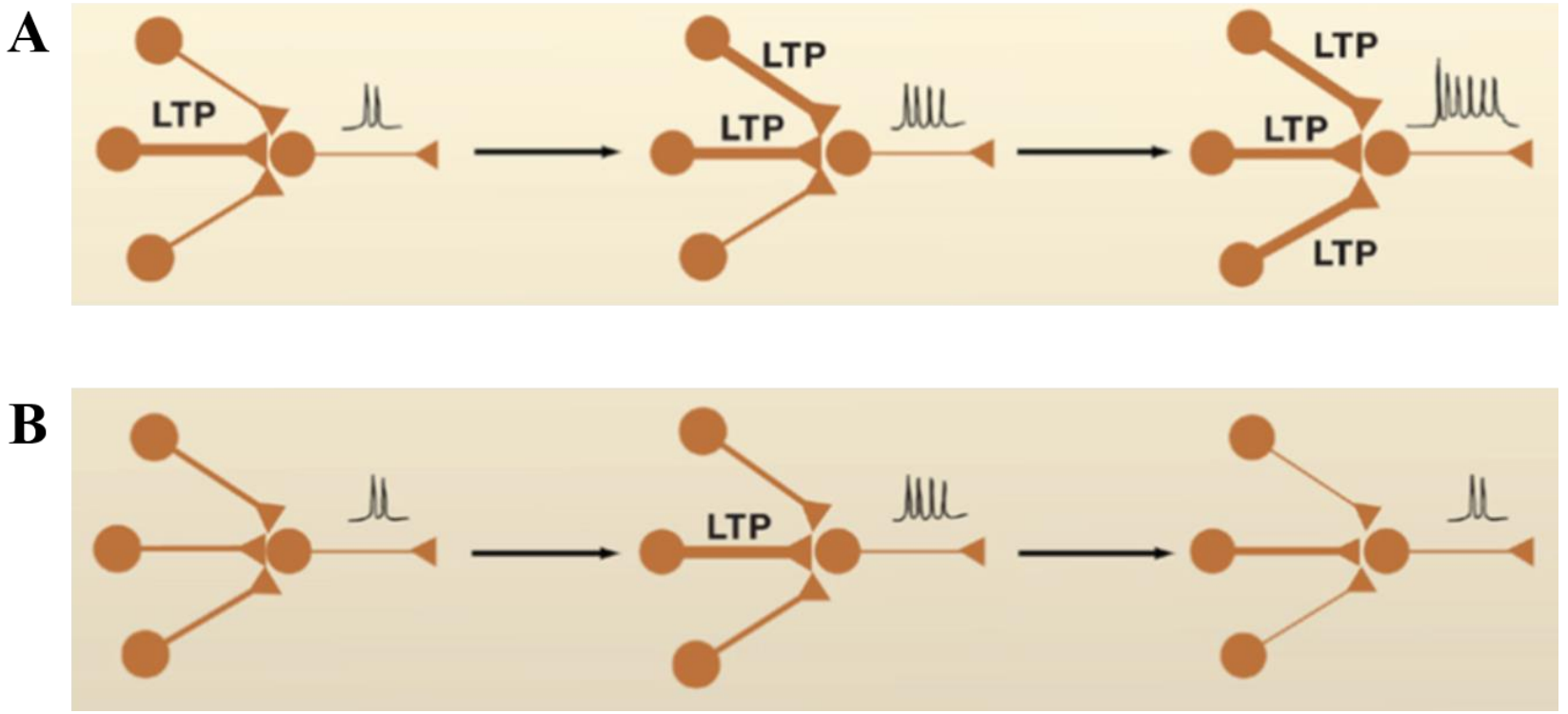

Figure 1.2: *Pictorial representation of global homeostatic regulation. Picture A demonstrates runaway LTP formation as afferent input increases LTP induction in surrounding neurons. Picture B demonstrates global homeostatic regulation following afferent input. Here, LTP induction in one neuron results in overall compensation in neuronal activity in surrounding neurons. Note overall change in size of neurons from left to right in pictures A and B.*

Reprinted from Cell, Gina G. Turrigiano. The Self-Turning Neuron: Synaptic scaling of Excitatory Synapses, 135(3), 422-435, Copyright (2015) with permission from Elsevier.

Bidirectional compensatory changes in overall synaptic strength was first identified in cultured neocortical neurons (Turrigiano et al., 1998). Here, chronic blockade of neocortical culture activity increased the amplitude of miniature excitatory postsynaptic potentials (mEPSP) used to index the overall strength of synaptic transmission between neurons. Conversely, blocking Gamma-aminobutyric acid (GABA) mediated inhibition that normally increases neuronal activity, reduced mEPSP amplitudes to baseline levels at 48-hours follow-up. These observations suggest that compensatory modulation of overall synaptic strength between neurons stabilise synaptic plasticity following manipulation of synaptic activity (Desai et al., 2002; Gainey et al., 2009; Kim & Tsien, 2008; Turrigiano et al., 1998).

Similar findings have been demonstrated in vivo and in vitro in motor networks (Knogler et al., 2010), spinal neurons (O'Brien et al., 1998), and the visual cortex (Desai et al., 2002; Goel & Lee, 2007). In the motor network of the developing zebra fish, tetrodotoxin (TTX) induced activity deprivation upscaled glutamatergic mEPSPs by 25 % at three to four days follow-up, whereas Tumor Necrosis Factor α (TNFα) induced increase in activity downscaled mEPSPs by 20 % while maintaining motor neuron activity patterns in swimming behaviour (Knogler et al., 2010). Likewise, spinal neurons incubated for one week in glutamate receptor antagonist (blocks excitatory synaptic transmission) increased the amplitude of mEPSPs, whereas glycine receptor antagonists (increases excitatory

synaptic activity) reduced the amplitude of mEPSPs as synaptic plasticity (i.e., LTP – LTD) dynamics remained unchanged (O'Brien et al., 1998). Such findings have been extended to the visual cortex where two days of visual deprivation increased the amplitude of mEPSPs in layer 2/3 of the mouse visual cortex (Goel & Lee, 2007). These changes reversed following one day of light exposure. Together, these findings extend early observations of Turrigiano et al. (1998) and indicate compensatory changes in overall synaptic strength between neurons underpin regulation of synaptic plasticity following incoming stimuli.

While there is ample evidence to support global homeostatic regulation within neural circuits (Desai et al., 2002; Goel & Lee, 2007; Kim & Tsien, 2008; Lissin et al., 1998; O'Brien et al., 1998), the timescale difference between global homeostatic regulation and rapid LTP – LTD formation limits generalisation of findings to all neural networks (Wu & Yamaguchi, 2006; Zenke et al., 2013). As a result, studies raise the possibility that other rapid forms of homeostatic plasticity must exist to regulate synaptic plasticity that take place within seconds to minutes (Wu & Yamaguchi, 2006; Zenke et al., 2013). Following these reports, a large body of evidence have demonstrated rapid forms of homeostatic plasticity, termed local homeostatic plasticity at pre- and post-synaptic junctions between individual neurons.

1.3.2 Local homeostatic plasticity

Unlike global homeostatic plasticity (Goel & Lee, 2007; Knogler et al., 2010; O'Brien et al., 1998), local homeostatic plasticity involves rapid compensatory changes to neuronal excitability at pre- and post-synaptic junctions that operate from minutes to hours (Frank et al., 2006; Hou et al., 2011; Ibata et al., 2008; Ju et al., 2004; Sutton et al., 2006; Wang et al., 2012). The clearest examples of local homeostatic plasticity was reported at the neuromuscular junction (NMJ) of Drosophila (Frank et al., 2006) and in cultured hippocampal neurons (Hou et al., 2011). In the Drosophila NMJ, increased pre-synaptic neurotransmitter release was observed 10 minutes following pharmacological blockade of post-synaptic glutamate receptors (Frank et al., 2006). Similarly, cultured hippocampal neurons reduced AMPA receptors at the post-synaptic terminal 30-minutes following increased pre-synaptic activity, whereas reduced glutamate release at the pre-synaptic terminal increased AMPA insertion at the post-synaptic terminal (Hou et al., 2011). These findings indicate that in addition to global homeostatic regulation, some neurons have potential for rapid compensatory changes at pre- and post-synaptic junctions.

While homeostatic regulation at pre- and post-synaptic junctions have been well documented, the underlying cellular mechanisms remain largely equivocal (Figure 1.3) (Ibata et al., 2008; Turrigiano, 2007, 2012; Turrigiano et al., 1995; Vitureira et al., 2012). For instance, microperfusion of TTX along with NMDA antagonist APV [(2R)-amino-5-

phosphonopentanoate] to local synapses increased AMPA receptor expression at the silenced post-synaptic terminal, suggesting AMPA receptor trafficking and accumulation at post-synaptic terminals can lead to local homeostatic regulation (Sutton et al., 2006). However, in another study, local perfusion of TTX failed to alter AMPA receptor expression at the post-synaptic terminal (Ibata et al., 2008). Instead, local homeostatic regulation was observed through modulation of calcium influx and release at the pre-synaptic terminal. Several studies have reported similar observations suggesting the probability of neurotransmitter release at presynaptic terminals also has a role in local homeostatic regulation (Frank, 2014; Jeans et al., 2017; Lazarevic et al., 2013; Muller & Davis, 2012; Ngodup et al., 2015; Subramanian, 2011; Weyhersmuller et al., 2011; Zhao et al., 2011). These reports suggest local homeostatic regulation takes place either by AMPA receptor trafficking and accumulation at post-synaptic terminals and / or by neurotransmitter release at pre-synaptic terminals. However, whether these mechanisms interact and contribute to local homeostatic regulation at the same pre- and post-synaptic junction or are restricted to particular pre- and post-synaptic junctions needs further investigation.

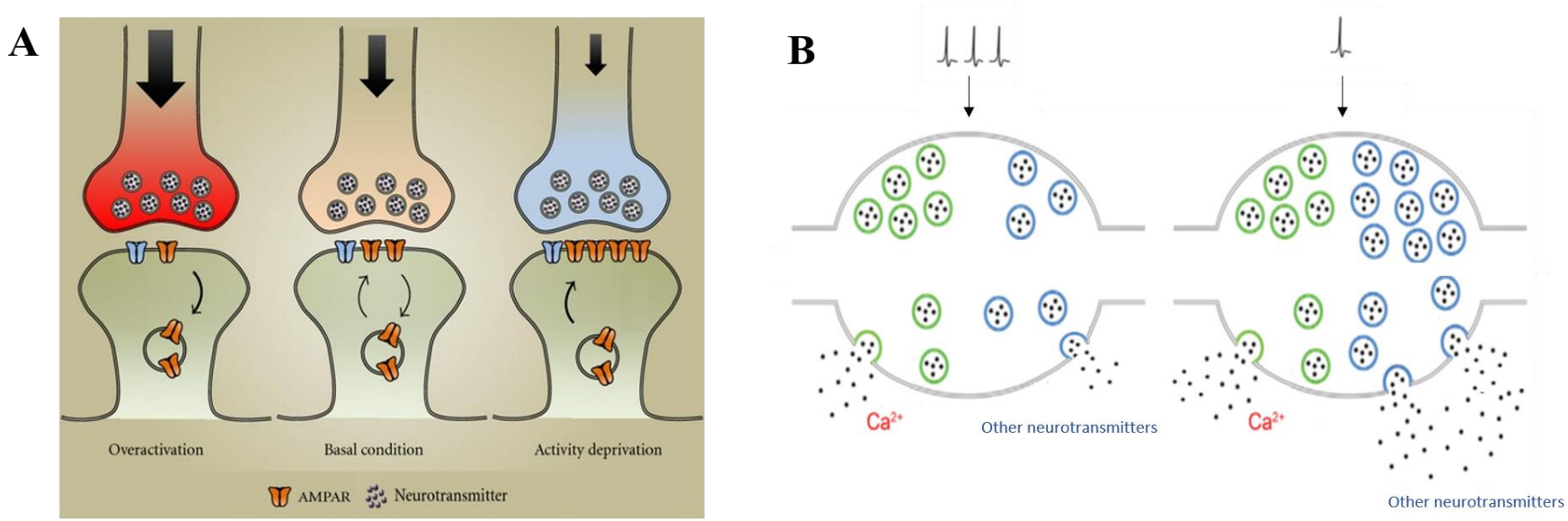

Figure 1.3: *Pictorial representation of local homeostatic regulation. Picture A demonstrates local homeostatic regulation induced via AMPA receptor trafficking at the post-synaptic terminal in a single neuron, whereas picture B demonstrates local homeostatic regulation by neurotransmitter release at the pre-synaptic terminal. Note the state of activation in picture A, and the stimuli applied to the pre-synaptic terminal in picture B.*

Picture A reprinted from Neural Plasticity, Guan Wang, James Gilbert, and Heng-Ye man. AMPA Receptor Trafficking in Homeostatic Synaptic Plasticity: Functional and Signaling Cascades, Article ID: 825364, Copyright (2015) with permission from Hindawi Publishing Corporation.
Picture B reprinted from Cell, Alexander F. Jeans, Fran C, van Heusden, Bashayer Al-Mubarak, Zahid Padamsey, and Nigel J. Emptage. Homeostatic Presynaptic Plasticity Is Specifically Regulated by P/Q-type Ca^{2+} Channels at Mammalian Hippocampal Synapses, 21(2), 341-350, Copyright (2015) with permission from Cell Press.

1.3.3 Summary

At present, studies suggest homeostatic mechanisms regulate synaptic plasticity either by modifying the overall synaptic strength or by inducing local compensatory changes at pre- and post-synaptic junctions. However, as the field of homeostatic plasticity is still in its infancy, several questions remain. For example, whether global and local forms of homeostatic regulation function together or separately within neural networks remain unanswered. Second, cellular mechanisms that underpin homeostatic regulation are unclear as some studies suggest AMPA trafficking and accumulation at post-synaptic terminals contribute to homeostatic regulation while others indicate neurotransmitter release at pre-synaptic terminals regulate synaptic activity. Finally, the majority of studies exploring homeostatic plasticity are limited to computational modeling studies, cultured neurons, and animal models. Whether similar homeostatic mechanisms operate in humans is yet to be definitively determined as existing studies are limited to non-invasive brain stimulation (NIBS) techniques that do not directly measure LTP and LTD but rather infer LTP – LTD-like effects. The following sections discuss the evidence for homeostatic plasticity in humans.

1.4 Homeostatic plasticity in humans

Following observations in seminal studies, numerous pharmacological studies provide evidence for NIBS induced LTP- and LTD-like plasticity such that homeostatic regulation can be inferred in humans (Kapogiannis & Wassermann, 2008; Nitsche et al., 2012; Schwenkreis et al., 2005; Soundara Rajan et al., 2017; Wankerl et al., 2010). For instance, NMDA receptor agonist D-Cycloserine enhanced cortical excitability following excitatory NIBS (Nitsche et al., 2004), while NMDA receptor antagonist dextromethorophane reduced cortical excitability following excitatory NIBS (Liebetanz et al., 2002; Nitsche et al., 2003a). Similar findings have been reported following AMPA receptor agonist ketamine (Di Lazzaro et al., 2003) and calcium channel antagonist nimodipine (Wankerl et al., 2010). Here, NIBS induced excitability was increased and eliminated respectively. Together, these findings suggest NIBS techniques target mechanisms of LTP and LTD, and can be used to inform homeostatic mechanisms expressed through LTP – LTD-like dynamics in the human brain (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015).

In accordance with pharmacological studies and principles of homeostatic regulation, NIBS techniques have been applied using a priming-test paradigm to explore homeostatic plasticity in the human brain (Bienenstock et al., 1982; Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). Here, the ‘priming’ NIBS technique is used to modulate prior cortical activity, while the following ‘test’ NIBS technique is used to demonstrate a

homeostatic response characterised as the plasticity response opposite the 'priming' NIBS technique (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). An overview of how excitatory and inhibitory NIBS techniques are combined using a priming-test paradigm to elicit a homeostatic response is provided in Table 1.1.

Table 1.1. Principles underpinning combinations of excitatory (induces LTP-like plasticity), and inhibitory (induces LTD-like plasticity) non-invasive brain stimulation to induce and elicit homeostatic plasticity in the human brain.

Priming	+	**Test**	**Result**
Excitatory NIBS^{+}		Excitatory NIBS^{+}	LTD induction$^{\downarrow}$
Inhibitory NIBS^{-}		Inhibitory NIBS^{-}	LTP induction$^{\uparrow}$
Excitatory NIBS^{+}		Inhibitory NIBS^{-}	Enhanced LTD$^{\downarrow\downarrow}$
Inhibitory NIBS^{-}		Excitatory NIBS^{+}	Enhanced LTP$^{\uparrow\uparrow}$

LTD: long-term depression LTP: long-term potentiation; NIBS: non-invasive brain stimulation. Note symbols used to denote excitatory (+) and inhibitory (-) NIBS to induce LTP (↑) or LTD (↓). Two arrows indicate enhanced LTP (↑↑) or LTD (↓↓). Tables in the following sections will use these symbols to indicate type of NIBS (i.e., excitatory or inhibitory) and the direction of induced effect (LTP or LTD).

Based upon the principles highlighted in Table 1.1., NIBS techniques including transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and paired associative stimulation (PAS) have been used to explore homeostatic plasticity in the human brain. Each of these techniques is discussed in the following section, that has

been divided into two parts: first, the role of each technique during homeostatic plasticity assessment or induction is described in section 1.4.1; second, evidence supporting the use of these techniques (in line with the principles highlighted in Table 1.1) to explore homeostatic regulation in the human brain is discussed in section 1.4.2.

1.4.1 Utility of TMS, tDCS, and PAS to explore homeostatic plasticity in the human brain

In general, there are three basic forms of NIBS techniques used to explore homeostatic plasticity in the human brain. First, there is transcranial magnetic stimulation (TMS), which is a safe, non-invasive brain stimulation technique used to assess or induce homeostatic plasticity in the human brain (Barker et al., 1985; Klein et al., 2015; Rossi et al., 2009; Sauvé & Crowther, 2014). Transcranial magnetic stimulation is based upon Faraday's law of electromagnetic induction, where an electrical current in a TMS coil produces a changing magnetic field that induces a secondary electrical current within the human brain (Barker et al., 1985; Di Lazzaro et al., 2004; Di Lazzaro et al., 2017; Sauvé & Crowther, 2014; Vidal-Dourado et al., 2014).

When delivered as a single form of stimulation (termed '*single-pulse TMS*'), the secondary electrical current generated during TMS produces a descending volley that travels down the corticospinal tract to the target muscle, and elicits a muscle response termed a *Motor*

evoked potential (MEP; Figure 1.4) (Di Lazzaro, 2013; Di Lazzaro et al., 2001; Klomjai et al., 2015; Rossi et al., 2009; Wassermann, 1998). An MEP is a measure of corticospinal excitability (Di Lazzaro et al., 2017; Rossini et al., 2015; Rothwell et al., 1999), and has been commonly used to index homeostatic plasticity in the human brain. In particular, the difference in peak-to-peak MEP amplitude before and after NIBS applied in a priming-test paradigm has been used to represent LTP- or-LTD-like changes during homeostatic plasticity assessment (Rossini et al., 2015; Rossini et al., 1991; Sauvé & Crowther, 2014). For example, an increase in MEP amplitude following high levels of LTD-like plasticity induced using two successive inhibitory NIBS techniques is interpreted to indicate LTP-like changes. Conversely, a reduction in MEP amplitude following high levels of LTP-like plasticity induced using two successive excitatory NIBS techniques indicate LTD-like changes (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). In this way, the difference in MEP amplitudes before and after NIBS is thought to provide an index of homeostatic plasticity in the human brain.

While single pulse TMS is used to assess homeostatic plasticity, repetitive TMS (rTMS) pulses have been used to elicit a homeostatic response through induction of LTP-or LTD-like plasticity (Di Lazzaro et al., 2010; Fitzgerald et al., 2006; Klomjai et al., 2015). In conventional rTMS paradigms, low (≤ 1 Hz) frequency rTMS reduce cortical excitability through LTD-like changes, whereas high (≥ 5 Hz) frequency rTMS increase cortical

excitability through LTP-like changes (Di Lazzaro et al., 2008; Maeda et al., 2000b; Matheson et al., 2016). However, while there is general consensus on low- and high-frequency rTMS induced effects, substantial variability in the size of the induced effect is commonly reported when these paradigms are applied alone (Hamada et al., 2013). For instance, 50 % of participants (n=32) reduced cortical excitability (indexed as reduced MEP amplitudes) following inhibitory rTMS (Strigaro et al., 2016), while 22 out of 30 participants increased cortical excitability (indexed as increased MEP amplitudes) following excitatory rTMS (Hinder et al., 2014). Similar findings were reported by Nettekoven et al. (2017) and Maeda et al. (2000) where only 44 % (n=16), and 34 % (n=36) of all participants responded to excitatory and inhibitory rTMS respectively (Maeda et al., 2000a; Nettekoven et al., 2015). These findings suggest rTMS induced LTP-or LTD-like plasticity vary in healthy individuals and may affect the homeostatic response elicited through inhibitory or excitatory rTMS. Importantly, studies of variability in the homeostatic response to rTMS protocols have yet to be conducted, and it is unclear whether a similar degree of variability is present when rTMS is used to elicit a homeostatic response as when used in isolation.

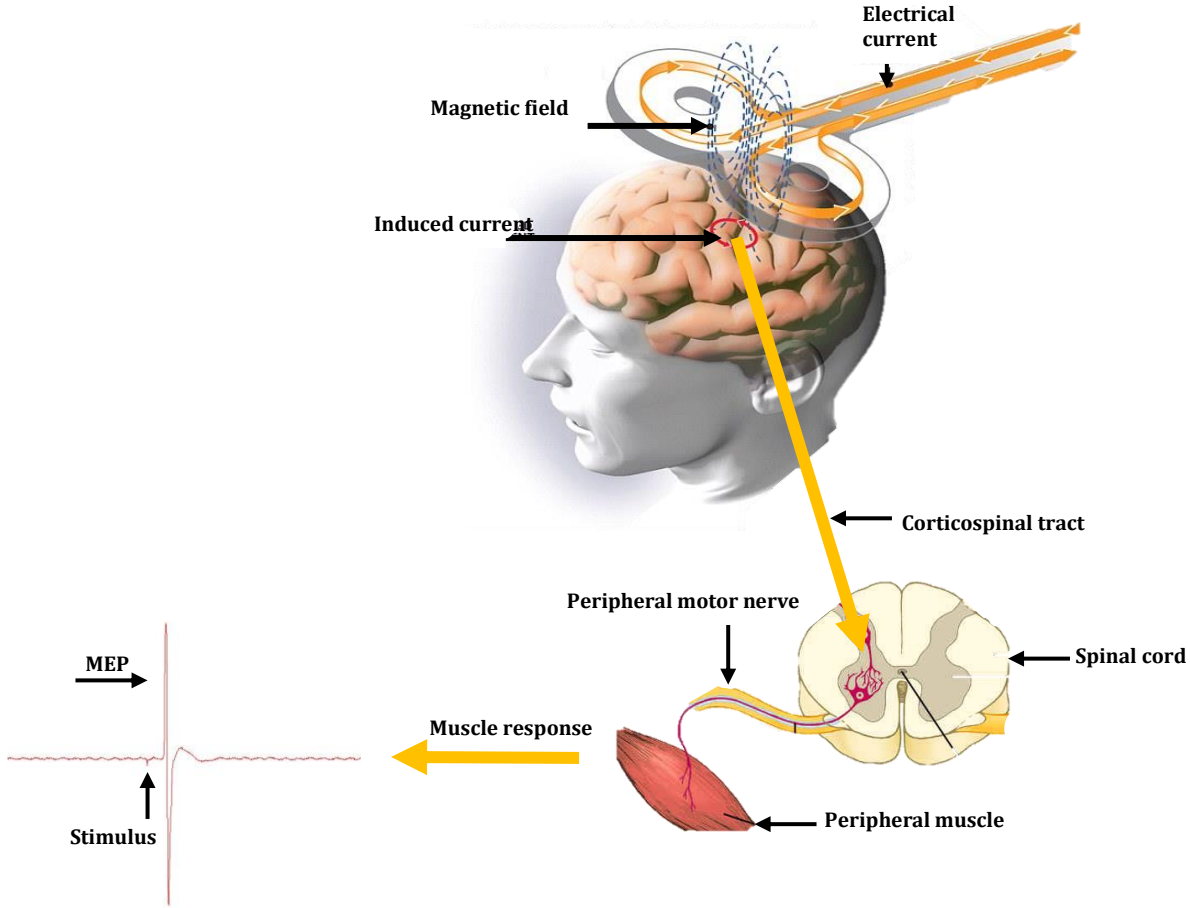

Figure 1.4. *Pictorial representation of transcranial magnetic stimulation (TMS) in the human brain. Transcranial magnetic stimulation (TMS) is performed using an electromagnetic TMS coil attached to a high-voltage discharge system. The magnetic field produced through the TMS coil lasts for approximately 100 µs, and produces a pulse (2 Tesla) strong enough to depolarise cortical neurons. Depolarisation of cortical neurons produces a descending volley that travels down the corticospinal tract, and results in a muscle twitch. This muscle twitch, recorded as a motor evoked potential (MEP), is used to index corticospinal excitability (in particular, primary motor cortex excitability), and homeostatic plasticity in humans.*

A second NIBS technique used to investigate homeostatic plasticity in the human brain involves low amplitude (≤1 – 2 mA) transcranial direct current stimulation (tDCS) applied through electrodes placed on the scalp (Figure 1.5) (Caytak et al., 2015; Nitsche et al., 2008). Due to weak direct currents, tDCS does not cause neural firing, but rather is thought to induce LTP-or LTD-like plasticity through shift in electric charge distribution within cortical neurons (Creutzfeldt et al., 1962; Fregni & Pascual-Leone, 2007; Lang et al., 2005; Nitsche & Paulus, 2000; Purpura & McMurtry, 1965; Wagner et al., 2007). Typically, two different forms of tDCS, termed anodal and cathodal tDCS have been used to induce LTP- and LTD-like plasticity respectively (Nitsche et al., 2008; Nitsche et al., 2007a; Nitsche & Paulus, 2000).

Anodal and cathodal tDCS induced LTP-and LTD-like plasticity is dependent upon several parameters (Nitsche et al., 2008; Nitsche et al., 2005; Poreisz et al., 2007). First, large current densities induce stronger LTP-and LTD-like plasticity, while small current densities lead to weaker LTP-and LTD-like plasticity (Iyer et al., 2005; Nitsche et al., 2008; Nitsche & Paulus, 2000). Second, with the current density held constant, longer stimulation duration result in longer periods of LTP-or LTD-like plasticity, while shorter stimulation duration result in shorter periods of LTP-or LTD-like changes (Nitsche et al., 2003c; Nitsche & Paulus, 2001). While both current density and duration have been shown to result in tDCS induced LTP-and LTD-like plasticity (Nitsche et al., 2008), studies

recommend modulating current duration over current density, as high current densities result in cutaneous pain and reduced focality during tDCS stimulation (Merrill et al., 2005; Minhas et al., 2011; Nitsche et al., 2003b).

Another important determinant of the direction of tDCS induced plasticity is the position of scalp electrodes, as the position of the stimulating electrodes govern current flow and electrical field distribution (Figure 1.5) (Bikson et al., 2010; DaSilva et al., 2011; Moliadze et al., 2010; Wagner et al., 2007). For instance, with one electrode fixed on the M1, varying the location of the reference electrode on different regions of the brain was shown to alter electrical field distribution, and thereby the induced LTP – LTD-like effect (Datta et al., 2010; Wagner et al., 2007). Further, significant change in cortical excitability thought to index LTP-and LTD-like plasticity was observed at the M1 only during a motor cortex – forehead electrode placement as opposed to a motor cortex – chin montage (DaSilva et al., 2011; Nitsche & Paulus, 2000; Priori et al., 1998). These data suggest that with the right parameters and electrode placement, tDCS can be used to induce LTP-and LTD-like plasticity (Antal et al., 2004; Antal et al., 2007; Nitsche et al., 2007a), and this in-turn can be implemented to explore homeostatic plasticity within the human brain (Antal et al., 2008a; Fricke et al., 2011; Nitsche & Paulus, 2000).

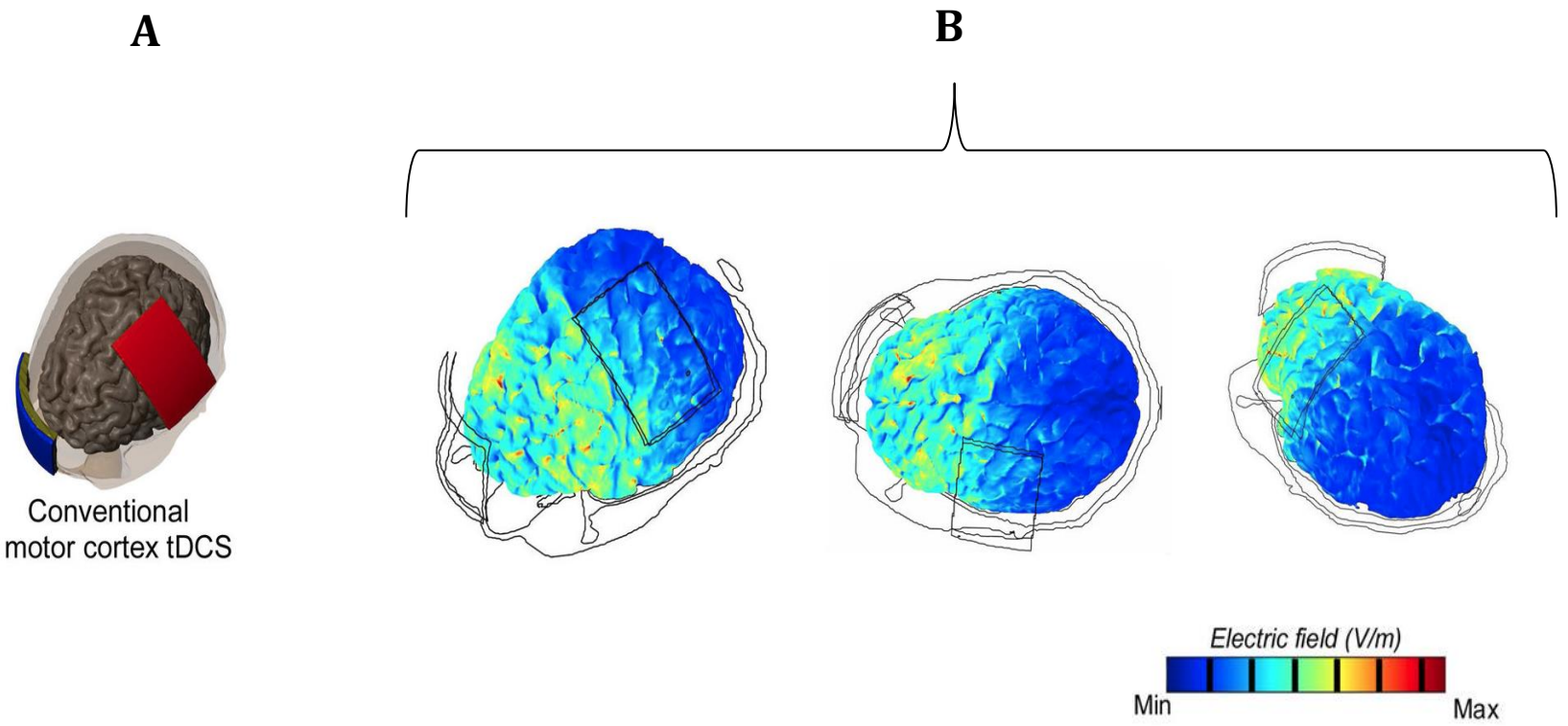

Figure 1.5. *Pictorial representation of transcranial direct current stimulation (tDCS) in the human brain. Picture A represents a typical primary motor cortex – forehead montage. Picture B represents the surface magnitude plots of the induced electrical field from different views when tDCS is applied using a primary motor cortex – forehead montage. Note the electrical field induced (refer to colours on the 'Electric field' scale) after tDCS stimulation.*

Reprinted from Neuroimage, Abhishek Datta, Marom Bikson, and Felipe Fregni. Transcranial direct current stimulation in patients with skull defects and skull plates: high-resolution computational FEM study of factors altering cortical current flow, 54(2), 1268-1278, Copyright (2010) with permission from Elsevier.

The third NIBS technique is paired associative stimulation (PAS), which involves pairing two stimulation protocols to induce LTP-or LTD-like plasticity (Classen et al., 2004; Lee et al., 2017; Stefan et al., 2000; Wolters et al., 2003). Typically, peripheral electrical stimulation applied to a peripheral nerve has been paired with TMS to the corresponding contralateral hemisphere (Figure 1.6) (Arai et al., 2011; Stefan et al., 2002; Stefan et al., 2000). The inter-stimulus interval (ISI) between PES and TMS is critical to PAS induced LTP – LTD-like plasticity, such that if the ascending afferent stimulus from peripheral nerve stimulation is in advance of TMS produced descending volley, the neural circuit undergoes excitation, and LTP-like plasticity is induced (Stefan et al., 2000; Wolters et al., 2003; Wolters et al., 2005). However, if the order of these events is reversed, the neural circuit undergoes inhibition, and LTD-like plasticity is induced (Müller-Dahlhaus et al., 2010; Stefan et al., 2000; Thirugnanasambandam et al., 2011; Wolters et al., 2003). More specifically, since PAS induced LTP – LTD-like plasticity is contingent upon the order of peripheral or cortical stimulation, specific ISIs are proposed to induce LTP-and LTD-like plasticity (Stefan et al., 2000; Wolters et al., 2003).

Following the original experiments, PAS at an ISI of 25 ms ($PAS_{25\ ms}$) has been commonly used to induce LTP-like plasticity, while PAS at an ISI of 10 ms ($PAS_{10\ ms}$) is recommended to induce LTD-like plasticity (Carson & Kennedy, 2013; Classen et al., 2004; Fathi et al., 2010; Huber et al., 2008; Ilic et al., 2009). However, use of fixed ISIs to

induce LTP – LTD-like plasticity is debatable, as studies suggest PAS induced LTP – LTD-like plasticity does not only occur over a single ISI, but rather across multiple ISIs (Carson & Kennedy, 2013; Dileone et al., 2010; Weise et al., 2011; Weise et al., 2006; Wolters et al., 2003). For example, when assessed with ISIs ranging from -10, 0, 5, 10, 15, 20, 25, 35, and 50 ms, LTP-like plasticity was observed not only with an ISI of 25 ms, but also with ISIs above 20 ms (Wolters et al., 2003). Similar observations were noted for LTD-like plasticity, where inhibitory effects were observed with ISIs of 10 ms, and -10, 0, and 5 ms (Wolters et al., 2003). However, contrary to these observations, Kang et al. (2011), and Dileone et al. (2010) report no change in LTP – LTD-like plasticity following PAS with ISIs of 10, 25, and 100 ms.

These discrepancies could be explained by considerable inter-individual variability between subjects classified as 'responders' and 'non-responders' (Krivanekova et al., 2011; Muller-Dahlhaus et al., 2008; Murase et al., 2015). Indeed, inter-individual variability is high during PAS as only 52 % of the total sample (n=27) demonstrated typical $PAS_{25\ ms}$ induced LTP-like plasticity (Muller-Dahlhaus et al., 2008). Similar observations were noted by Huber et al. (2008) where $PAS_{25\ ms}$ not only induced LTP-like plasticity, but also induced LTD-like plasticity in some participants (26 %). In the same study, LTD-inducing $PAS_{10\ ms}$ was shown to induce LTP-like plasticity in 18 % of participants (Huber et al., 2008), suggesting inconsistency in $PAS_{25\ ms}$ and $PAS_{10\ ms}$ induced LTP – LTD-like

plasticity may be explained by high inter-individual variability within and between studies (Lahr et al., 2016; Lopez-Alonso et al., 2014; Muller-Dahlhaus et al., 2008). Whether the same variability exists when PAS is applied in a priming-test paradigm to elicit a homeostatic response is unknown. Despite this, 15 studies have used PAS induced LTP – LTD-like plasticity to explore homeostatic plasticity in the human brain (primary motor cortex: six studies; primary somatosensory cortex: one study; inter-regional cortical networks: two studies; learning: six studies) (Karabanov et al., 2015).

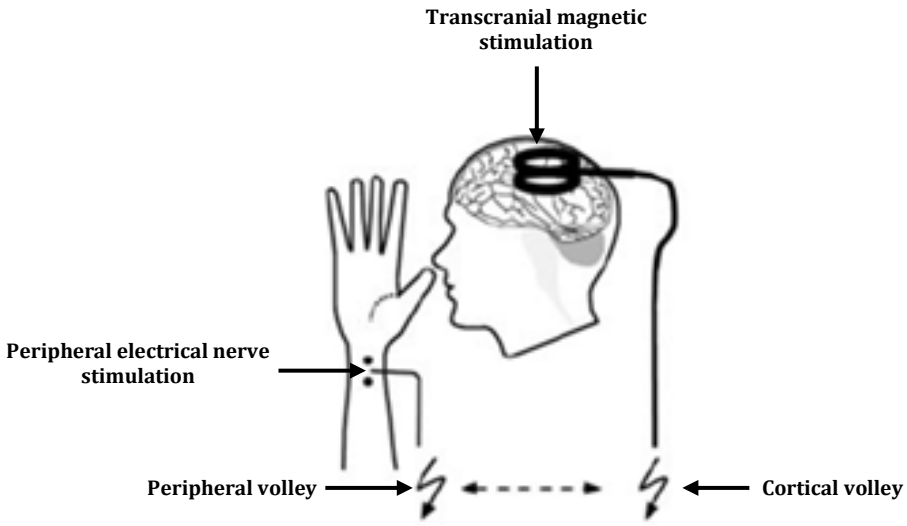

Figure 1.6. *Pictorial representation of paired associative stimulation in the human brain. Transcranial magnetic simulation (TMS) is applied (to the primary motor cortex in the picture) immediately before or after peripheral electrical nerve stimulation to a peripheral nerve (median nerve in the picture). Synaptic plasticity is modulated by timing the volleys generated from both peripheral electrical nerve stimulation, and cortical TMS. If the peripheral volley arrives before TMS induced volley is generated, long-term potentiation-like plasticity is induced, conversely, a reversal of stimulation order induces long-term depression-like plasticity.*

1.4.2 TMS, tDCS, and PAS to investigate homeostatic plasticity

The previous section addressed how TMS, tDCS, and PAS are appropriate to asses (single-pulse TMS only) or induce homeostatic plasticity in the human brain. The following sections provide a critical review of studies that have used TMS, tDCS, and PAS in combination (in line with the principles highlighted in Table 1.1) to elicit a homeostatic response in the primary motor cortex (M1), and in brain regions outside the M1.

1.4.2.1 Homeostatic plasticity in the primary motor cortex

The majority of research on homeostatic plasticity in humans is limited to the M1. Table 1.2 summarises all 17 cross-sectional studies that have used various forms of TMS, tDCS and PAS to investigate homeostatic plasticity in the human M1.

The clearest example is provided by Fricke and coworkers (2011) who are the only research group to have systematically investigated the impact of stimulation duration and inter-stimulus period between priming and test NIBS on the induction of homeostatic response in the human M1 (Fricke et al., 2011). This study suggests homeostatic plasticity is best elicited in the M1 when two blocks of anodal tDCS for 7- and 5-minutes with a 3-minute interval is delivered in a priming-test paradigm as opposed to two blocks of 5-minutes tDCS or 7- and 5-minutes tDCS with a 1, 10, 20, and 30-minute break between tDCS blocks (Fricke et al., 2011). Here, the first block of 7-mintue anodal tDCS (primer)

increased cortical excitability (observed as an increase in MEP amplitudes indicating LTP-like plasticity). Following the application of a second block of 5-minute anodal tDCS after a 3-mintue interval (test stimulation), a homeostatic response was observed as cortical excitability was reduced (observed as a decrease in MEP amplitudes indicating LTD-like plasticity) (Fricke et al., 2011).

Similar findings have been observed following PAS protocols that induce LTP- and LTD-like plasticity (Muller et al., 2007). Here, MEP amplitudes used to index homeostatic plasticity was reduced when two LTP-like plasticity inducing PAS protocols were applied in succession to the M1 in 11 healthy individuals. Conversely, when primed using an LTD-like plasticity inducing PAS protocol, MEP amplitudes were increased, suggesting homeostatic regulation of LTD-like plasticity in the human M1 (Muller et al., 2007).

These findings have also been reported following different combinations of rTMS, tDCS or PAS. For instance, the application of anodal, and cathodal tDCS prior to rTMS (Lang et al., 2004; Siebner et al., 2004) or PAS (Nitsche et al., 2007b) was shown to result in a reversal of after-effects when compared to rTMS or PAS applied alone. However, while these findings are consistent with the principles of homeostatic regulation, six studies failed to observe a homeostatic response following high levels of LTP-or LTD-like

plasticity induced using rTMS and PAS (Delvendahl et al., 2010; Goldsworthy et al., 2012b; Mastroeni et al., 2013; Opie et al., 2017a; Sidhu et al., 2017; Todd et al., 2009). One explanation for this discrepancy could be the difference in sample size between studies (Faber & Fonseca, 2014). As highlighted in Table 1.2, studies that have observed homeostatic regulation following rTMS, tDCS or PAS have considerably smaller sample sizes relative to studies that did not observe homeostatic plasticity following high levels of LTP-or LTD-like plasticity. Therefore, it is possible that studies that have reported homeostatic regulation following rTMS, tDCS or PAS may have overestimated the magnitude of the homeostatic response leading to Type I error (Cohen, 1969; Hackshaw, 2008; Sedgwick, 2014). However, that findings of homeostatic regulation are consistent between 11 studies from different laboratories, and none of the studies (including those that did not observe homeostatic plasticity) have demonstrated a power calculation to estimate the sample size required to detect a true homeostatic response limits the current assumptions. Future studies exploring homeostatic plasticity in the human M1 should demonstrate sample size calculations to observe true homeostatic responses.

Alternatively, studies that did not observe homeostatic regulation used combinations of rTMS and PAS (Delvendahl et al., 2010; Goldsworthy et al., 2012b; Mastroeni et al., 2013; Opie et al., 2017a; Sidhu et al., 2017; Todd et al., 2009). None of these studies used anodal or cathodal tDCS. This difference in methodology could explain the inconsistency between

studies. Indeed, rTMS, tDCS, and PAS are known to induce LTP-and LTD-like plasticity through different cellular mechanisms discussed in section 1.4.1 (Nitsche & Paulus, 2011; Rossini et al., 2015; Stefan et al., 2000). Hence, it is likely that different combinations of rTMS, tDCS, and PAS have a differential effect on cortical excitability (Muller-Dahlhaus & Ziemann, 2015; Ziemann et al., 2008). Future studies exploring M1 homeostatic plasticity in larger populations using similar combinations of NIBS techniques is warranted.

Table 1.2. Studies investigating homeostatic plasticity using rTMS, tDCS, and / or PAS in the human primary motor cortex of healthy individuals.

Study (year)	N	Priming / Test protocol	Priming / Test protocol duration (mins)	Outcome measure	Findings
Delvendahl et al. (2010)	38	0.1 Hz $rTMS^{-}$ / $PAS_{25\ ms}{}^{+}$	41.6 / 15	MEP amplitude	•
		0.1 Hz $rTMS^{-}$ / $PAS_{10\ ms}{}^{-}$	41.6 / 15	MEP amplitude	•
Gamboa et al. (2011)	16	$cTBS^{-}$ / $cTBS^{-}$	0.7 / 0.7	MEP amplitude	↑
		$iTBS^{+}$ / $iTBS^{+}$	3.2 / 3.2	MEP amplitude	↓
Goldsworthy et al. (2012)	22	$cTBS^{-}$ / $cTBS^{-}$	0.7 / 0.7	MEP amplitude	↓
Hamada et al. (2008)	10	5 ms QPS^{+} / Short ISI QPS^{+}	10 / 30	MEP amplitude	↓
		5 ms QPS^{+} / Long ISI QPS^{-}	10 / 30	MEP amplitude	↓↓
		50 ms QPS^{-} / Short ISI QPS^{+}	10 / 30	MEP amplitude	↑↑
		50 ms QPS^{-} / Long ISI QPS^{-}	10 / 30	MEP amplitude	↑
Huang et al. (2010)	8 – 7	$iTBS^{+}$ / $cTBS^{-}$	3.3 / 0.3	MEP amplitude	↓
		$cTBS^{-}$ / $iTBS^{+}$	0.3 / 3.3	MEP amplitude	↑
Iezzi et al. (2011)	10	5 Hz $rTMS_{subthreshold}$ / $iTBS^{+}$	9.3 / 2.7	MEP amplitude	↑↑
		5 Hz $rTMS_{subthreshold}$ / $cTBS^{-}$	9.3 / 0.7	MEP amplitude	↓↓
Iyer et al. (2003)	26	6 Hz $rTMS^{+}$ / 1 Hz $rTMS^{-}$	10 / 10	MEP amplitude	↓↓
Lang et al. (2004)	10	Anodal $tDCS^{+}$ / 5 Hz $rTMS^{+}$	10 / 0.3	MEP amplitude	↓↓
		Cathodal $tDCS^{-}$ / 5 Hz $rTMS^{+}$	10 / 0.3	MEP amplitude	↑↑
Mastroeni et al. (2013)	29	$cTBS^{-}$ / $cTBS^{-}$	0.6 / 0.6	MEP amplitude	↓
		$cTBS^{-}$ / $iTBS^{+}$	0.6 / 3.2	MEP amplitude	↑
		$iTBS^{+}$ / $iTBS^{+}$	3.2 / 3.2	MEP amplitude	↑

Muller et al. (2007)	11	PAS_{LTD}^{-} / PAS_{LTP}^{+}	15 / 15	MEP amplitude	↑↑
		PAS_{LTP}^{+} / PAS_{LTP}^{+}	15 / 15	MEP amplitude	↓
Ni et al. (2014)	14	$cTBS^{-}$ / PAS_{LTP}^{+}	0.2 / 30	MEP amplitude	↑↑
		$cTBS^{-}$ / PAS_{LTD}^{-}	0.2 / 30	MEP amplitude	↑
Nitsche et al. (2007)	6 – 12	Anodal $tDCS^{+}$ / PAS_{LTP}^{+}	7 / 7, 15, and 30	MEP amplitude	↓
		Cathodal $tDCS^{-}$ / PAS_{LTP}^{+}	7 / 7, 15, and 30	MEP amplitude	↑
Opie et al (2017)	30	$cTBS^{-}$ / $iTBS^{+}$	0.7 / 3.2	MEP amplitude	↑↑
		$iTBS^{+}$ / $iTBS^{+}$	3.2 / 3.2	MEP amplitude	↓
		Sham / $iTBS^{+}$	3.2 / 3.2	MEP amplitude	↑
Rothkegel et al. (2010)	14	/ 5 Hz $rTMS_{\text{in blocks}}^{+}$	8	MEP amplitude	↑↑
		/ 5 Hz $rTMS_{\text{continued}}^{+}$	4	MEP amplitude	↓
Sidhu et al (2017)	15	PAS_{LTD}^{-} / $PAS_{\text{LTP at 10 mins}}^{+}$	15 / 15	MEP amplitude	↓
		PAS_{LTP}^{+} / $PAS_{\text{LTP at 10 mins}}^{+}$	15 / 15	MEP amplitude	•
		PAS_{LTD}^{-} / $PAS_{\text{LTP at 30 mins}}^{+}$	15 / 15	MEP amplitude	↑↑
		PAS_{LTP}^{+} / $PAS_{\text{LTP at 30 mins}}^{+}$	15 / 15	MEP amplitude	•
Siebner et al. (2004)	8	Anodal $tDCS^{+}$ / 1 Hz $rTMS^{-}$	10 / 15	MEP amplitude	↓
		Cathodal $tDCS^{-}$ / 1 Hz $rTMS^{-}$	10 / 15	MEP amplitude	↑
Todd et al. (2009)	28	2 Hz $rTMS^{-}$ / $cTBS^{-}$	0.5 / 0.7	MEP amplitude	•
		6 Hz $rTMS^{+}$ / $cTBS^{-}$	0.5 / 0.7	MEP amplitude	•
		$iTBS^{+}$ / $cTBS^{-}$	0.2 / 0.7	MEP amplitude	↓

cTBS: continuous theta burst stimulation; LTD: long-term depression; LTP: long-term potentiation; M1: Primary motor cortex; MEP: Motor evoked potential; Mins: minutes; N: number of participants; cTBS: continuous theta-burst stimulation; iTBS: intermittent theta-burst stimulation; PAS: paired

associative stimulation; QPS: quadripulse transcranial magnetic stimulation; rTMS: repetitive transcranial magnetic stimulation; tDCS: transcranial direct current stimulation; • no difference in the outcome measure. Note symbols used to denote excitatory (+) and inhibitory (-) NIBS to induce LTP (↑) or LTD (↓). Two arrows indicate enhanced LTP (↑↑) or LTD (↓↓).

1.4.2.2 Homeostatic plasticity outside the M1

At present, a total of nine cross-sectional studies have explored homeostatic plasticity in brain regions outside the M1 (Table 1.3). Findings in these studies are consistent with the 11 studies that have demonstrated homeostatic regulation in the M1 (Bliem et al., 2008; Bocci et al., 2014).

The first study to demonstrate homeostatic regulation outside the M1 was conducted by Bliem and colleagues (2008) at the primary somatosensory cortex (S1) (Bliem et al., 2008). Indexed using the amplitude of somatosensory evoked potentials (SEPs), LTP- and LTD-like changes in S1 was assessed following a combination of PAS, and a LTP-like plasticity inducing excitatory peripheral high frequency stimulation (pHFS) protocol. PAS and pHFS were applied in a priming-test paradigm for 15- and 20- minutes respectively (Bliem et al., 2008). PAS induced LTD-like plasticity when followed by excitatory pHFS resulted in LTP-like changes expressed as an increase in SEP amplitude. Conversely, PAS induced LTP-like plasticity when followed by excitatory pHFS resulted in LTD-like changes expressed as a decrease in SEP amplitude (Bliem et al., 2008). Similar findings were observed in the primary visual cortex (V1) where anodal and cathodal tDCS applied for 20-minutes prior to low- (1 Hz) and high (5 Hz)- frequency rTMS reversed the after-effects when compared to rTMS applied alone (Bocci et al., 2014).

Homeostatic plasticity has also been reported following NIBS techniques applied to two different brain regions (Hamada et al., 2009; Popa et al., 2013; Potter-Nerger et al., 2009). For instance, inhibitory (26-minutes duration) and excitatory (9-minutes duration) rTMS to the left dorsal premotor cortex reversed the aftereffects of PAS (13-minutes duration) on the M1 (Potter-Nerger et al., 2009). Likewise, inhibitory priming of the cerebellum for 0.6 minutes increased PAS (2-minutes duration) induced LTP-like plasticity in the M1 (Popa et al., 2013), while the direction of LTP-or LTD-like plasticity in the M1 was determined following excitatory or inhibitory priming of the supplementary motor area (Hamada et al., 2009). When taken together, these findings suggest that NIBS induced LTP-or LTD-like plasticity is modulated by homeostatic mechanisms that are not only limited to the M1, but also extend to cortical regions outside the M1 (Table 1.3).

While there is evidence to support homeostatic regulation in and between different brain regions, evidence is conflicting for intracortical networks known to influence synaptic plasticity mechanisms in the human brain (Doeltgen & Ridding, 2011; Huang et al., 2017; Murakami et al., 2012; Siebner et al., 2004). Two studies (Doeltgen & Ridding, 2011; Siebner et al., 2004) report no homeostatic changes within short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF) networks following NIBS applied in a priming-testing paradigm. However, Murakami et al. (2012) contradict

these findings, and suggest homeostatic plasticity within SICI networks following repetitive TMS applied in a priming-testing paradigm (Murakami et al., 2012).

One explanation for this discrepancy is the difference in methodology between studies. To detect subtle changes in SICI and SICF networks, studies recommend measuring SICI and SICF at varying stimulus intensities (Chen et al., 1998). However, Siebner et al. (2004) and Doeltgen & Ridding (2011) used fixed stimulation intensities at 80 % and 70 % of active motor threshold, while Murakami et al. (2012) used stimulation intensities from 70 to 100 % of active motor threshold. This difference in fixed and varying stimulation intensities may explain the disagreement in findings between studies. Future studies using varying stimulus intensities to explore homeostatic plasticity within intracortical networks is needed.

Table 1.3. Studies investigating homeostatic plasticity using rTMS, tDCS, and / or PAS in brain regions outside the human M1 in healthy individuals.

Study (year)	N	Priming / Test protocol	Priming / Test protocol duration (mins)	Outcome measure	Findings
Bliem et al. (2008)	19	PAS_{LTP}^{+} / $pHFS^{+}$	15 / 20	SEP amplitude	↓
		PAS_{LTD}^{-} / $pHFS^{+}$	15 / 20	SEP amplitude	↑
Bocci et al. (2014)	10	Anodal $tDCS^{+}$ / 5 Hz $rTMS^{+}$	20 / 1	VEP amplitude	↓
		Cathodal $tDCS^{-}$ / 1 Hz $rTMS^{-}$	20 / 20	VEP amplitude	↑
Doeltgen et al. (2011)	14	$iTBS^{+}$ / $cTBS^{-}$	3.2 / 0.7	SICI	•
				SICF	•
Hamada et al. (2009)	9	5 ms QPS to SMA^{+} / Short ISI QPS^{+}	10 / 30	MEP amplitude	↓
		5 ms QPS to SMA^{+} / Long ISI QPS^{-}	10 / 30	MEP amplitude	↓
		50 ms QPS to SMA^{-} / Short ISI QPS^{+}	10 / 30	MEP amplitude	↑
		50 ms QPS to SMA^{-} / Long ISI QPS^{-}	10 / 30	MEP amplitude	↑
Murakami et al. (2012)	14	$cTBS^{-}$ / $cTBS^{-}$	0.6 / 0.6	SICI	↑
		$iTBS^{+}$ / $iTBS^{+}$	3.3 / 3.3	SICI	↓
		$cTBS^{-}$ / $iTBS^{+}$	0.6 / 3.3	SICI	•
		$iTBS^{+}$ / $cTBS^{-}$	3.3 / 0.6	SICI	•
Popa et al. (2013)	24	cTBS to $cerebellum^{-}$ / PAS_{LTP} to $M1^{+}$	0.6 / 2	MEP amplitude	↑
Potter-Nerger et al. (2009)	11	5 Hz rTMS to left DPC^{+} / PAS_{LTP}^{+}	9 / 13	MEP amplitude	↓
		1 Hz rTMS to left DPC^{-} / PAS_{LTD}^{-}	26 / 13	MEP amplitude	↑
Ragert et al. (2009)	34	1 Hz rTMS right $M1^{-}$ / iTBS left $M1^{+}$	20 / 3.2	MEP amplitude	↓
Siebner et al. (2004)	8	Anodal $tDCS^{+}$ / 1 Hz $rTMS^{-}$	10 / 15	SICI	•
				SICF	•

	Cathodal $tDCS^{-}$ / 1 Hz $rTMS^{-}$	10 / 15	SICI	•
			SICF	•

cTBS: continuous theta burst stimulation; DPC: dorsal premotor cortex; LTD: long-term depression; LTP: long-term potentiation; M1: Primary motor cortex; MEP: Motor evoked potential; Mins: minutes; N: number of participants; PAS: paired associative stimulation; pHFS: peripheral high frequency stimulation; rTMS: repetitive transcranial magnetic stimulation; SEP: somatosensory evoked potential; SMA: supplementary motor area; tDCS: transcranial direct current stimulation; VEP: visual evoked potential; • no difference in the outcome measure. Note symbols used to denote excitatory (+) and inhibitory (-) NIBS to induce LTP (↑) or LTD (↓). Two arrows indicate enhanced LTP (↑↑) or LTD (↓↓).

1.4.3 Summary

A broad range of NIBS techniques have been used successfully to investigate and demonstrate homeostatic plasticity in the human brain. However, studies exploring homeostatic plasticity in the human brain are mainly limited to cross-sectional study designs in the M1. As a result, whether M1 homeostatic plasticity can be reliably induced and assessed over time using NIBS techniques is unknown. Study one (chapter two) was, therefore, designed to address this gap in literature to determine the test-retest reliability of M1 homeostatic regulation, induced and assessed using non-invasive brain stimulation over time.

1.5 Homeostatic plasticity in pathological populations

As discussed in the previous section, data on homeostatic plasticity in the healthy human brain is available from 26 studies (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). However, there is much less information on homeostatic plasticity in individuals with pathology, in particular, in individuals experiencing pain (Table 1.4). At present, seven cross-sectional studies have explored homeostatic plasticity in writer's cramp and chronic migraine with sample sizes ranging from eight to 14 (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). Despite small sample sizes, findings from these studies are consistent, and suggest impaired homeostatic plasticity in writer's cramp, and chronic migraine may have a role in the pathophysiology of these conditions, and

contribute to motor dysfunction and unwanted sensory experiences (Antal et al., 2008b; Brighina et al., 2005; Brighina et al., 2002; Cosentino et al., 2014b; Kang et al., 2011; Quartarone & Pisani, 2011; Quartarone et al., 2005).

Writer's cramp is associated with abnormal, involuntary movements, and one influential hypothesis is deficient homeostatic control of LTP formation (Kang et al., 2011; Quartarone et al., 2003; Quartarone et al., 2008; Quartarone et al., 2006). This notion is supported by two studies that have explored homeostatic plasticity in eight to 10 individuals with writer's cramp (Table 1.4) (Kang et al., 2011; Quartarone et al., 2005). For example, Quartarone et al., (2005) probed M1 homeostatic plasticity using anodal and cathodal tDCS, and inhibitory rTMS applied for 10-and -15-minutes duration respectively (Quartarone et al., 2005) in eight patients with writer's cramp (mean ± standard deviation for history of writer's cramp: 10 ± 10 years), and eight age-matched healthy controls. Following cathodal tDCS priming, inhibitory rTMS resulted in a marked increase in MEP amplitude, while anodal tDCS priming reduced MEP amplitudes in healthy controls. These findings are consistent with studies that have used NIBS in similar priming-test paradigms to probe homeostatic plasticity in the healthy brain (Lang et al., 2004; Siebner et al., 2004). However, in contrast, individuals with writer's cramp failed to respond to tDCS (anodal and cathodal) primed inhibitory rTMS, suggesting impairment in M1 homeostatic regulation. Similar findings were reported by Kang and colleagues (2011) who

investigated homeostatic plasticity during motor learning in 10 individuals with writer's cramp (mean ± standard deviation for history of writer's cramp: 6.7 ± 6.6 years), and 10 age-matched healthy controls (Kang et al., 2011). Here, PAS induced LTP – LTD-like plasticity for 15-minutes was used to prime 30-minutes of rapid thumb abductions, and probe homeostatic plasticity. In line with rules of homeostatic regulation, PAS induced LTP-like plasticity applied prior to rapid thumb abductions reduced motor learning, while PAS induced LTD-like plasticity applied prior to rapid thumb abductions increased motor learning in age-matched healthy controls. However, individuals with writer's cramp failed to reduce motor learning when rapid thumb abductions were primed using PAS induced LTP-like plasticity. This deficiency in homeostatic control was later correlated with the clinical severity of writer's cramp (Kang et al., 2011). Together, these findings suggest impaired homeostatic plasticity in writer's cramp such that LTD-like plasticity is not induced when LTP-like plasticity is high. As excessive increases in cortical excitability cannot be regulated, impaired homeostatic plasticity is hypothesised to contribute to uncontrolled muscle function, and the pathophysiology of writer's cramp (Kang et al., 2011; Quartarone et al., 2003; Quartarone et al., 2008; Quartarone et al., 2005). A pictorial representation of normal and impaired homeostatic plasticity in healthy individuals and those with writer's cramp following NIBS applied in a priming-test paradigm is provided in Figure 1.7.

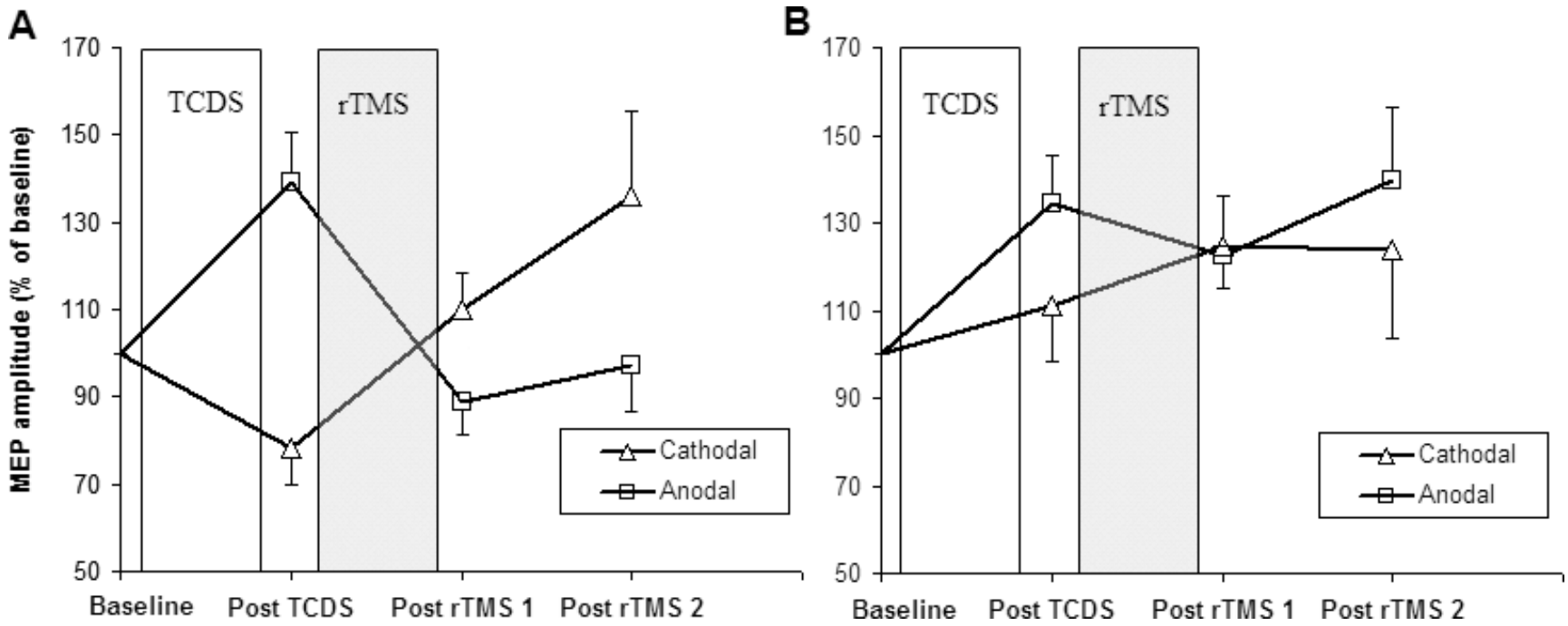

Figure 1.7. *Change in motor evoked potential (MEP) amplitude used to index M1 homeostatic plasticity following tDCS and rTMS applied in a priming-test paradigm. Picture A represents change in MEP amplitude following tDCS and rTMS in healthy individuals. Pictures B corresponds to impaired homeostatic regulation in individuals with writer's cramp. Note the difference in the direction of MEP amplitudes following tDCS and rTMS between Pictures A and B.*

Reprinted from Brain, Angelo Quartarone, Vincenzo Rizzo, Sergio Bagnato, Francesca Morgante, Antonio Sant'Angelo, Marcelo Romano, Domenica Crupi, Paolo Girlanda, John C. Rothwell, and Hartwig R. Siebner. Homeostatic-like plasticity of the primary motor hand area is impaired in focal hand dystonia, 128(Pt. 8), 1943-1950, Copyright (2005) with permission from Oxford University Press.

In addition to studies on homeostatic plasticity in individuals with writer's cramp, the other pathological population within which homeostatic plasticity has been investigated is chronic migraine (Antal et al., 2008b; Brighina et al., 2005; Brighina et al., 2010; Cosentino et al., 2014b; Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). Consistent findings between five studies that have explored homeostatic plasticity in individuals with chronic migraine indicate homeostatic impairment in these individuals (Table 1.4). For example, by using tDCS and rTMS in a priming-test paradigm for 10-and 0.3-minutes, Antal et al. (2008b) explored M1 homeostatic plasticity in 13 chronic migraineurs (mean ± standard deviation for migraine history: 14 ± 13 years), and 13 age-sex matched healthy controls. Both chronic migraineurs, and healthy controls displayed similar increases in MEP amplitudes following cathodal tDCS primed excitatory rTMS. However, when exposed to anodal tDCS primed excitatory rTMS, only healthy controls displayed a reduction in MEP amplitudes. Similar impairments in M1 homeostatic plasticity was observed by Cosentino et al. (2014) in 10 chronic migraineurs (mean ± standard deviation for migraine history: 15.5 ± 6.8 years) where failure to regulate high levels of LTP-like plasticity was observed specifically before the onset of a migraine attack. This impairment in homeostatic regulation prior to migraine onset is thought to predispose chronic migraineurs to headache recurrence. However, that Cosentino et al. (2014) did not assess the same individual during different phases of the migraine cycle limits the establishment of a cause and effect relationship between observed homeostatic

impairment and its role in triggering migraine attacks (or headache recurrence). Nonetheless, these findings suggest impaired homeostatic plasticity in chronic migraineurs characterised by a failure to regulate high levels of LTP-like plasticity in the M1 (Antal et al., 2008b; Brighina et al., 2011; Brighina et al., 2010; Cosentino et al., 2014b). A pictorial representation of impaired homeostatic regulation in individuals with chronic migraine is provided in Figure 1.8.

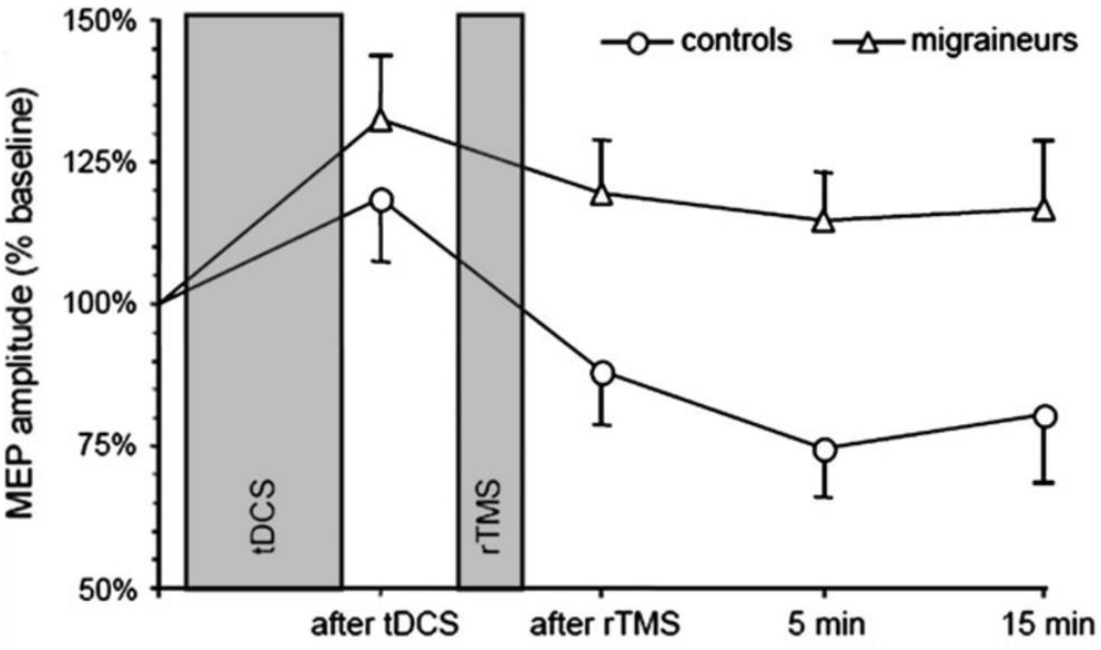

Figure 1.8. *Change in motor evoked potential (MEP) amplitude used to index M1 homeostatic plasticity following tDCS and rTMS applied in a priming-test paradigm in healthy controls (circles) and chronic migraineurs (triangles). Note the difference in the direction of MEP amplitudes following rTMS at the follow-up time-points.*

Reprinted from Cerebral Cortex, Andrea Antal, Nicolas Lang, Klara Boros, Michael Nitsche, Hartwig R. Siebner, and Walter Paulus. Homeostatic metaplasticity of the motor

cortex is altered during headache-free intervals in migraine with aura, 18(11), 2701-2705, Copyright (2008) with permission from Oxford University Press.

1.5.1 Summary

In summary, despite small sample sizes, consistent reports of impaired M1 homeostatic plasticity in pathological populations (i.e., writer's cramp, and chronic migraine) suggest uncontrolled levels of LTP-like plasticity is associated with motor dysfunction and altered sensory experiences. Likewise, homeostatic plasticity may be impaired in other clinical populations such as chronic musculoskeletal pain where synaptic plasticity has been shown to be altered, and motor dysfunction present. Thus, study two (chapter three) aimed to explore and understand the role of M1 homeostatic plasticity during pain, using a chronic musculoskeletal pain population i.e., people with non-specific chronic low back pain (cLBP).

Table 1.4. Studies investigating homeostatic plasticity in pathological populations in humans.

Study (year)	N	Priming / Test protocol	Priming / Test protocol duration (mins)	Outcome measure	Findings
Antal et al. (2008)	13 migraineurs 13 healthy controls	Cathodal $tDCS^{-}$ / 5 Hz $rTMS^{+}$	10 / 0.3	MEP amplitude	Migraineurs: ↑ Controls: ↑
		Anodal $tDCS^{+}$ / 5 Hz $rTMS^{+}$	10 / 0.3	MEP amplitude	Migraineurs: ↑ Controls: ↓
Brighina et al. (2011)	18 migraineurs 18 healthy controls	/ 5 Hz $rTMS^{+}$	/ 14.3	MEP amplitude	Migraineurs: ↓ Controls: ↑
Brighina et al. (2005)	9 migraineurs 8 healthy controls	/ 1 Hz $rTMS^{-}$	/ 15	MEP amplitude	Migraineurs: ↑ Controls: ↓
Brighina et al. (2002)	13 migraineurs 15 healthy controls	/ 1 Hz $rTMS^{-}$	/ 15	VEP amplitude	Migraineurs: ↑ Controls: ↓
Cosentino et al. (2014)	10 migraineurs 20 healthy controls	/ 5 Hz rTMS Interictal^{+}	/ 10.2	MEP amplitude	Migraineurs: • Controls: ↑
		/ 5 Hz rTMS Preictal^{+}	/ 10.2		Migraineurs: ↑ (excessive) Controls: ↑
		/ 5 Hz rTMS Ictal^{+}	/ 10.2		Migraineurs: ↓ Controls: ↑
		/ 5 Hz rTMS Postictal^{+}	/ 10.2		Migraineurs: ↓ Controls: ↑

Kang et al. (2011)	10 writer's cramp 10 healthy controls	PAS_{LTP}^{+} / Thumb abduction^{+}	15 / 30	Peak acceleration	Writer's cramp: ↑ Controls: ↓
		PAS_{LTD}^{-} / Thumb abduction^{+}	15 / 30		Writer's cramp: • Controls: ↑
Quartarone et al. (2005)	8 writer's cramp 8 healthy controls	Anodal tDCS^{+} / 1 Hz rTMS^{-}	10 / 15	MEP amplitude	Writer's cramp: ↑ Controls: ↓
		Cathodal tDCS^{-} / 1 Hz rTMS^{-}	10 / 15		Writer's cramp: • Controls: ↑

MEP: motor evoked potential; rTMS: repetitive transcranial magnetic stimulation; tDCS: Transcranial direct current stimulation; VEP: visual evoked potential; • no

1.6 Synaptic plasticity and musculoskeletal pain

Alternatively, as synaptic plasticity is known to be altered during chronic musculoskeletal pain, it is possible that the development of these changes may be explained by an impairment in homeostatic mechanisms that regulate synaptic plasticity and prevent overexpression of LTP-and LTD-like plasticity. However, as identified in section 1.5, all studies in pathological populations are limited to cross-sectional study designs. With such limitations, it is not possible to determine whether impaired homeostatic plasticity leads to altered synaptic plasticity or vice versa. Thus, the following sections discuss altered synaptic plasticity during chronic musculoskeletal pain, and the potential role of homeostatic plasticity in such individuals.

1.6.1 Altered synaptic plasticity during chronic musculoskeletal pain

Several studies have demonstrated altered synaptic plasticity in people with chronic musculoskeletal pain (Apkarian, 2011; Apkarian et al., 2009; Apkarian et al., 2011; Baliki et al., 2008; Baliki et al., 2011). These studies characterise altered synaptic plasticity in chronic musculoskeletal pain as unwanted brain reorganisation that underpins muscle dysfunction, and heightened pain sensitivity (Doyon & Benali, 2005; Flor, 2002; Flor, 2003; May, 2008). For example, in individuals with prolonged complex regional pain syndrome (CRPS), cortical representation of fingers one and five in the M1 was reduced in size and shifted towards the direction of the adjacent cortical lip representation when

compared to cortical representation of fingers from the contralateral, unaffected side (Maihöfner et al., 2003). These findings indicate distorted cortical representation of the affected fingers, which was later shown to be correlated with pain intensity and sensitivity to noxious stimuli (Maihofner et al., 2004).

A similar change in M1 topography has been reported in individuals with cLBP following cortical maps made with TMS. Here, a shift in the center of gravity of cortical maps corresponding to the deep multifidus and superficial longissimus muscles was correlated with reduced lower back muscle control, pain intensity, and disability (Schabrun et al., 2017b; Tsao et al., 2011b; Tsao et al., 2008). These observations were also reported in individuals with persistent elbow pain where shift in the center of gravity of cortical maps corresponding to the extensor carpi radialis brevis and extensor digitorum muscles were associated with higher pain intensity during rest, and at six months follow up (Figure 1.9) (Schabrun et al., 2015c). These studies provide evidence that altered synaptic plasticity occurs in people with chronic musculoskeletal pain. One interpretation is that these altered changes, characterised by distorted representation of body parts in the cortex may explain muscle dysfunction, and heightened pain sensitivity in individuals with chronic musculoskeletal pain (Schabrun et al., 2015c; Tsao et al., 2011a; Tsao et al., 2008).

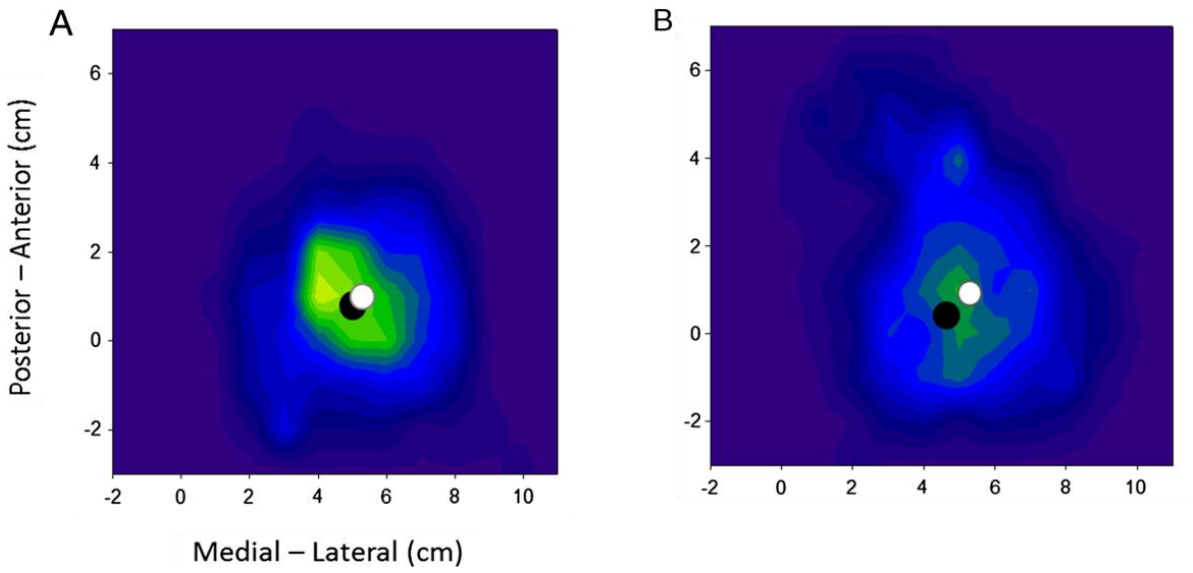

Figure 1.9. *A pictorial representation of the center of gravity for the extensor digitorum (black circles), and the extensor carpi radialis brevis (white circles) muscle from individuals with persistent elbow pain (A), and healthy controls (B). Note the distance between the white and black circles in pictures A and B.*

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Importantly, it is noteworthy that in addition to altered synaptic plasticity in the M1, several studies have demonstrated altered synaptic plasticity in brain regions outside the M1 in individuals with chronic musculoskeletal pain (Abu-Saad Huijer, 2010; Apkarian et al., 2011; Baliki et al., 2008; Flor, 2002; Gustin et al., 2012; Kuner & Flor, 2017). Together, these brain regions dubbed the 'pain neuromatrix', are argued to influence pain perception and experience (Apkarian, 2011; Apkarian et al., 2009; Apkarian et al., 2011; Moseley, 2003). However, that only one study has investigated homeostatic plasticity in brain

regions outside the M1 in any clinical population (Brighina et al., 2002; Muller-Dahlhaus & Ziemann, 2015), and that no study has established a tool to explore the relationship between homeostatic regulation and the pain neuromatrix limits its exploration in the current thesis.

In summary, as synaptic plasticity is altered in the M1 and influences clinical presentations in chronic musculoskeletal pain, homeostatic mechanisms that regulate, monitor, and stabilise alterations in synaptic plasticity may prevent these changes (Abraham, 2008; Abraham & Bear, 1996; Antal et al., 2008b; Bear, 2003; Cosentino et al., 2014b). However, at present, only one study has explored homeostatic plasticity in cortical areas outside the M1 during pathology (Table 1.4), and no studies have explored homeostatic regulation when musculoskeletal pain develops or persists. Thus, the role of homeostatic plasticity during pain is unknown.

1.6.2 Homeostatic regulation during pain

To date, no published studies have explored homeostatic plasticity as pain develops, peaks, and resolves (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). Several reasons explain this potential gap in the field. First, to explore homeostatic plasticity during pain over time, patients must first be assessed at baseline to determine if change in homeostatic regulation occurs as pain develops, peaks, and resolves. However, it is not possible to

obtain baseline data from individuals with clinical pain syndromes, as patients are generally only identified once pain is experienced (Apkarian et al., 2009; Loeser & Treede, 2008; Treede et al., 2015). Second, as patients are identified only when pain is experienced, patients may develop confounders to research through exposure to treatments, medication use, and other physical and psychological comorbidities (Akobeng, 2008; Brookhart et al., 2010; Jackson et al., 2006). Since it is unethical to stop or regulate treatments and medication use when patients are enrolled in a study, data quality is compromised by these confounders (Brookhart et al., 2010; Emanuel et al., 2000). While studies attempt to regulate these confounders through well-defined inclusion and exclusion criteria, rigorous pre-screening, and post-hoc sub-group analysis, generalisability of findings is often limited (Cleophas & Zwinderman, 2007; Jager et al., 2008).

In response to these ethical and methodological issues, experimental pain models that mimic the temporal dynamics of pain similar to that observed in patient populations provide a valuable means to assess pain in a controlled manner (Olesen et al., 2012; Reddy et al., 2012). Researchers currently use chemicals, electrical current, heat, pressure or exercise to induce experimental pain (Graven-Nielsen et al., 2002; Reddy et al., 2012; Staahl & Drewes, 2004; Svensson et al., 1997). However, despite the many options, these experimental pain models induce pain only for a short period of time (i.e., nociception

lasting ≤ 2hours) (Olesen et al., 2012; Reddy et al., 2012), thus, limiting their usefulness for homeostatic plasticity assessment as pain develops, peaks, and resolves.

Recently, repeated injection of nerve growth factor (NGF) has been used to mimic mechanisms potentially involved in the development of pain (Hayashi et al., 2013; Lewin & Mendell, 1993; Petty et al., 1994; Schabrun et al., 2016). Defined as a naturally occurring neurotrophic protein responsible for mediating inflammatory and immune responses in the adult nervous system, NGF is injected in small bursts to manifest muscle pain over 12 to 14 days (Lewin & Mendell, 1993; Petty et al., 1994; Reddy et al., 2012). As NGF induced muscle pain lasts for days, it provides a realistic model to investigate the temporal dynamics of homeostatic plasticity during the onset, and progressive development of pain whilst obtaining baseline data prior to pain onset, and controlling for confounders that compromise data quality (Hayashi et al., 2013; Schabrun et al., 2016). Thus, study three (chapter four) aimed to address the role of M1 homeostatic regulation as muscle pain, induced using NGF as an experimental pain model, developed, peaked, and resolved.

1.7 Study rationale

The literature and primary research data discussed in the introduction provides indicative evidence of synaptic plasticity and homeostatic regulation following afferent input. However, synaptic plasticity has also been shown to be altered in individuals experiencing pain. Despite this, homeostatic mechanisms that regulate synaptic plasticity has not been investigated in any depth during pain. Further, studies investigating homeostatic plasticity in individuals with pathology are currently limited to neurological populations with writer's cramp, and chronic migraine. These studies suggest M1 homeostatic plasticity is impaired in individuals with writer's cramp, and chronic migraine, and may have a role in the pathophysiology of these conditions. As similar clinical presentations are observed in individuals with chronic musculoskeletal pain, it is possible that homeostatic plasticity is impaired during pain. Thus, the broad goal of this thesis was to explore the effect of pain, using a clinical chronic musculoskeletal pain population and an experimental pain model, on homeostatic plasticity in the M1. This aim was achieved by three studies that addressed each knowledge gap identified in the introduction.

1.7.1 Study one

As identified in section 1.4 and Table 1.2, the majority of studies have explored the use of NIBS techniques to study homeostatic plasticity in the human M1. However, these studies are limited to cross sectional study designs that have not established whether NIBS can be

used to reliably induce and assess M1 homeostatic plasticity. Therefore, the aim of study one (chapter two) was to explore the test-retest reliability of NIBS used to induce and assess homeostatic plasticity in the human M1. This was achieved by conducting a longitudinal study on healthy volunteers over 48 hours, 7 days, and 2 weeks. Two successive blocks of anodal tDCS for 7 and 5-minutes, with a 3-minute interval was used to explore M1 homeostatic plasticity over 48 hours, 7 days, and 2 weeks, as this protocol has been suggested to best elicit a homeostatic response in the human M1 (Fricke et al., 2011).

1.7.2 Study two

As identified in section 1.5, studies exploring homeostatic plasticity in writer's cramp, and chronic migraine suggest impaired M1 homeostatic regulation may contribute to motor dysfunction and altered sensory experiences. As individuals with chronic musculoskeletal pain are known to experience similar clinical symptoms, study two (chapter three) aimed to investigate homeostatic plasticity in the M1 of individuals with non-specific cLBP. Here, two successive blocks of anodal tDCS for 7 and 5-minutes, with a 3-minute interval was used to explore M1 homeostatic plasticity in individuals with non-specific chronic low back pain (cLBP), and healthy, pain-free, controls. Pain intensity and duration were also recorded to determine an association (if any) between impairment in M1 homeostatic plasticity (if found), and pain profile of individuals with non-specific cLBP.

1.7.3 Study three

As identified in section 1.6, synaptic plasticity is altered in individuals with chronic musculoskeletal pain. As homeostatic regulation ensures stability in synaptic plasticity, it is possible that an impairment in homeostatic regulation could lead to the development of altered synaptic plasticity in individuals with chronic musculoskeletal pain or vice-versa. Therefore, the aim of study three (chapter four), was to explore the temporal dynamics of homeostatic plasticity as pain developed, peaked, and resolved. Nerve growth factor (NGF) was injected in small amounts to induce progressively developing muscle pain (Andresen et al., 2014; Hayashi et al., 2013; Schabrun et al., 2016), while homeostatic plasticity was explored in the M1 using two successive blocks of anodal tDCS applied for 7 and 5-minutes, with a 3-minute interval. To further explore M1 homeostatic plasticity during pain, participants filled in an online questionnaire addressing pain intensity, muscle soreness, level of disability, and sleep quality every alternative day from day 0 to day 21.

Chapter 2: Test-retest reliability of homeostatic plasticity induced and assessed using non-invasive brain stimulation in the human primary motor cortex

As highlighted in chapter one, present studies exploring homeostatic plasticity in humans are limited to cross-sectional study designs. Therefore, there is the need to explore whether non-invasive brain stimulation can be used to induce and assess homeostatic plasticity over time. The aim of this paper was to explore inter-session reliability in M1 homeostatic plasticity induced and assessed using non-invasive brain stimulation at intervals of 48 hours, 7 days, and 2 weeks. The content of this chapter has been published in ***Thapa, Tribikram.***, *& Schabrun, M. Siobhan. (2018). Test-Restest Reliability of Homeostatic Plasticity in the Human Primary Motor Cortex. Neural Plasticity. 2018(9). doi: 10.1155/2018/6207508.* A copy of the publication is provided in appendix A.

2.1 Abstract

Homeostatic plasticity regulates synaptic activity by preventing uncontrolled increases (long-term potentiation) or decreases (long-term depression) in synaptic efficacy. Homeostatic plasticity can be induced and assessed in the human primary motor cortex (M1) using non-invasive brain stimulation. However, the reliability of this methodology has not been investigated. Here, we examined the test-retest reliability of homeostatic plasticity induced and assessed in M1 using non-invasive brain stimulation in ten, right-handed, healthy volunteers on days 0, 2, 7, and 14. Homeostatic plasticity was induced in left M1 using two blocks of anodal transcranial direct current stimulation (tDCS) applied for 7-min and 5-min, separated by a 3-min interval. Fifteen motor evoked potentials to single pulse transcranial magnetic stimulation recorded at baseline, between the two blocks of anodal tDCS and at 0-min, 10-min, and 20-min follow-up were used to assess homeostatic plasticity. Test-retest reliability was evaluated using intraclass correlation coefficients (ICCs). Moderate-to-good test-retest reliability was observed for the M1 homeostatic plasticity response at all follow-up time-points (0-min, 10-min, and 20-min, ICC range: 0.43-0.67) at intervals up to two weeks. The greatest reliability was observed when the homeostatic response was assessed at 10-min follow-up (ICC >0.61). These data suggest M1 homeostatic plasticity can be reliably induced and assessed in healthy individuals using two blocks of anodal tDCS at intervals of 48 hours, 7 days, and 2 weeks.

2.2 Introduction

Synaptic plasticity is fundamental to learning and memory in the human brain. However, synaptic plasticity operates via a positive feedback loop and as a result, has the potential to destabilise neural networks through excessive synaptic strengthening (long-term potentiation-like effects, LTP) or excessive synaptic weakening (long-term depression-like effects, LTD) (Ziemann & Siebner, 2008). To avoid destabilisation, LTP and LTD-like changes are subject to homeostatic plasticity mechanisms that maintain neural activity within an optimal physiological range.

Homeostatic plasticity is theorised to rely on the 'sliding threshold' rule, such that the threshold for the induction of LTP or LTD is dependent on activity in the post-synaptic neuron; high post-synaptic activity favours LTD, whereas low post-synaptic activity favours LTP (Bear, 2003; Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). Although early studies investigating homeostatic plasticity occurred in slice preparations *in vitro*, a growing body of research has used non-invasive brain stimulation to investigate this mechanism in the human cortex (Bear, 2003; Gisabella et al., 2003; Huang et al., 1992; Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015; Turrigiano, 2007; Turrigiano, 1999). Typically, one non-invasive brain stimulation protocol is used to 'prime' (or condition) the synaptic effects of a subsequent non-invasive brain stimulation protocol and, LTP and LTD-like effects are indexed using transcranial magnetic stimulation (TMS). For

example, when a 5-min block of anodal transcranial direct current stimulation (tDCS) is preceded at short interval (3-min) by an additional 7-min block of anodal tDCS, the LTP-like (facilitatory) effect of anodal tDCS on the primary motor cortex (M1) is reversed toward LTD (observed as a reduction in corticomotor excitability to TMS) (Fricke et al., 2011). Similarly, preconditioning of a 1 Hz repetitive transcranial magnetic stimulation (rTMS) paradigm (that has no overt effect on corticomotor excitability when applied alone) with anodal tDCS produces LTD-like (inhibitory) effects, whereas preconditioning with cathodal tDCS produces LTP-like (facilitatory) effects (Siebner et al., 2004).

Non-invasive brain stimulation used to evaluate homeostatic plasticity have mainly been limited to the M1 in pathological conditions including writer's cramp, migraine, and chronic pain (Antal et al., 2008b; Cosentino et al., 2014b; Kang et al., 2011; Thapa et al., 2018a). No study has explored homeostatic plasticity in other brain regions that could be involved in pathology including the primary somatosensory cortex or the dorsolateral prefrontal cortex. Studies that have explored homeostatic plasticity in the M1 demonstrate impaired homeostatic control in writer's cramp, migraine, and chronic pain such that the threshold for synaptic plasticity fails to favour the induction of LTD when post-synaptic activity is high. For instance, in individuals with writer's cramp, a single block of anodal tDCS increases corticomotor excitability consistent with the response observed in healthy controls. However, application of a subsequent block of 1 Hz rTMS fails to reverse

corticomotor excitability toward LTD in this population (Quartarone et al., 2005). Additional studies have provided evidence of paradoxical facilitation in both the visual cortex and M1 of individuals with migraine, observed as an increase in visual cortex and M1 excitability in response to 1 Hz rTMS (in contrast to a reduction in the excitability of both cortices in healthy controls) (Brighina et al., 2005; Brighina et al., 2002).

Studies comparing M1 homeostatic plasticity between healthy individuals and those with pathology have been limited to cross-sectional designs, despite conditions such as migraine and low back pain being cyclical in nature (Cosentino et al., 2014b; Thapa et al., 2018a). To allow longitudinal evaluation of homeostatic plasticity, as well as detailed evaluation of the relationship between impaired homeostatic plasticity and symptom status, it is necessary to determine whether homeostatic plasticity can be reliably induced and assessed over time. To our knowledge, no study has investigated the reliability of M1 homeostatic plasticity. Here we aimed to determine the test-retest reliability of M1 homeostatic plasticity, induced and assessed using non-invasive brain stimulation, at intervals of 48 hours, 7 days, and 2 weeks.

2.3 Methods and materials

2.3.1 Subjects

As no previous multi-day studies of homeostatic plasticity exist, a sample size calculation was performed using best available data of MEP amplitudes recorded from healthy individuals at 0-min, 10-min, and 20-min following an identical double tDCS protocol used to induce and assess homeostatic plasticity in M1 (effect size estimates of 0.4, alpha of 0.05, and power of 0.8) (Thapa et al., 2018a). Using these parameters, ten participants were required to evaluate the test-retest reliability of non-invasive brain stimulation to induce and assess M1 homeostatic plasticity at intervals of 48 hours, 7 days, and 2 weeks. Accordingly, ten, right-handed, healthy volunteers (mean ± standard deviation age: 23 ± 5 years, 5 males) were recruited. Handedness was assessed using the Edinburgh handedness questionnaire (Oldfield, 1971). All participants were required to meet inclusion criteria as per transcranial magnetic stimulation (TMS) safety guidelines (i.e. no history of epilepsy, absence of metal implants in the skull) (Keel et al., 2001). Individuals with a history of neurological, musculoskeletal, upper limb or psychiatric conditions were excluded. A verbal and written description of the experimental procedures was provided to all participants. Written, informed consent was obtained before testing. The study was approved by the institutional Human Research Ethics Committee (Approval number: H10184) and performed in accordance with the Declaration of Helsinki.

2.3.2 Experimental design

Based on intervals used in previous TMS reliability studies (Uy et al., 2002), corticomotor excitability was assessed, and plasticity induced in M1, on day 0, 2, 7, and 14. Participants were seated comfortably with their right hand and arm at rest for each test session. To evaluate change in corticomotor excitability across days, 15 motor evoked potentials (MEPs) to single pulse transcranial magnetic stimulation (TMS) were recorded at 120 % of resting motor threshold (rMT) at the beginning of each test session. 15 MEPs were recorded as previous studies demonstrate good-to-excellent within and between session reliability for corticomotor excitability assessment (Bastani & Jaberzadeh, 2012; Chang et al., 2016; Christie et al., 2007; Doeltgen et al., 2009; Kamen, 2004). To account for any potential changes in corticomotor excitability occurring across days that could influence the homeostatic response, and to ensure a baseline level of corticomotor excitability that was consistent between individuals immediately prior to homeostatic plasticity induction, a further 15 MEPs were recorded immediately prior to the induction of homeostatic plasticity (timepoint 'baseline') at an intensity sufficient to evoke an average MEP of 1 mV peak-to-peak amplitude (S_{1mV}). This methodology is standard in studies of homeostatic plasticity (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). Homeostatic plasticity was induced in M1 using two blocks of anodal transcranial direct current stimulation (tDCS) applied for 7-min and 5-min respectively, and separated by a 3-min rest period ('double tDCS protocol'). This protocol has been used previously to

induce homeostatic plasticity in human M1 (Fricke et al., 2011; Thapa et al., 2018a). Corticomotor excitability in response to tDCS was monitored by recording 15 MEPs at S_{1mV} during the 3-min rest period between the two tDCS blocks (timepoint 'between'), and at 0-min, 10-min, and 20-min follow-up (see Figure 2.1). The number of MEPs was selected based on previous studies that have demonstrated good – excellent reliability when 15 MEPs are used to assess corticomotor excitability within-and-between sessions (Bastani & Jaberzadeh, 2012; Chang et al., 2016; Christie et al., 2007; Doeltgen et al., 2009; Groppa et al., 2012).

2.3.3 Assessment of corticomotor excitability

Single pulse transcranial magnetic stimulation (TMS) was delivered using a Magstim 200 stimulator (Magstim Co., Ltd., Dyfed, UK) and a standard 70 mm figure-of-eight coil. The coil was held over the left hemisphere, at a 45º angle to the sagittal plane to induce current in the posterior-anterior direction. Optimal coil position was determined by systematically moving the coil in 1 cm increments and locating the site that evoked the maximum response at the lowest stimulator intensity from the relaxed abductor pollicis brevis (APB) muscle (termed the 'hot-spot'). A soft-tip pen was used to mark the hotspot to allow accurate coil and tDCS electrode re-positioning within and between testing sessions. Participants were requested to precisely re-mark their hotspot using a mirror and a soft-tipped pen or if required, with assistance from a second person, on the days they did not

attend the laboratory for testing. Surface electromyography was recorded using surface dual electrodes (Ag-AgCl, Noraxon dual electrodes, inter-electrode distance 2.0 cm) placed in a belly-tendon montage over the relaxed APB muscle (Antal et al., 2008b; Fricke et al., 2011; Quartarone et al., 2005). The ground electrode was positioned over the ipsilateral olecranon. Raw EMG signals were amplified (1000 times), bandpass filtered 20-1000 Hz, and sampled at 2000 Hz (CED 1401 AD, Cambridge Electronic Design, Cambridge, United Kingdom) using Signal software (CED, version 5.08 x 86). To evaluate change in corticomotor excitability across days, 15 motor evoked potentials (MEP) were recorded at 120 % of resting motor threshold at the APB hot-spot. Resting motor threshold (rMT) was defined as the minimum TMS intensity required to elicit at least five MEPs ≥ 50 μV in ten consecutive trials from the resting APB muscle (Rossini et al., 1994).

2.3.4 Induction and monitoring of M1 synaptic and homeostatic plasticity

A battery driven, ramp controlled, constant current stimulator (DC-Stimulator Plus, NeuroConn, Ilmenau, Germany) delivered two blocks of excitatory, anodal transcranial direct current stimulation (tDCS) to the left primary motor cortex (M1). The left M1 was targeted to control for hand dominance, as only right-handed individuals were included in this study. The first anodal tDCS block lasted for 7-min and the second for 5-min. The two blocks were separated by a 3-min rest period. Rubber electrodes, placed in NaCl soaked sponges (5 x 7 cm) were positioned over the hot-spot corresponding to the right APB

muscle (anode) as determined above, and over the contralateral supraorbital region (cathode). Electrodes were fixed in position with two adjustable rubber straps. Current intensity was ramped up (0 mA – 1 mA) and down (1 mA – 0 mA) over ten seconds at the start and end of stimulation (Nitsche et al., 2008). Single-pulse TMS was used to monitor corticomotor excitability in response to the first and second block of anodal tDCS. This was achieved by setting the stimulator intensity to S_{1mV} at the previously determined optimal scalp site.

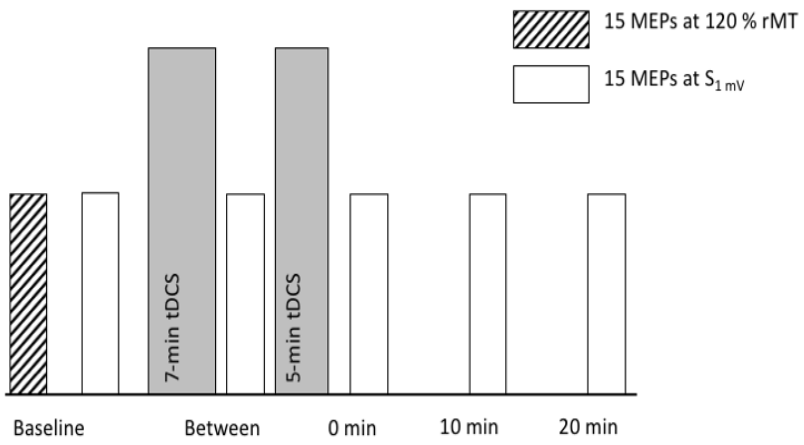


Figure 2.1 *Experimental protocol for days 0, 2, 7, and 14. Corticomotor excitability was assessed at the beginning of each test session using 15 motor evoked potentials (MEPs) recorded at 120 % of resting motor threshold. To ensure a consistent level of baseline corticomotor excitability across subjects prior to the induction of plasticity, a further 15 MEPs were recorded at an intensity sufficient to elicit an average MEP of 1 mV peak-to-peak amplitude (S_{1mV}) immediately before the first block of 7-min anodal transcranial direct current stimulation (tDCS). This intensity was kept consistent for the remainder of the test session. Plasticity was induced using a 7-min block of anodal tDCS, followed by a second 5-min block of anodal tDCS, separated by a 3-min rest period. Fifteen MEPs were recorded at S_{1mV} between the two blocks of anodal tDCS, and at 0-min, 10-min, and 20-min follow-up.*

2.3.5 Data analysis

Data are presented as means and standard deviations (SD) in text, tables, and figures. Statistical analyses were conducted using SPSS software for windows, version 22.

Data distribution was assessed using the Shapiro-Wilk test. A one-way repeated measures ANOVA with factor day (0, 2, 7, 14) was performed to compare i) resting motor threshold, ii) TMS intensity used to elicit S_{1mV}, and iii) corticomotor excitability (recorded at 120 % rMT), between days. To examine the change in corticomotor response following the first block of anodal tDCS across days, the amplitude of the MEP at timepoint 'between' was calculated as a proportion of the MEP amplitude at 'baseline' and analysed using a one-way repeated measures ANOVA with factor 'day'. To examine the change in corticomotor response to the double tDCS protocol across days, the amplitude of the MEP at each of the follow-up time-points (0-min, 10-min, and 20-min) was calculated as a proportion of the MEP amplitude at time-points 'baseline' and 'between', and analysed using a one-way repeated measures ANOVA with factor 'day'. This analysis was performed as the magnitude of the homeostatic response is likely to be dependent on corticomotor excitability at 'baseline', and the amount of facilitation achieved following the first block of anodal tDCS (i.e., timepoint 'between'). Bonferroni post-hoc tests corrected for multiple comparisons were performed where appropriate. The Greenhouse-Geisser method was used to correct for non-sphericity. Effect sizes from the one-way repeated measures

ANOVA are reported using partial eta squared (η^2_p). Cohen's benchmarks were used to define small (η^2_p=0.01), medium (η^2_p=0.06), and large effect sizes (η^2_p=0.14) (Cohen, 1969; Richardson, 2011).

An intraclass correlation coefficient model ($ICC_{3,k}$) was used to evaluate the test-retest reliability of the i) resting motor threshold, ii) TMS intensity used to elicit $S_{1mV,}$ iii) corticomotor excitability (recorded at 120 % rMT), iv) corticomotor response to the first block of anodal tDCS, and v) the corticomotor (homeostatic) response recorded at 0-min, 10-min, and 20-min after the second block of anodal tDCS, across days 0, 2, 7, and 14. The $ICC_{3,k}$ model was used to determine consistency between variables across days by accounting for fixed effects from the rater and random effects from study participants (McGraw & Wong, 1996; Schambra et al., 2015). ICC scores $\leq$ 0.20 were considered poor, 0.21 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 good, and $\geq$ 0.81 excellent (Matamala et al., 2018).

2.4. Results

2.4.1 Corticomotor excitability, and homeostatic plasticity in healthy individuals at intervals of 48 hours, 7 days, and 2 weeks

All data had normal distribution. There was no difference in resting motor threshold ($F_{2,16}$=0.3, P=0.7, η^2_p=0.03), the TMS intensity used to elicit S_{1mV} ($F_{3,27}$=0.4, P=0.7, η^2_p=0.04) or corticomotor excitability (assessed at 120 % rMT, $F_{2,16}$=0.4, P=0.6, η^2_p=0.05) between days (Table 2.1).

The magnitude of the increase in MEP amplitude following the first block of anodal tDCS was not different between days (corticomotor excitability at timepoint 'between' calculated as a proportion of the MEP amplitude at 'baseline'; $F_{3,27}$=0.4, P=0.8, η^2_p=0.04; Figure 2.2 and 2.3). Similarly, the magnitude of the decrease in MEP amplitude following the second block of anodal tDCS was not different between days at all follow-up time-points (corticomotor excitability at time-points 0-min, 10-min, and 20-min calculated as a proportion of the MEP amplitude at timepoint 'baseline'; 0-min: $F_{2,16}$=0.5, P=0.5, η^2_p=0.06; 10-min: $F_{3,27}$=1.7, P=0.2, η^2_p=0.16; 20-min: $F_{3,27}$=0.8, P=0.5, η^2_p=0.08; and corticomotor excitability at time-points 0-min, 10-min, and 20-min calculated as a proportion of the MEP amplitude at timepoint 'between'; 0-min: $F_{3,27}$=1.2, P=0.3, η^2_p=0.12; 10-min: $F_{3,27}$=1.3, P=0.3, η^2_p=0.13; 20-min: $F_{3,27}$=1.2, P=0.3, η^2_p=0.12; Figure 2.2 and 2.3).

Small effect sizes were observed for rMT (η^2_p=0.03), the TMS intensity used to elicit S_{1mV} (η^2_p=0.04), corticomotor excitability (assessed at 120 % rMT, η^2_p=0.05), and the corticomotor response to the first block of anodal tDCS (η^2_p=0.04). Medium-to-large effect sizes were observed for homeostatic responses to the double tDCS protocol when normalised to 'baseline' (0-min: η^2_p=0.06; 10-min: η^2_p=0.16; 20-min: η^2_p=0.08), and timepoint 'between' (0-min: η^2_p=0.12; 10-min: η^2_p=0.13; 20-min: η^2_p=0.12).

2.4.2 Test-retest reliability

Excellent test-retest reliability was observed for rMT (ICC=0.92, 95% CI 0.76 to 0.98; Table 2.1) and the TMS intensity used to elicit S_{1mV} (ICC=0.95, 95% CI 0.87 to 0.99; Table 2.1) across days. Moderate-to-good reliability was observed for corticomotor excitability assessed at 120 % rMT across days (ICC=0.80, 95% CI 0.47 to 0.94; Table 2.1).

The corticomotor response to the first block of anodal tDCS (ICC=0.41, 95% CI -0.72 to 0.84; Table 2.1), and homeostatic responses to the double tDCS protocol at all follow-up time-points across days, demonstrated moderate-to-good-reliability when data were normalised to timepoint 'baseline' (0-min: ICC=0.58, 95% CI -0.01 to 0.88; 10-min: ICC=0.61, 95% CI -0.03 to 0.89; 20-min: ICC=0.43, 95% CI -0.67 to 0.85; Table 2.1). Similarly, moderate-to-good-reliability was observed at all follow-up time-points across days, when homeostatic responses were normalised to timepoint 'between' (0-min:

ICC=0.61, 95% CI -0.03 to 0.89; 10-min: ICC=0.67, 95% CI 0.12 to 0.91; 20-min: ICC=0.60, 95% CI -0.06 to 0.89; Table 2.1). The highest ICCs were observed for the homeostatic plasticity response recorded at 10-min follow-up across days, (normalised to 'baseline' ICC=0.61, 95% CI -0.03 to 0.89; normalised to 'between' ICC=0.67, 95% CI 0.12 to 0.91; Table 2.1).

Table 2.1. Test-retest reliability (intraclass correlation coefficient [ICC]) estimates for cortical measures recorded across days 0, 2, 7, and 14.

Cortical measures	Cortical measures across days				ICC (95% CI)
	Day 0 (mean+SD)	Day 2 (mean+SD)	Day 7 (mean+SD)	Day 14 (mean+SD)	
rMT (% maximum stimulator output)	44 ± 7	45 ± 6	45 ± 7	44 ± 6	0.92 (0.76 – 0.98)
S_{1mV} (% maximum stimulator output)	54 ± 9	55 ± 11	56 ± 12	55 ± 12	0.95 (0.87 – 0.99)
Corticomotor excitability (mV)	1.0 ± 0.5	1.2 ± 0.9	1.0 ± 0.8	1.1 ± 0.9	0.80 (0.47 – 0.94)
Corticomotor $response_{baseline}$ (mV)	1.4 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.4 ± 0.5	0.41 (-0.72 – 0.84)
Homeostatic $response_{baseline}$ 0 min (mV)	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.4	0.8 ± 0.3	0.58 (-0.01 – 0.88)
Homeostatic $response_{baseline}$ 10 min (mV)	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.6 ± 0.1	0.61 (-0.03 – 0.89)
Homeostatic $response_{baseline}$ 20 min (mV)	0.8 ± 0.4	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.43 (-0.67 – 0.85)
Homeostatic $response_{between}$ 0 min (mV)	0.6 ± 0.1	0.6 ± 0.2	0.8 ± 0.4	0.7 ± 0.4	0.61 (-0.03 – 0.89)
Homeostatic $response_{between}$ 10 min (mV)	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.3	0.5 ± 0.2	0.67 (0.12 – 0.91)
Homeostatic $response_{between}$ 20 min (mV)	0.6 ± 0.3	0.5 ± 0.2	0.7 ± 0.3	0.6 ± 0.2	0.60 (-0.06 – 0.89)

Cortical measures: i) resting motor threshold (rMT), ii) transcranial magnetic stimulator (TMS) intensity needed to elicit an average peak-to-peak MEP amplitude of 1mV(S_{1mV}), iii) corticomotor excitability (motor evoked potential (MEP) amplitude recorded at 120% of rMT), iv) the corticomotor response to the first block of anodal tDCS normalised to baseline (corticomotor $response_{baseline}$), and v) the corticomotor (homeostatic) response to the second

block of anodal tDCS normalised to 'baseline' (homeostatic response$_{baseline}$), and 'between' (homeostatic response$_{between}$) at 0-min, 10-min, and 20-min follow-up.

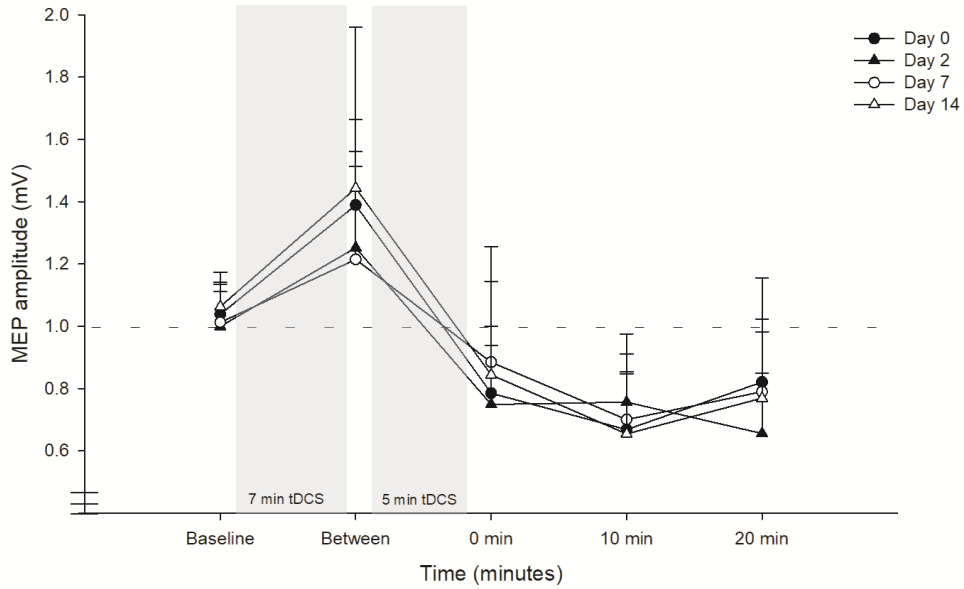


Figure 2.2 *Group data (mean + SD) for motor evoked potential (MEP) amplitude before the double tDCS protocol ('baseline'), after the first block of anodal tDCS ('between'), and at 0-min, 10-min and 20-min follow-up on day 0, 2, 7, and 14.*

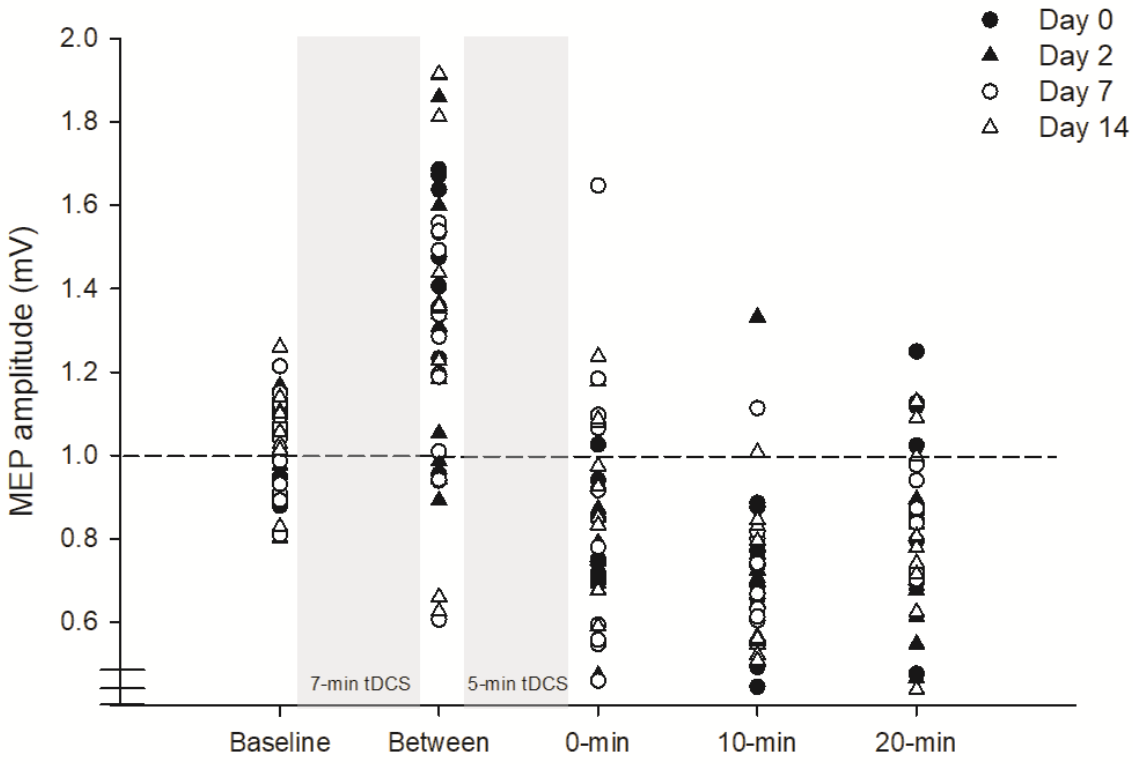


Figure 2.3 *Motor evoked potential (MEP) amplitudes recorded from each participant before the double tDCS protocol ('baseline'), after the first block of anodal tDCS ('between'), and at 0-min, 10-min and 20-min follow-up on day 0, 2, 7, and 14.*

2.5 Discussion

This study is the first to examine the test-retest reliability of M1 homeostatic plasticity, induced and assessed using non-invasive brain stimulation, in the healthy human brain. The corticomotor response to single, and double, anodal tDCS demonstrated moderate-to-good test-retest reliability in healthy individuals over intervals up to two weeks. These data suggest M1 homeostatic plasticity can be reliably induced and assessed over 48 hours, 7 days, and 2 weeks using two blocks of anodal tDCS and single pulse TMS respectively.

This finding provides a foundation for the longitudinal evaluation of M1 homeostatic plasticity in humans using the double tDCS protocol.

Homeostatic plasticity regulates neuronal firing rates in the human brain and ensures neuronal activity is maintained within a stable physiological range (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). The Bienenstock-Cooper-Munro (BCM) theory of homeostatic plasticity proposes that neuronal firing rates are regulated based on the history of post-synaptic activity, such that high levels of neuronal activity reduce the threshold for LTD-induction and promote LTD-like plasticity (synaptic weakening, lower firing rates), while low levels of neuronal activity reduce the threshold for LTP-induction and promote LTP-like plasticity (synaptic strengthening, higher firing rates) (Bear, 2003; Turrigiano & Nelson, 2004).

Consistent with the BCM theory, studies exploring homeostatic plasticity using repetitive tetanic stimulation (Abraham, 2008; Turrigiano, 2007, 2012; Turrigiano & Nelson, 2004), and non-invasive brain stimulation (Karabanov & Siebner, 2012; Karabanov et al., 2015; Lang et al., 2004; Muller-Dahlhaus & Ziemann, 2015; Sidhu et al., 2017) have shown that neuronal activity is modified based on the level of postsynaptic activity (Christova et al., 2015; Fujiyama et al., 2017; Opie et al., 2017b). For example, studies have shown that two blocks of anodal tDCS produce effects on M1 that follow a time-dependent rule consistent

with homeostatic mechanisms (Fricke et al., 2011). Specifically, when 7-min of anodal tDCS is followed at 3-min interval by a second, 5-min block of anodal tDCS, the increase in corticomotor excitability observed with 7-min anodal tDCS applied alone is reversed toward inhibition (Fricke et al., 2011). The nature of this response mimics the homeostatic rule of a threshold that slides to favour the induction of LTD-like effects (inhibitory response after the second block of anodal tDCS) when post-synaptic activity is high (following the first block of anodal tDCS) (Bear, 2003; Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015).

Our data confirm the direction and time-course of these effects in the healthy brain (increased corticomotor excitability in response to a single 7-min block of anodal tDCS; decreased corticomotor excitability in response to double tDCS), and extend previous work by demonstrating moderate-to-good test-retest reliability with medium-to-large effect sizes when homeostatic plasticity is induced and assessed using non-invasive brain stimulation at intervals of 48 hours, 7 days, and 2 weeks. Specifically, moderate-to-good test-retest reliability with medium-to-large effect sizes was observed when the magnitude of the homeostatic response was considered relative to 'baseline,' (all ICC $\geq$ 0.43; all $\eta^2_p \geq 0.06$; Table 2.1), and when the magnitude of the response was considered relative to the level of facilitation produced following the first block of anodal tDCS (all ICC $\geq$ 0.60; all $\eta^2_p \geq$ 0.12; Table 2.1). The greatest test-retest reliability (ICC $\geq$ 0.61) with the largest effect size

($\eta^2_p \geq 0.13$) was observed when the homeostatic response was evaluated at 10-mins follow-up.

The current data also provide further evidence that resting motor threshold (ICC=0.92, 95% CI 0.76 to 0.98), and corticomotor excitability (ICC=0.80, 95% CI 0.47 to 0.94) are reliable at intervals of 48 hours, 7 days, and 2 weeks. This finding is in agreement with previous studies. For example, Malcolm et al. (2006) reported high reliability in motor thresholds (ICC=0.90–0.97) in healthy volunteers over a period of two weeks (Malcolm et al., 2006). Further, good reliability (ICC ≥0.75) for cortical excitability measures (resting motor threshold, TMS input-output curves, MEP amplitude, and cortical silent period) have been reported across two testing sessions, each one week apart, in healthy volunteers (Liu & Au-Yeung, 2014). As changes in resting motor threshold and / or baseline corticomotor excitability are likely to influence the homeostatic response, reliability of these measures over time is an important consideration in the assessment of homeostatic plasticity in humans (Brighina et al., 2011; Cosentino et al., 2014b; Karabanov et al., 2015).

Previous studies have used a range of non-invasive brain stimulation protocols to probe M1 homeostatic plasticity in both healthy and clinical populations (Antal et al., 2008b; Kang et al., 2011; Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). In people

with non-specific chronic low back pain (cLBP), homeostatic plasticity was assessed in M1 using a double tDCS protocol identical to that investigated here (Thapa et al., 2018a). The authors demonstrated impaired homeostatic plasticity in this population characterised by a failure to reverse high corticomotor excitability (induced by the first block of tDCS) towards inhibition (following the second block of tDCS). Using 5 Hz trains of repetitive TMS, impaired homeostatic plasticity has been reported in individuals with episodic migraine during the preictal and post-ictal stages of the migraine cycle (Cosentino et al., 2014b). Although data was obtained from different individuals at different stages of the migraine cycle (i.e. the study did not utilise a repeated-measures design), impaired homeostatic plasticity was theorised to contribute to headache recurrence, and migraine transformation from an episodic to a chronic condition (Cosentino et al., 2014b). Similar observations were reported in the M1 of individuals with writer's cramp where patients failed to reverse high corticomotor excitability toward inhibition when 1 Hz rTMS was primed by anodal tDCS (Quartarone et al., 2005). Impaired M1 homeostatic plasticity in writer's cramp was later reported to correlate with the severity of symptoms and hypothesised to contribute to aberrant sensorimotor plasticity in this condition (Kang et al., 2011). These data have been interpreted to suggest that impaired homeostatic plasticity may play a role in the pathogenesis of some clinical conditions. Further exploration of these findings using longitudinal and repeated measures study designs are needed to confirm these hypotheses.

It is noteworthy that some studies using repeated non-invasive brain stimulation techniques have demonstrated non-homeostatic interactions in the human M1, where cumulative (rather than opposite) LTP or LTD-like effects are induced (Huang et al., 2011; Jung & Ziemann, 2009; Karabanov et al., 2015). For example, the application of two successive inhibitory continuous theta-burst stimulation protocols results in long-lasting MEP depression and not a reversal towards facilitation as would be hypothesised by the BCM theory (Goldsworthy et al., 2015; Goldsworthy et al., 2012a). These data suggest that in addition to homeostatic mechanisms, non-homeostatic interactions might also shape non-invasive brain stimulation induced LTP-LTD-like effects. Future studies exploring the interplay between homeostatic and non-homeostatic mechanisms over time are warranted in healthy and pathological populations.

This study has several limitations. First, test-restest reliability in M1 homeostatic plasticity was assessed in one direction only i.e., with a facilitatory priming protocol (anodal tDCS). This approach was selected as previous studies in pathological conditions have shown failure to induce LTD when post-synaptic activity is high (Antal et al., 2008b; Kang et al., 2011). However, since the polarity and magnitude of synaptic plasticity varies as a function of activation history in the post-synaptic neuron, future studies should seek to determine whether inhibitory priming protocols (e.g. cathodal tDCS) are also reliable over time.

Second, this study did not assess homeostatic plasticity in intracortical inhibitory or facilitatory networks. As tDCS is known to influence intracortical activity (Biabani et al., 2017; Nitsche et al., 2008; Nitsche et al., 2003c; Nitsche & Paulus, 2011), and homeostatic impairment has been demonstrated in intra-cortical inhibitory and facilitatory networks in individuals with migraine (Brighina et al., 2011; Brighina et al., 2005; Brighina et al., 2010; Brighina et al., 2002), future studies should investigate homeostatic regulation in these networks over time. Third, although this study used non-invasive brain stimulation methods similar to previous studies in this field (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015), tDCS applied to M1 using electrodes of 5 x 7 cm^2 may have resulted in current spread to surrounding cortical regions (Bastani & Jaberzadeh, 2013; DaSilva et al., 2011; Nitsche et al., 2008). Future studies should extend current findings using high density tDCS, as these approaches induce local rather than diffused tDCS effects (Datta et al., 2010). Finally, our findings are limited to homeostatic plasticity in the healthy M1 using a double tDCS protocol. Further research is needed to determine the test-retest reliability of homeostatic plasticity induced using other non-invasive brain stimulation methodologies in M1, as well as homeostatic plasticity induced in other brain regions relevant to different pathologies (Bliem et al., 2008; Bocci et al., 2014; Jones et al., 2016; Wolters et al., 2005).

2.6. Conclusion

These data demonstrate that M1 homeostatic plasticity induced using two blocks of anodal tDCS and assessed using single pulse TMS, has moderate-to-good reliability at intervals of 48 hours, 7 days, and 2 weeks, with the greatest reliability observed when the homeostatic response is assessed at 10-min follow-up. These findings provide a foundation for the assessment of homeostatic plasticity in the M1 using repeated measures and longitudinal study designs in humans.

Chapter 3: Disruption of cortical synaptic homeostasis in individuals with chronic low back pain

As identified in chapter one, no study has explored homeostatic plasticity in any chronic musculoskeletal pain condition despite similar clinical presentations to writer's cramp and chronic migraine, where homeostatic plasticity is impaired. Therefore, the aim of this chapter was to explore homeostatic plasticity in individuals with a chronic musculoskeletal pain condition i.e., in individuals suffering from non-specific chronic low back pain. The content of this chapter has been published in ***Thapa, T.***, *Graven-Nielsen, T., Chipchase, L. S., & Schabrun, S.M. (2018). Disruption of cortical synaptic homeostasis in individuals with chronic low back pain. Clinical Neurophysiology. 129(5), 1090-1096. doi: 10.1016/j.clinph.2018.01.060.* A copy of this publication is provided in appendix B. The editorial to this chapter is provided in appendix C

3.1 Abstract

Homeostatic plasticity mechanisms regulate synaptic plasticity in the human brain. Impaired homeostatic plasticity may contribute to maladaptive synaptic plasticity and symptom persistence in chronic musculoskeletal pain. We examined homeostatic plasticity in 50 individuals with chronic low back pain (cLBP), and 25 healthy, pain-free, controls. A single block (7-min) of anodal transcranial direct current stimulation ('single tDCS'), or two subsequent blocks (7-min and 5-min separated by 3-min rest; 'double tDCS'), were randomised across two experimental sessions to confirm an excitatory response to tDCS applied alone, and to evaluate homeostatic plasticity, respectively. Corticomotor excitability was assessed in the corticomotor representation of the first dorsal interosseous muscle by transcranial magnetic stimulation-induced motor evoked potentials (MEPs) recorded before and at 0-min, 10-min, 20-min, and 30-min following each tDCS protocol. Compared with baseline, MEP amplitudes increased at all time points in both groups following the single tDCS protocol ($P<0.003$). Following the double tDCS protocol, MEP amplitudes decreased in healthy, pain-free, controls at all time-points compared with baseline ($P<0.01$), and were unchanged in the cLBP group ($P\geq0.1$). These data indicate impaired homeostatic plasticity in the primary motor cortex of individuals with cLBP.

3.2 Introduction

Non-specific chronic low back pain (cLBP) is a prevalent and disabling musculoskeletal condition with few effective treatments (Balague et al., 2012). Although precise mechanisms remain unclear, structural and functional reorganisation of the sensorimotor cortex has been identified in non-specific cLBP, and is associated with pain severity, pain duration, and movement dysfunction (Kregel et al., 2015; Masse-Alarie et al., 2016). Cortical reorganisation in non-specific cLBP is hypothesised to be a marker of maladaptive synaptic plasticity, and this concept provides the foundation for contemporary theories of pain persistence (Moseley & Flor, 2012). Importantly, synaptic plasticity is regulated by homeostatic mechanisms (termed *homeostatic plasticity*), that if impaired, could explain aberrant synaptic plasticity and potentially symptom persistence in non-specific cLBP. Despite this, a pathophysiological role for changes in homeostatic plasticity has been overlooked in musculoskeletal pain.

Hebbian or use-dependent synaptic plasticity involves the expression of lasting changes in synaptic efficacy underpinned by long-term potentiation (LTP; synaptic strengthening) and long-term depression (LTD; synaptic weakening) (Hebb, 1949). However, synaptic plasticity relies on a positive feedback loop that left unchecked would lead to either too much strengthening and excessive neuronal excitability (LTP), or too much weakening and neuronal silencing (LTD) (Karabanov et al., 2015). In the healthy brain, homeostatic

plasticity mechanisms enforce stability and maintain brain excitability within a normal range by shifting the threshold for LTP and LTD based on the history of synaptic activity. For example, the threshold of a synapse with a history of high excitability will shift to favour induction of LTD (Bienenstock et al., 1982).

Homeostatic plasticity can be assessed in humans using non-invasive brain stimulation. For example, in healthy individuals a homeostatic response is elicited when two blocks of excitatory brain stimulation are applied at short intervals (Fricke et al., 2011). Homeostatic plasticity is observed as an increase in cortical excitability following the first block of excitatory stimuli (synaptic strengthening) that is reversed towards inhibition (synaptic weakening) when the second block of excitatory stimuli is applied after a few minutes. In this way, the brain corrects for exposure to excessive levels of excitation and prevents aberrant synaptic plasticity (Karabanov et al., 2015; Murakami et al., 2012).

Evidence from neurological conditions such as migraine and writer's cramp suggest a link between impaired homeostatic plasticity and symptoms. For example, these individuals exhibit reorganisation of the sensorimotor cortex (Jia & Yu, 2017; Schabrun et al., 2009a) as well as excessive cortical excitability (Brighina et al., 2005; Quartarone et al., 2003; Welch, 2003). Impaired homeostatic plasticity is hypothesised to contribute to abnormal cortical reorganisation and sensorimotor symptoms in these conditions as a result of

inappropriate and excessive LTP-like effects resulting from a failure to shift the threshold towards LTD when excitability is high (Brighina et al., 2005; Kang et al., 2011; Quartarone et al., 2008; Quartarone & Pisani, 2011). A comparable failure of homeostatic control in cLBP could explain similar observations of maladaptive cortical reorganisation and symptom persistence in this population yet, no study has investigated homeostatic plasticity in musculoskeletal disorders.

Importantly, impaired homeostatic plasticity has been shown to be generalised throughout the sensorimotor system and is not restricted to the cortical representations of affected muscles (Antal et al., 2008b; Brighina et al., 2011; Quartarone et al., 2008; Quartarone et al., 2005). For instance, in migraine, impaired homeostatic plasticity is not restricted to the visual cortex and is also observed in M1 representations of 'unaffected' hand muscles (Antal et al., 2008b; Brighina et al., 2011; Cosentino et al., 2014b). Similarly, impaired homeostatic plasticity is present in the 'unaffected' median and ulnar innervated muscles in writer's cramp (Kang et al., 2011; Quartarone et al., 2008). Together, these findings indicate a global impairment in homeostatic plasticity that has been suggested to provide evidence for a primary role of impaired homeostatic plasticity in the pathophysiology of these conditions (Antal et al., 2008b; Brighina et al., 2011; Kang et al., 2011; Quartarone & Pisani, 2011).

This study aimed to investigate homeostatic plasticity in the primary motor cortex representation of 'unaffected' hand muscles in individuals with non-specific cLBP and healthy, pain-free, controls. Similar to findings in individuals with migraine and writer's cramp, it was hypothesised that individuals with cLBP would fail to display a reversal of excitation towards inhibition following a second block of excitatory non-invasive brain stimulation consistent with global impairment in homeostatic control. A secondary aim was to examine the relationship between the magnitude of impaired homeostatic plasticity (if present) and the intensity and duration of cLBP.

3.3 Methods and materials

3.3.1 Participants

To determine a sufficient sample size to detect a difference in homeostatic plasticity between those with and without cLBP should one exist (aim 1), a power calculation was performed using a conservative effect size estimate of 0.2, an alpha of 0.05 and a power of 0.8. Using these parameters, 24 individuals were required in each group. However, to ensure there was also sufficient power to examine a relationship between impaired homeostatic plasticity and pain in the cLBP group, should one exist (aim 2), a second power calculation using an r value of 0.4, an alpha of 0.05 and power of 0.8 was performed. Using these parameters, a sample size of 47 individuals with cLBP was required. Thus, 50 individuals with cLBP (mean ± standard deviation age: 45 ± 16 years, 26 men), and 25

healthy, pain-free, controls (age: 43 ± 17 years; 13 men) were recruited. Chronic, non-specific low back pain was defined as the presence of continuous back pain lasting three months or more that was not due to a diagnosable pathology. A verbally administered 11-point numerical rating scale (NRS) anchored with 'no pain' at zero and 'worst pain possible' at 10, was used to determine pain intensity in the week prior to, and on the day of, testing. Participants were excluded if they presented with low back pain due to lumbar surgery, fracture, lumbar puncture, malignancy, infection, facet denervation, neuropathic or mixed pain (where pain radiated below the gluteal fold), and healthy, pain-free, controls were excluded if they had a history of cLBP, or any musculoskeletal pain condition. Any participant with a history of major circulatory, neurological, psychiatric, respiratory or cardiac diseases, who was taking central nervous system acting medication, or who presented with a cognitive deficit that impaired the ability to understand instructions or provide informed consent was excluded. All participants were required to meet inclusion criteria as per the transcranial magnetic stimulation (TMS) safety guidelines (i.e. no history of epilepsy, absence of metal implants in the skull) (Keel et al., 2001). Participant characteristics are summarised in Table 3.1.

A verbal and written description of the experimental procedures was provided to all participants. Written, informed consent was obtained before testing. The study was

approved by the institutional Human Research Ethics Committee (Approval number: H10184) and performed in accordance with the Declaration of Helsinki.

3.3.2 Experimental design

All participants attended two experimental sessions in random order. Experimental sessions were separated by at least seven days to avoid carry over effects as previous studies have demonstrated tDCS when applied for 7 and 5-minutes with a 3-minute interval induce effects that persist for up to 60-minutes (Fricke et al., 2011; Nitsche et al., 2007b). Participants received a single 7-min block of anodal transcranial direct current stimulation (tDCS) applied to the primary motor cortex (M1) in one session to confirm the existence of an excitatory response ('single tDCS' protocol; Figure 3.1A). In a separate session, participants were exposed to two blocks of anodal tDCS (7-min and 5-min separated by a 3-min rest period) to investigate homeostatic plasticity in M1 ('double tDCS'; Figure 3.1B). This protocol has been shown to induce homeostatic plasticity (observed as a reduction in motor evoked potential (MEP) amplitude in response to TMS) in healthy individuals (Fricke et al., 2011). In both sessions, MEPs were elicited using single pulse TMS and recorded from the first dorsal interosseous (FDI) muscle ipsilateral to the side of worst pain in individuals with non-specific cLBP and the matched side for healthy, pain-free, controls. The FDI muscle was chosen to allow investigation of global impairment in homeostatic plasticity consistent with previous studies in writer's cramp and chronic

migraine (Antal et al., 2008b; Quartarone et al., 2005). Motor evoked potentials were recorded at baseline, and immediately following each experimental protocol at 0-min, 10-min, 20-min, and 30-min follow-up (Cavaleri et al., 2017). Participants were comfortably seated with their hand and arm at rest throughout both sessions. No participant reported muscle spasm or discomfort during testing.

3.3.3 Assessment of corticomotor excitability

Motor evoked potentials were recorded using surface dual electrodes (Ag-AgCl, Noraxon dual electrodes, product #272S, inter-electrode distance 2.0 cm) placed in a belly-tendon montage on the relaxed FDI muscle (Antal et al., 2008b; Fricke et al., 2011; Quartarone et al., 2005). The ground electrode was positioned on the olecranon process. Raw EMG signals were amplified (1000 times), bandpass filtered within a range of 20 Hz (high-pass) to 1 kHz (low-pass) and sampled at 2 kHz (CED 1401 AD, Cambridge Electronic Design, Cambridge, United Kingdom) via acquisition software (CED, version 5.08 x 86). The MEP peak-to-peak amplitude was extracted and averaged for analysis.

A standard 70 mm figure-of-eight coil connected to a magnetic stimulator (Magstim 200, Magstim Co. Ltd. Dyfed, UK) was used to provide single-pulse TMS. The coil was positioned tangentially to the scalp with the handle pointing posterolaterally at a 45˚ angle from the mid-sagittal plane. This orientation is optimal for the induction of posterior-to-

anterior (PA) directed current for trans-synaptic activation of horizontal cortical connections in M1 (Bashir et al., 2013; Brasil-Neto et al., 1992). The optimal site (hotspot) for eliciting MEPs from the relaxed FDI was determined before each experimental session by systematically moving the coil in 1 cm increments around the motor cortex. The hotspot was marked with a pen to allow accurate coil positioning. The stimulation intensity for TMS was adjusted to elicit an MEP amplitude of 1 mV peak-to-peak in the relaxed FDI at baseline, and this intensity was kept consistent throughout each test session. The average amplitude of 30 MEPs was used for analysis at each time-point.

3.3.4 Transcranial direct current stimulation

In both experiments, a battery driven direct current stimulator (DC-Stimulator Plus, NeuroConn, Ilmenau, Germany) was used to deliver a constant current of 1 mA through saline-soaked sponge electrodes (surface 7 x 5 cm). The active electrode (anode) was positioned over the motor cortical representation (hotspot) for FDI as determined by TMS in each participant. The reference electrode (cathode) was positioned over the contralateral supraorbital region. Electrodes were fixed with two adjustable rubber straps around the head. Stimulation in this montage has been reported to increase cortical excitability in the underlying M1 that outlasts the stimulation period by 20 – 60 min (Fricke et al., 2011). Current was ramped up and down over 10 s at the start and end of stimulation to avoid

startling participants by alternating current transients that cause immediate neuronal firing during tDCS (Nitsche et al., 2008).

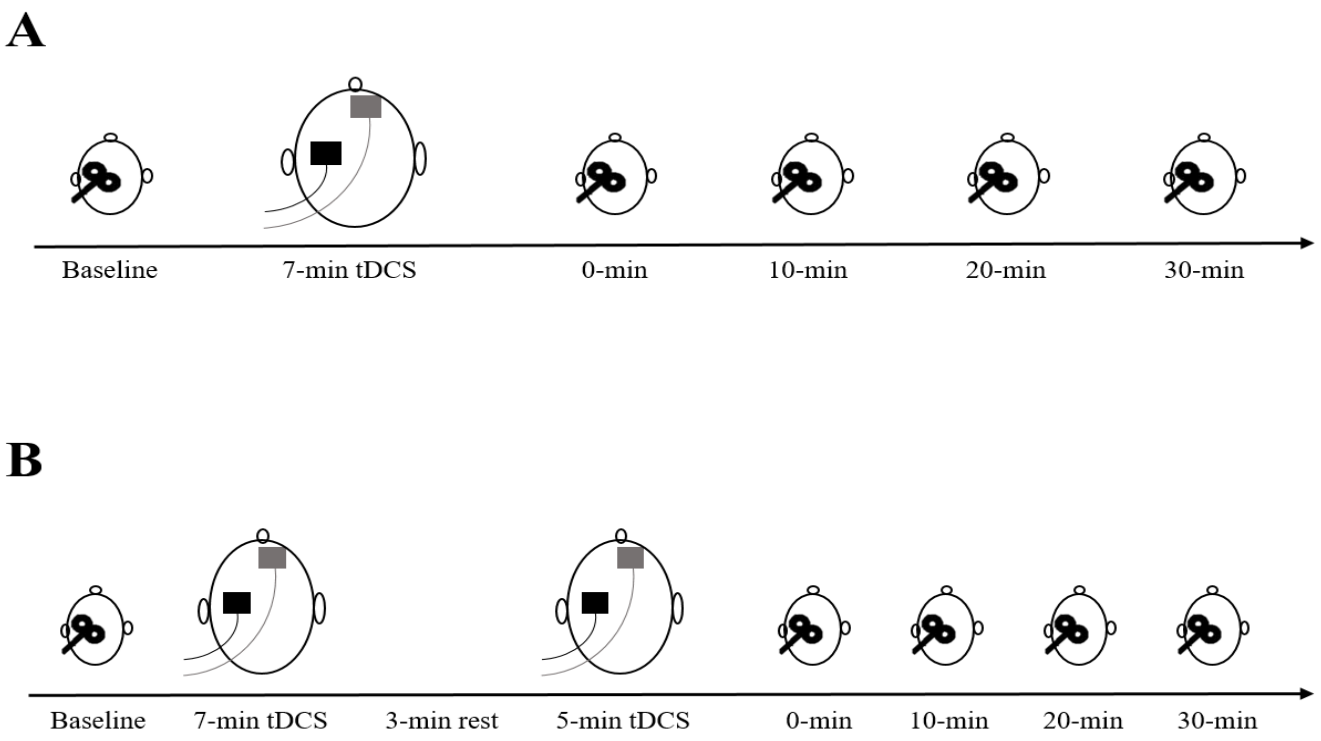


Figure 3.1. *Experimental protocol**: A)** Single tDCS protocol: Anodal tDCS was applied to the primary motor cortex contralateral to the side of worst pain in individuals with cLBP and the matched side for healthy, pain-free, controls in a single, 7-min block.* ***B)*** *Double tDCS protocol: Anodal tDCS was applied to the primary motor cortex contralateral to the side of worst pain in individuals with cLBP and the matched side for healthy, pain-free, controls for a 7-min block followed by a second 5-min block separated by a 3-min rest period. Motor evoked potentials (elicited using transcranial magnetic stimulation) were recorded at baseline and at 0-min, 10-min, 20-min, and 30-min follow-up in each experiment.*

3.3.5 Data analysis

Data are presented as means and standard deviations (SD) throughout the text. For all analyses, SPSS software for windows, version 22 was used. A two-way analysis of variance (ANOVA) was performed to examine the TMS intensity used to elicit a 1 mV MEP at baseline between groups (cLBP and pain-free controls) and protocols (single vs. double tDCS). To examine the effect of each tDCS protocol (single vs. double) on raw (non-normalised) MEP amplitudes, separate two-way repeated measures ANOVAs were conducted for each protocol with factors 'Group' (cLBP vs. pain-free controls), and 'Time' (baseline, 0, 10, 20, and 30-minutes). Where appropriate, post-hoc testing was performed using t-tests with Bonferroni corrections for multiple comparisons. The Greenhouse-Geisser method was used to correct for non-sphericity as required. A Pearson product-moment correlation coefficient was used to assess the relationship between the magnitude of homeostatic plasticity (MEP amplitude) at each time-point after the double tDCS protocol and pain intensity, and pain duration, respectively. For all statistical tests a P-value of <0.05 was considered significant.

3.4 Results

Table 3.1. Participant characteristics (mean ± standard deviation)

Demographics	Chronic low back pain	Pain-free controls
N	50	25
Age (years)	45 ± 16	43 ± 17
Male:Female	26:24	13:12
Side of worst pain (L:R)	22:28	-
Side of hemisphere tested (L:R)	28:22	14:11
Pain on day of testing (NRS)	4.9 ± 2.8	-
Pain in the week before (NRS)	4.1 ± 2.6	-
History of back pain (years)	12.7 ± 14.4	
Pain medication*	9	-

*N: total number of participants; L: left; R: right; NRS: numerical rating scale; * taking paracetamol as required.*

3.4.1 TMS intensity at baseline

In the single tDCS protocol, the baseline TMS intensity (percent of maximum stimulator output) used to elicit MEPs of 1 mV peak-to-peak amplitude was 58 ± 15 % and 58 ± 13 % in the cLBP and healthy, pain-free, control groups respectively. In the double tDCS protocol, the TMS intensity used to elicit MEPs of 1 mV peak-to-peak was 57 ± 14 %, and 56 ± 15 % in the cLBP and healthy, pain-free, control groups respectively. There was no

difference in the baseline TMS intensity between groups ($F_{1,73}$=0.0, P=0.8) or protocols ($F_{1,73}$=3.8, P=0.1).

3.4.2 Single tDCS protocol

MEP amplitude increased in both groups following the single tDCS protocol (Figure 3.2A; Table 3.2). There was a main effect of time ($F_{4,292}$=6.7, P<0.001), but no main effect of group ($F_{1,292}$=0.0, P=0.9) and no interaction effect ($F_{4,292}$=1.4, P=0.2). Compared with baseline, MEP amplitudes increased at all time points in both groups following 7-min of anodal tDCS (post-hoc all: t>3.6, P<0.003; Figure 3.3A). Sixty-eight per cent of individuals with cLBP, and 76% of healthy, pain-free individuals exhibited an excitatory response (increased MEP amplitude relative to baseline) following the single tDCS protocol (Figure 3.4A).

3.4.3 Double tDCS protocol

In the double tDCS protocol, the size of the MEP amplitude over time was dependent on the presence or absence of cLBP (Figure 3.2B; Table 3.2). The ANOVA demonstrated no main effect of time ($F_{4,292}$=2.4, P=0.1) but a main effect of group ($F_{1,292}$=37.9, P<0.001) and an interaction effect between group and time ($F_{4,292}$=7.4, P<0.001). Compared with baseline, healthy, pain-free, controls displayed a reduction in MEP amplitude at all time-points following the double tDCS protocol (post-hoc vs. baseline; 0 min: t=3.4, P=0.01; 10 min: t=4.4, P<0.001; 20 min: t=4.3, P<0.001; 30 min: t=3.4, P=0.01; Figure 3.3B). In

contrast, individuals with cLBP demonstrated no change in MEP amplitude over time (post-hoc vs baseline; 0 min: t=1.1, *P*=0.9; 10 min: t=1.5, *P*=0.7; 20 min: t=1.8, *P*=0.5; 30 min: t=2.5, *P*=0.1; Figure 3.3B). When individuals with and without cLBP were compared at each time-point, there was no difference in the MEP amplitude at baseline (post hoc: t=0.5; *P*=0.7). However, MEP amplitudes were higher in the cLBP group when compared to healthy, pain-free, controls at all other time-points (0-min, 10-min, 20-min, and 30-min; post hoc all: t>4.3, *P*<0.001; Figure 3.3B).

Thirty-two per cent of individuals with cLBP and 72 % of healthy, pain-free individuals displayed a normal homeostatic plasticity response (decreased MEP amplitude relative to baseline) following the double tDCS protocol (Figure 3.4B).

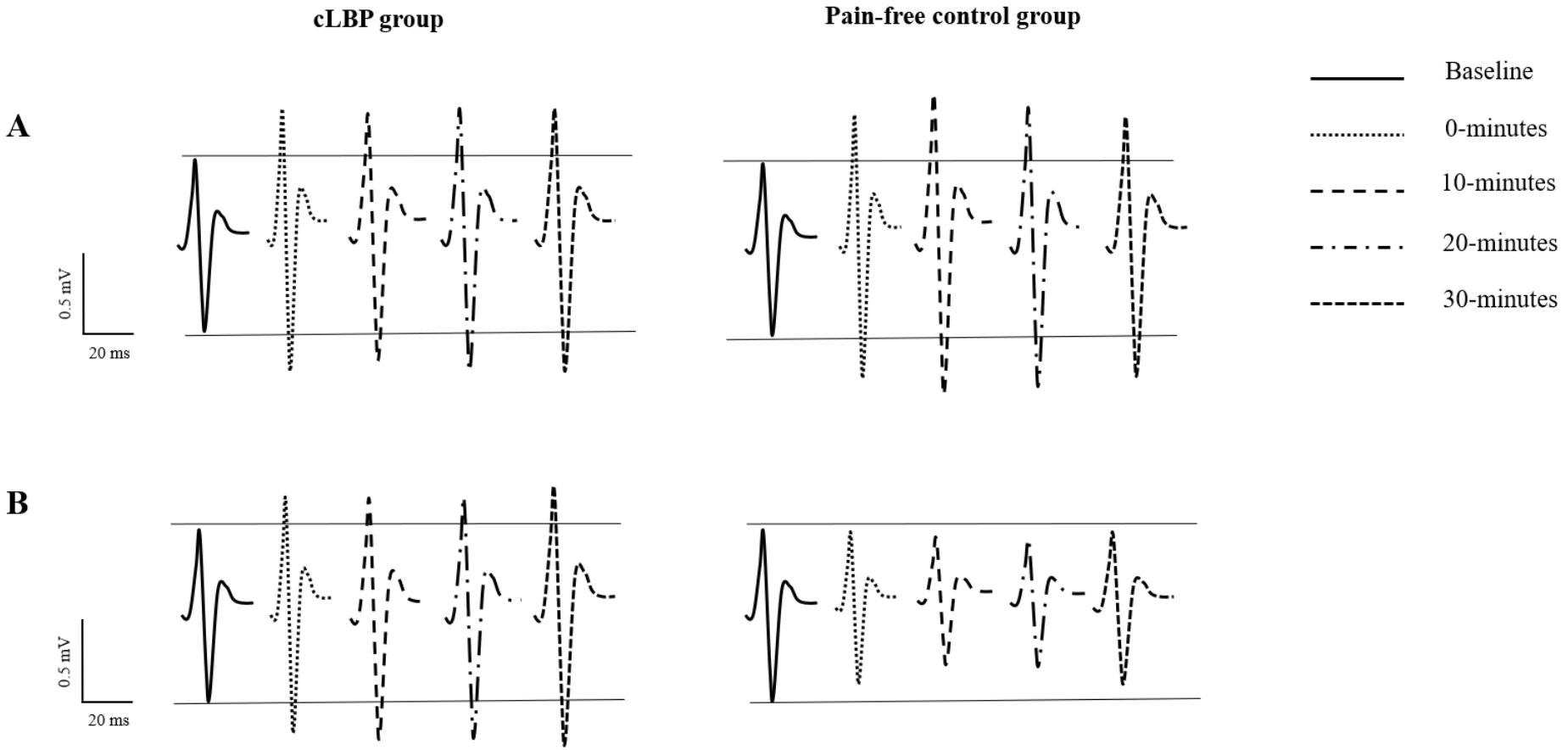


Figure 3.2. *Grand average raw MEP traces obtained at each time-point from participants in the cLBP and the healthy, pain-free, control group in response to the single (**A**), and double (**B**) tDCS protocols.*

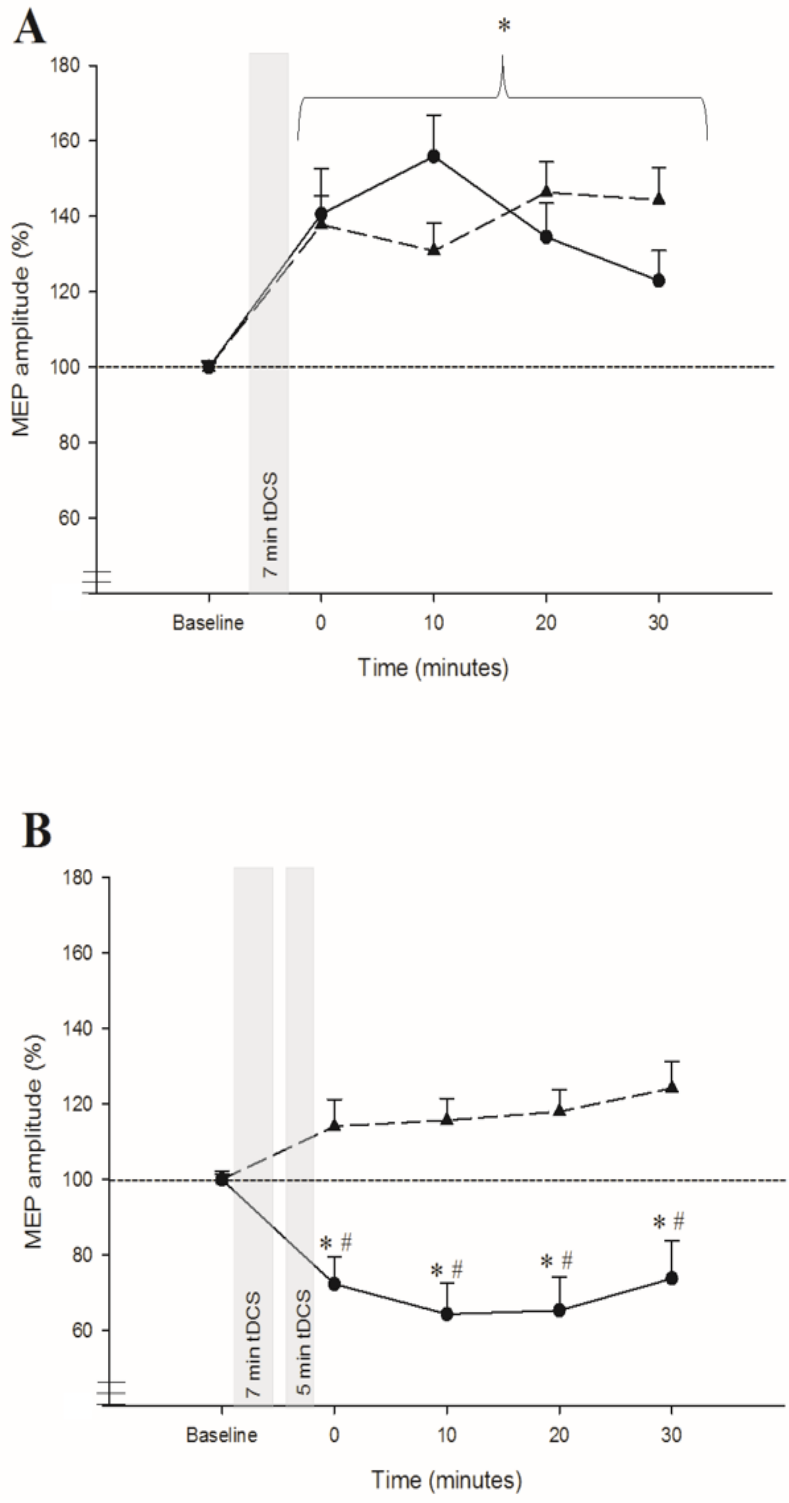


Figure 3.3. *Mean (+ SEM) motor evoked potential (MEP) amplitudes normalised to the baseline MEP amplitude (100%) in the cLBP (n=50, closed triangles), and healthy, pain-free, control (n=25, closed circles) group in response to the single* ***(A)****, and double* ***(B)*** *tDCS*

*protocols. MEP amplitudes increased in both groups following the single tDCS protocol (main effect of time: P<0.003). In the double tDCS protocol, the MEP amplitude was reduced at all time-points compared to baseline only in the healthy, pain-free, control group (*P all<0.01 relative to baseline). The cLBP group demonstrated no change in MEP amplitude compared to baseline (#P all<0.001 relative to cLBP group at the same time-point).*

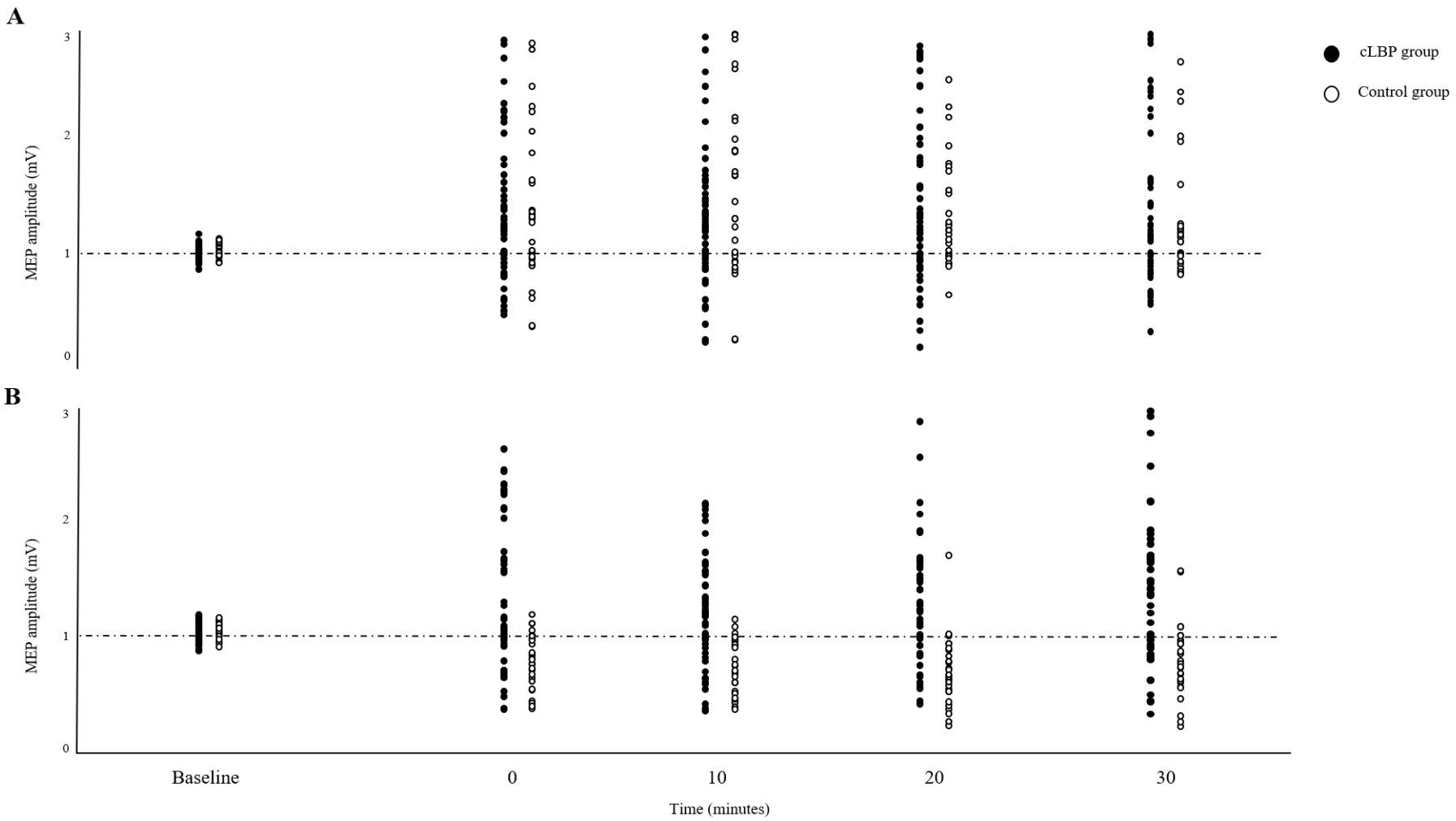


Figure 3.4. *Motor evoked potential (MEP) amplitude for each individual in the chronic low back pain (cLBP, closed circles) and healthy, pain-free, control (open circles) group at each time-point in response to the single (A) and double (B) transcranial direct current stimulation (tDCS) protocols. Each circle represents the average MEP of 30 recordings at each time-point.*

Table 3.2. Group data (mean ± standard deviation) for motor evoked potential amplitude (mV) recorded at each time-point (baseline, 0-min, 10-min, 20-min, and 30 min follow-up) in the chronic low back pain, and healthy, pain-free, control groups in response to the single and double transcranial direct current stimulation (tDCS) protocols.

		Baseline	0 min	10 min	20 min	30 min
cLBP	**Single tDCS**	1.0 ± 0.1	1.4 ± 0.7*	1.3 ± 0.7*	1.4 ± 0.8*	1.4 ± 0.9*
	Double tDCS	1.0 ± 0.1	1.2 ± 0.6	1.2 ± 0.5	1.2 ± 0.5	1.3 ± 0.6
Controls	**Single tDCS**	1.0 ± 0.1	1.4 ± 0.9*	1.6 ± 0.9*	1.4 ± 0.6*	1.3 ± 0.5*
	Double tDCS	1.0 ± 0.1	0.7 ± 0.3$^{*\#}$	0.6 ± 0.3$^{*\#}$	0.6 ± 0.3$^{*\#}$	0.7 ± 0.4$^{*\#}$

cLBP: chronic low back pain. Significant difference relative to baseline ($^{*}P<0.01$) or the cLBP group at the same time-point ($^{\#}P<0.001$).

3.4.4 Relationship between the intensity and duration of cLBP and impairment in homeostatic plasticity

Neither NRS scores of pain intensity (all time-points: r<0.2, n=50, P>0.1) nor pain duration (all time-points: r<0.1, n=50, P>0.5) were correlated with the change in MEP amplitude in individuals with cLBP after the double tDCS protocol.

3.5 Discussion

This study is the first to investigate homeostatic plasticity in musculoskeletal pain. Although individuals with and without cLBP displayed typical increases in corticomotor excitability in response to the single tDCS protocol, only healthy, pain-free, controls demonstrated a reversal of excitation towards inhibition following the double tDCS protocol consistent with normal homeostatic control. These novel data suggest a disruption of homeostatic plasticity in the primary motor cortex of individuals with cLBP that is present regardless of pain intensity or pain duration, and is not restricted to the representation of painful muscles. This mechanism could explain observations of maladaptive synaptic plasticity in cLBP, and could provide a pathophysiological mechanism to explain pain persistence in this condition.

Homeostatic plasticity is an essential form of plasticity in the human brain that ensures neuronal activity is maintained within a stable physiological range (Murakami et al., 2012; Ziemann & Siebner, 2008). Originally described by Bienenstock, Cooper and Munro, homeostatic plasticity prevents uncontrolled increases or decreases in synaptic efficacy by linking the effectiveness of LTP and LTD processes to the level of activity in the postsynaptic neuron (Bienenstock et al., 1982). When activity is high, LTP processes are less effective, favouring LTD and synaptic weakening. Similarly, when postsynaptic activity is low, LTD processes are less effective, favouring LTP and synaptic

strengthening. It has previously been argued that changes in the amplitude of the MEP to transcranial magnetic stimulation after successive blocks of excitatory non-invasive brain stimulation reflect changes in the efficacy of synaptic relays within the corticomotor pathway (Siebner & Rothwell, 2003), and that reversal of the direction of these changes (towards inhibition) can be used to assess homeostatic plasticity (Fricke et al., 2011; Quartarone et al., 2005).

Using this model, the present data suggest individuals with cLBP have impaired homeostatic plasticity that manifests as a failure to regulate increases in corticomotor excitability since excitability is not reversed towards inhibition when postsynaptic activity is high. Failure to regulate synaptic plasticity in individuals with cLBP could lead to a disproportionately high rate of synaptic strengthening that in turn, produces abnormally high cortical excitability and maladaptive reorganisation of brain regions. Indeed, studies have shown increased cortical excitability (Kregel et al., 2015; Wand et al., 2011; Zhuo, 2008), reduced GABAergic inhibition (Janetzki et al., 2016; Schliessbach et al., 2017), and enlarged representations of the back muscles that are posteriorly shifted and show greater overlap in cLBP (Schabrun et al., 2015b; Tsao et al., 2011b; Tsao et al., 2008) when compared with those of healthy controls. These cortical changes are associated with pain severity, impaired postural control, and reduced coordination of trunk muscles (Janetzki et al., 2016; Tsao et al., 2011b; Tsao et al., 2008). In addition, the threshold where mechanical

pressure turns to pain is lower in people with cLBP than controls (Giesbrecht & Battié, 2005; Giesecke et al., 2004; Imamura et al., 2013; Kobayashi et al., 2009), and people with cLBP exhibit reduced nociceptive withdrawal reflex thresholds (Biurrun Manresa et al., 2013), enlarged reflex receptive fields (Biurrun Manresa et al., 2013; Neziri et al., 2011), facilitated temporal summation (Biurrun Manresa et al., 2013), and increased S1 excitability (Flor et al., 1997; Kong et al., 2013). Together, these findings indicate an increase in spinal and cortical excitability in cLBP that could be explained by excessive synaptic strengthening as a result of impaired homeostatic control.

Although no studies have examined homeostatic plasticity in musculoskeletal pain, evidence from neurological populations supports this hypothesis. For example, writer's cramp is characterised by increased M1 excitability (Abbruzzese et al., 2001; Quartarone et al., 2003), reduced GABAergic inhibition (Gallea et al., 2017; Hallett, 2011; Siebner et al., 1999), and enlarged and overlapped M1 representations of the hand muscles (Schabrun et al., 2009b) – cortical changes that are similar to those reported in cLBP. Several studies have shown impaired homeostatic plasticity in writer's cramp that is hypothesised to underpin the increased excitability and enlarged cortical representations observed in this condition (Kang et al., 2011; Quartarone et al., 2008; Quartarone & Pisani, 2011; Quartarone et al., 2005). Specifically, the failure of homeostatic plasticity to prevent the positive feedback cycle of synaptic plasticity is believed to produce unchecked increases

in synaptic strength that consolidate maladaptive cortical reorganisation as well as the pathological sensorimotor interactions and movement patterns that manifest in writer's cramp (Quartarone & Pisani, 2011). Notably, excessive synaptic strengthening in writer's cramp is not restricted to the circuits clinically affected by dystonia, but is generalised throughout the sensorimotor system (Antal et al., 2008b; Quartarone et al., 2008) – a finding consistent with the current observations in cLBP. This finding is also consistent with previous reports of a generalised alteration in cortical excitability that extends beyond the cortical representation of painful muscles in chronic pain conditions (termed 'pain-motor integration'), including cLBP (Flor et al., 1997; Juottonen et al., 2002; Schwenkreis et al., 2003; Tsao et al., 2011b; Tsao et al., 2008). As such, it is plausible that impaired homeostatic plasticity may also influence pain-motor integration in cLBP, driving increased cortical excitability, representational shifts, reinforcement of unwanted movement patterns (poor postural control, and coordination), and unpleasant sensory experiences.

Accordingly, should future studies confirm the relevance of impaired homeostatic plasticity to cLBP, therapies that seek to target neuroplasticity in persistent pain may need to target homeostatic, rather than synaptic, plasticity mechanisms. For example, previous studies have shown that patterned peripheral electrical stimulation applied to reduce cortical excitability (induce synaptic weakening; LTD) in writer's cramp where

homeostatic plasticity is known to be impaired, improves cortical organisation and reduces symptoms (Schabrun et al., 2009a). Similar treatments, designed specifically to counter excessive synaptic strengthening, could also be effective in cLBP. Further, an impaired ability to control increases in cortical excitability in cLBP may suggest that commonly used treatments known to promote synaptic strengthening (e.g. motor retraining, exercise) may require reconsideration in this population to avoid reinforcing aberrant synaptic plasticity, and inducing detrimental effects on symptoms. Further work is required to test these hypotheses.

Examination of individual level data revealed a portion of healthy individuals (38%) who displayed impaired homeostatic plasticity, and a portion of cLBP individuals (32%) whose homeostatic control was normal. Although the reasons for this are unclear it is possible that impaired homeostatic plasticity in healthy individuals could predispose to the development of chronic pain in future. However, longitudinal exploration of this mechanism within the same individual is needed to understand the relevance of individual variability in both the healthy and diseased brain. Notably, there was no association between the magnitude of impaired homeostatic plasticity and pain intensity or pain duration. However, all participants were experiencing LBP that had persisted for a substantial period of time (average duration of 12.7 ± 14.4 years). It is possible that homeostatic impairment develops in the sub-acute or early phases of cLBP and thus, was

already present, regardless of small differences in symptom status in individuals tested in this study. Investigation of homeostatic plasticity in other brain areas, and during the acute, sub-acute, and early phases of cLBP is warranted.

This study has several limitations. As the first exploration of this mechanism in cLBP we utilised a cross-sectional design. It is therefore not possible to determine whether homeostatic plasticity is impaired as a cause or a consequence of cLBP or to investigate the relationship between impaired homeostatic plasticity and fluctuations in pain over the clinical course of cLBP. Future studies should utilise longitudinal study designs with multiple measures of homeostatic plasticity and pain to provide comprehensive examination of this mechanism and the symptoms of cLBP. In the present study, the experimenter was not blinded to participant group and future studies should ensure blinding to reduce the risk of bias. In addition, our sample presented with a long history of cLBP. Different changes in homeostatic plasticity could be present in the acute or sub-acute phase of low back pain. Further studies are required to disentangle the relationship between different phases of cLBP and homeostatic plasticity mechanisms. Finally, this study did not include evaluation of spinal nociception (either through nociceptive withdrawal reflexes or laser evoked potentials). Previous studies have demonstrated spinal hyperexcitability in people with cLBP (Biurrun Manresa et al., 2013; Neziri et al., 2011). As transcranial magnetic stimulation provides an indication of excitability throughout the

corticomotor pathway it is possible that changes in spinal excitability in people with cLBP may have contributed to the current findings. It is unclear whether changes in spinal excitability may drive altered homeostatic plasticity through the upregulation of afferent input to the cortex or whether impaired homeostatic plasticity drives an increase in spinal excitability. Future studies should seek to clarify the interaction between spinal mechanisms and homeostatic plasticity in people with and without cLBP.

3.6 Conclusion

This study is the first to explore homeostatic plasticity in musculoskeletal pain conditions. These unique data suggest a disruption of synaptic homeostasis in individuals with cLBP that manifests as an inability to counter excessive increases in corticomotor excitability. Further research is required to determine whether impaired homeostatic plasticity drives maladaptive synaptic plasticity and pain persistence in cLBP.

Chapter 4: Aberrant synaptic plasticity in musculoskeletal pain: a failure of homeostatic control

Chapter three suggests homeostatic plasticity is impaired in individuals with chronic musculoskeletal pain. However, as findings from chapter three are limited to a cross-sectional study design, it is not possible to determine whether homeostatic plasticity is impaired as a cause or consequence of musculoskeletal pain. Therefore, chapter four aimed to address this limitation by assessing homeostatic plasticity during the development, persistence, and resolution of experimentally induced sustained muscle pain over 14 days. The content of this chapter is currently under review in the Journal of Pain.

4.1 Abstract

Aberrant synaptic plasticity is widely hypothesised to underpin chronic pain. Yet, synaptic plasticity is controlled and regulated by homeostatic mechanisms that have received limited attention in pain. Here, we aimed to investigate homeostatic plasticity in the human primary motor cortex (M1) in response to the progressive development, maintenance and resolution of pain over 14 days. Nerve-growth factor was injected into the right elbow extensor musculature of 21 healthy individuals on days 0, 2, and 4 to induce progressively developing, sustained muscle pain. Pain and disability were monitored until day 21. Homeostatic plasticity was induced in left M1 using two blocks of anodal transcranial direct current stimulation (tDCS) applied for 7-min and 5-min, and separated by a 3-min rest period. Motor-evoked potentials (MEP) assessed the homeostatic response on days 0, 2, 4, 6, and 14. On days 0 and 14, MEP amplitude was increased following the first block of tDCS (P<0.004), and decreased following the second block of tDCS (P<0.001), consistent with a normal homeostatic response. However, on days 2 (P=0.07) and 4 (P=0.7), the decrease in MEP amplitude after the second block of tDCS was attenuated, representing an impaired homeostatic response. These data provide new insight into the maladaptive plasticity hypothesis in pain. Our findings demonstrate a disturbance in homeostatic plasticity after 2 days of pain that could explain aberrant synaptic plasticity, and contribute to the pathogenesis of chronic musculoskeletal pain conditions.

4.2 Introduction

Synaptic plasticity plays a key role in neural adaptation, and is fundamental to memory, learning, and recovery after injury or illness (Joseph, 2013; Martin et al., 2000; Nudo, 2013; Ziemann & Siebner, 2008). A number of functional and structural mechanisms underpin synaptic plasticity in the human brain, including the dynamic expression of long-term potentiation (LTP) and long-term depression (LTD)-like changes in synaptic efficacy (Hebb, 1949; Joseph, 2013; Martin et al., 2000). Numerous studies postulate that aberrant synaptic plasticity contributes to the development of chronic pain (Apkarian, 2011; Apkarian et al., 2009; Apkarian et al., 2011; Baliki et al., 2011; Flor, 2003, 2008; Kuner & Flor, 2017; May, 2008; Morton et al., 2016). However, in addition to plasticity mechanisms that promote neural 'changeability', the human brain is governed by plasticity mechanisms that promote stability (Turrigiano, 2012; Turrigiano, 1999, 2006; Turrigiano & Nelson, 2000). These 'homeostatic' mechanisms prevent overexpression of LTP and LTD based on the principle of a 'sliding synaptic threshold', such that high post-synaptic activity elicits a compensatory response that biases the synaptic threshold towards LTD, and low post-synaptic activity biases the synaptic threshold towards LTP (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). Thus, homeostatic mechanisms are responsible for the control and regulation of synaptic plasticity and a disturbance in this mechanism could plausibly drive the aberrant synaptic plasticity observed in musculoskeletal pain conditions.

Despite the importance of homeostatic mechanisms to healthy brain function, there has been limited investigation of this mechanism in pain. However, studies in several chronic pain states including migraine (Antal et al., 2008b; Brighina et al., 2005; Brighina et al., 2002; Cosentino et al., 2014b), and low back pain suggest global disturbance in homeostatic control. This is observed as a reduction in the normal LTD-like response to experimental protocols shown to induce extended LTP-like effects in healthy individuals (i.e. two successive blocks of anodal transcranial direct current stimulation [tDCS] or high frequency repetitive transcranial magnetic stimulation [rTMS]). Impaired homeostatic modulation is hypothesised to contribute to abnormally high cortical excitability, aberrant cortical reorganisation, increased pain perception, and sensorimotor dysfunction in these chronic pain conditions (Brighina et al., 2005; Brighina et al., 2002; Cosentino et al., 2014b; Thapa et al., 2018a). While it is reasonable to assume that sustained pain may contribute to the global disruption of homeostatic control, there has been no longitudinal investigation of homeostatic plasticity in pain, in particular, in cortical areas corresponding to the affected muscle. How and when changes in homeostatic control develop in the transition to sustained pain or how they relate to the symptoms of pain is unknown. This information is essential to enhance our understanding of homeostatic plasticity in humans and to understand the impact of sustained pain on this fundamental neural mechanism.

Using a clinically-relevant, human pain model to induce progressively developing, sustained muscle pain, homeostatic plasticity was investigated in M1 using two successive blocks of anodal tDCS as pain developed, peaked, and resolved, over the course of 14 days. We hypothesised that several days of sustained pain would alter homeostatic plasticity (reduce the normal LTD-like response observed following two blocks of anodal tDCS in pain-free, healthy individuals (Fricke et al., 2011)) in the human M1.

4.3 Methods and materials

4.3.1 Participants

Twenty-one, right-handed, healthy individuals (mean ± standard deviation age: 23 ± 4 years, 12 males) participated. Handedness was assessed using the Edinburgh handedness questionnaire (Oldfield, 1971), and a transcranial magnetic stimulation (TMS) safety screening questionnaire was completed prior to study commencement (Keel et al., 2001). Individuals with a history of neurological, psychiatric, musculoskeletal or upper limb conditions were excluded. All participants received written and verbal description of experimental procedures and provided written informed consent consistent with the Declaration of Helsinki. Experimental procedures were approved by the local human research ethics committee (H10184).

4.3.2 Experimental design

Homeostatic plasticity was induced in M1 using two blocks of anodal tDCS applied for 7-min and 5-min respectively, separated by a 3-min rest period (Fricke et al., 2011; Thapa & Schabrun, 2018). Corticomotor excitability was monitored by recording 15 motor evoked potentials (MEPs) to single pulse transcranial magnetic stimulation (TMS) i) before tDCS, ii) between the two tDCS blocks (used to evaluate the *plasticity* response to a single block of anodal tDCS), and iii) 10-min after the last tDCS block (used to evaluate the *homeostatic* response; Figure 4.1A). We have previously shown that the homeostatic response induced and assessed using this tDCS protocol has moderate-to-good between session reliability in healthy individuals at intervals of 2, 7, and 14 days (ICC=0.67, 95% CI 0.12 to 0.91) with the greatest intra-session reliability observed at 10-min following the tDCS protocol (ICC > 0.61, 95% CI 0.03 to 0.89). Progressively developing, sustained muscle pain was induced by repeated injection of nerve growth factor (NGF) into the belly of the extensor carpi radials brevis muscle (m. ECRB) at the end of the experimental session on days 0, 2, and 4. The number of injections used was determined from previous studies where the duration of NGF induced sustained muscle pain was dependent upon the number of injections used (Bergin et al., 2015; Hayashi et al., 2013; Lewin et al., 2014; Petty et al., 1994; Schabrun et al., 2016). Homeostatic responses were assessed on days 0, 2, 4, 6, and 14. Homeostatic responses recorded on day 0 reflected normal homeostatic regulation in the absence of pain and served as a baseline comparison, while homeostatic plasticity assessed on days 2, 4, 6,

and 14 reflected homeostatic regulation during pain development and resolution. Pain severity, disability, and sleep quality were assessed online (via SurveyMonkey) every second day from day 1 to day 21 (Figure 4.1B). Following consent, participants were sent emails or text messages as reminders to fill in the questionnaires.

4.3.3 Induction of M1 homeostatic responses

Homeostatic responses were elicited in M1 using two blocks of anodal tDCS. This protocol has been used previously to investigate M1 homeostatic responses in both healthy and clinical populations (Fricke et al., 2011; Thapa et al., 2018a; Thapa & Schabrun, 2018). A battery driven, ramp controlled, constant current stimulator (DC-Stimulator Plus, NeuroConn, Ilmenau, Germany) was used to deliver anodal tDCS at an intensity of 1 mA. Current was ramped up and down over 10 seconds at the start and end of stimulation (Nitsche et al., 2008). Rubber electrodes, placed in sodium-chloride soaked sponges (5 x 7 cm), were positioned over the left M1 hot-spot corresponding to the right m. ECRB (anode; see below for hot-spot determination), and over the contralateral supraorbital region (cathode). Two adjustable rubber straps were used to fix the electrodes to the head.

4.3.4 Monitoring of corticomotor excitability

Single-pulse transcranial magnetic stimulation (TMS) was used to monitor corticomotor excitability in response to the first and second block of anodal tDCS. Transcranial magnetic

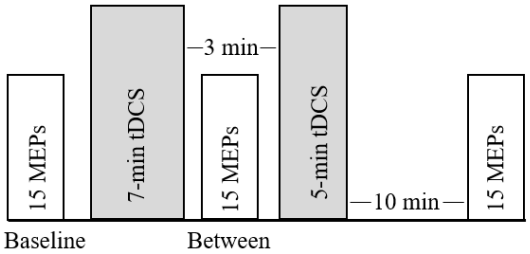
stimulation (TMS) was performed using a Magstim 200 stimulator (Magstim Co., Ltd., Dyfed, UK) with a monophasic current waveform. A 70mm figure-of-eight coil was positioned over the left hemisphere at a 45º angle from the sagittal plane. The optimal coil position was determined by locating the site at which the maximum muscle response from the relaxed right m. ECRB was evoked (termed the 'hot-spot'). A soft-tip pen was used to mark the hot-spot on the scalp for TMS coil and tDCS electrode re-positioning within and between sessions. On days not attending the laboratory for testing, participants were requested to precisely re-mark their hotspot using a mirror and a soft-tipped pen or if required, with assistance from a second person. Surface electromyography (EMG) was recorded from the right m. ECRB using disposable, surface electrodes (Ag-AgCl, Noraxon dual electrodes, inter-electrode distance 2.0 cm). The ground electrode was placed over the ipsilateral acromion. EMG signals were amplified (1000), bandpass filtered 20-1000 Hz and sampled at 2000 Hz (CED 1401 AD, Cambridge Electronic Design, Cambridge, United Kingdom) using Signal software (CED, version 5.08 x 86). All signals were stored on a computer for offline analysis. TMS intensity was adjusted at baseline on each day of testing to produce an average MEP of ~0.5 mV peak-to-peak amplitude in 15 trials (Burns et al., 2016; Cosentino et al., 2014b; Schabrun et al., 2016). A further 15 trials were recorded at the baseline TMS intensity between the two tDCS blocks to evaluate the response to the first tDCS protocol (*plasticity* response), and at 10-min follow-up to evaluate the response to the second tDCS protocol (*homeostatic* response).

4.3.5 Induction and assessment of sustained muscle pain

After cleaning the skin with alcohol, a dose of 5 μg (0.2 ml) sterile, recombinant human nerve growth factor (NGF; Lonza Australia Pty Ltd) was given as a bolus injection into the muscle belly of right m. ECRB on days 0, 2, and 4 using a 0.5-ml syringe with a disposable needle (31G). The injection site was located 5 cm distal, and 1 cm lateral from the lateral epicondyle along a line from the lateral epicondyle to the midline of the wrist (Bergin et al., 2015).

An online diary consisting of an 11-point numerical pain rating scale (NRS) anchored with ‘no pain’ at zero and ‘worst pain possible’ at 10, a modified 7-point Likert muscle soreness scale anchored with ‘a complete absence of soreness’ at zero and ‘severe muscle soreness, stiffness or weakness that limits the ability to move’ at 6 (Hayashi et al., 2013; Schabrun et al., 2016), the patient-rated tennis elbow evaluation questionnaire (PRTEEQ), and an 11-point numerical sleep rating scale anchored with ‘extremely poor sleep (shallow, unrefreshing)’ at zero and ‘excellent sleep (deep, refreshing)’ at 10, were administered every second day from day 1 to day 21 (Macdermid, 2005).

A



B

	Day																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
On-line diary		X		X		X		X		X		X		X		X		X		X		X
Assessment and induction of plasticity	X		X		X		X								X							
NGF injection	X		X		X																	

Figure 4.1. *Transcranial direct current stimulation (tDCS) protocol: two blocks of anodal tDCS (the first of 7-min duration and the second of 5-min duration, separated by a 3-min rest period) were applied to the primary motor cortex (M1) contralateral to the right (painful) extensor carpi radialis brevis (ECRB) muscle. Fifteen motor evoked potentials (elicited using transcranial magnetic stimulation) were recorded at baseline, during the 3-min rest period, and 10-min after the last block of tDCS.* ***B)*** *Every alternate day, from day 1 to day 21, participants completed an online diary consisting of an 11-point numerical pain rating scale, a modified 7 point Likert muscle soreness scale, the patient rated-tennis elbow evaluation questionnaire (PRTEEQ), and an 11-point numerical sleep rating scale. M1 plasticity was assessed and induced at the beginning of each experimental session on days 0, 2, 4, 6, and 14. Nerve growth factor (NGF) was injected into the belly of the right ECRB on days 0, 2, and 4.*

4.3.6 Data analyses

There was no missing data. For all analyses, SPSS software for windows, version 22 was used. A one way-repeated measures analysis of variance (ANOVA) was performed to examine differences in the i) TMS intensity used to elicit MEPs of 0.5 mV, and ii) the amplitude of the mean MEP, recorded at baseline with factor *day* (0, 2, 4, 6, and 14). To examine the change in the MEP amplitude in response to tDCS, a two-way repeated measures ANOVA was performed on raw data with factor *day* (0, 2, 4, 6, and 14), and *time* (baseline, between, 10-min). As the magnitude of the *homeostatic* response is likely to be influenced by the amount of facilitation achieved following the first block of anodal tDCS, data reflecting the *plasticity* (time-point 'between'), and *homeostatic* (time-point '10-min') responses were also analysed as ratio values (plasticity-ratio=$MEP_{between}/MEP_{baseline}$, homeostatic-ratio=$MEP_{10\text{-}min}/MEP_{between}$, respectively). Ratio data were analysed using a one-way repeated measures ANOVA with factor *day*.

A one-way repeated measures ANOVA was used to explore changes in pain severity, muscle soreness, sleep quality, and disability with factor *day* (1, 3, 5….21). Shapiro-Wilk tests were used to assess normality. Data that violated normality were log transformed. If normality was violated after transformation, a Friedman repeated measures ANOVA on ranks was conducted. The Greenhouse-Geisser method was used to correct for non-sphericity. Post-hoc tests were performed using either the Wilcoxon signed-rank test or

Bonferroni t-tests adjusted for multiple comparisons. Significance was set at $P<0.05$.

4.4 Results

4.4.1 TMS intensity and MEP amplitude at baseline

There was no difference in the TMS intensity required to elicit average MEPs of 0.5 mV peak-to-peak, or in the amplitude of the mean MEP recorded at baseline between days (TMS intensity: $\chi^2(4)=6.2$, $P=0.2$; MEPs at baseline: $F_{4,80}=2.1$, $P=0.09$; Table 4.1).

Table 4.1. Mean ± standard deviation (N=21) for i) transcranial magnetic stimulation (TMS) intensity (percent of maximum stimulator output, MSO) required to evoke a motor evoked potential (MEP) of 0.5 mV peak-to-peak amplitude at baseline and ii) MEP amplitude recorded at baseline (prior to tDCS), on each day.

Cortical measures	**Day 0**	**Day 2**	**Day 4**	**Day 6**	**Day 14**
TMS (% MSO)	41 ± 6	41 ± 6	43 ± 9	42 ± 7	42 ± 6
MEP (mV)	0.48 ± 0.1	0.46 ± 0.1	0.49 ± 0.1	0.50 ± 0.1	0.55 ± 0.1

4.4.2 NGF induced pain, muscle soreness, disability, and sleep quality

Pain ($\chi^2(11)=152.5$, $P<0.001$; Figure 4.2A) and muscle soreness ($\chi^2(11)=171$, $P<0.001$; Figure 4.2B) increased at day 1 (pain: z=-3.5, $P<0.001$, soreness: z=-3.8, $P<0.001$) and remained elevated from day 5 to day 15 (pain: all z>-2.9, $P<0.004$, soreness: z>-3.4, $P<0.001$) compared with day 0. Similarly, disability ($\chi^2(11)=163.7$, $P<0.001$; Figure 4.2C) was increased at day 1 (z=-3.7, $P<0.001$) and remained elevated from day 5 to day 15 (overall: z>-2.9, $P<0.003$) compared with day 0. There was no change in sleep quality across days ($F_{11,220}=1.9$, $P=0.1$; Figure 4.2D).

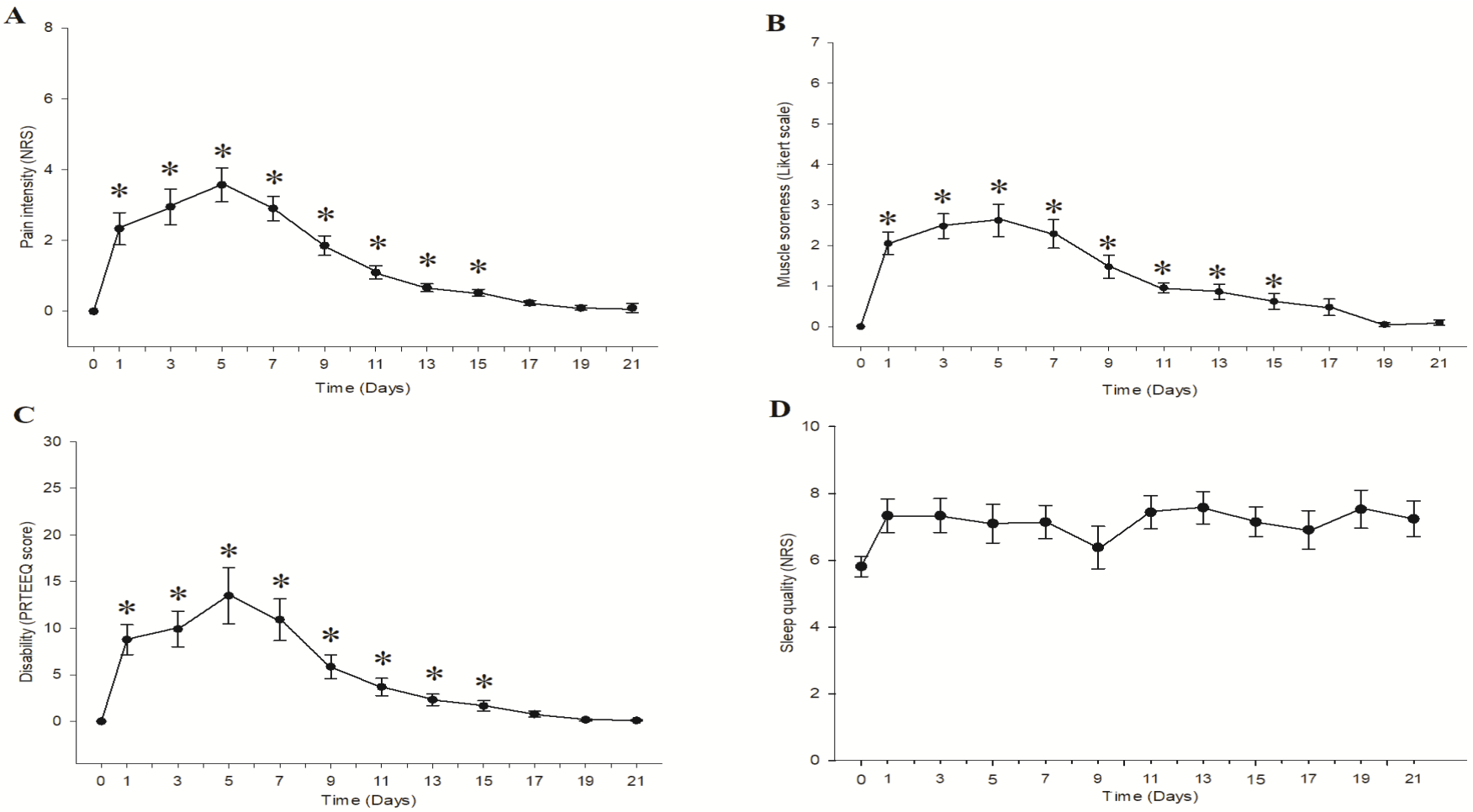


Figure 4.2. *Mean ± standard error (N=21) for (**A**) pain intensity (numerical rating scale scores), (**B**) muscle soreness (Likert scale scores), (**C**) disability (Patient Rated Tennis Elbow Evaluation Questionnaire score), and (**D**) sleep quality (numerical rating scale scores). Pain intensity, muscle soreness, and disability increased at day 1 and remained elevated at day 15 compared with day 0 (*P<0.004).*

4.4.3 The homeostatic response in M1 is altered after 2 days of sustained muscle pain

The progressive development of sustained muscle pain altered the MEP response to single and double tDCS (raw data; time x day interaction: $F_{8,160}$=3.5, P<0.001; Figure 4.3). A single block of anodal tDCS increased MEP amplitudes on days 0, 2, 4, and 14 (post-hoc baseline vs. between; P<0.004; *plasticity response-raw data*; Figure 4.3). The MEP amplitude was not significantly increased following single tDCS on day 6 (post-hoc baseline vs. between: P=0.09; *plasticity response-raw data*). However, examination of the ratio data for the *plasticity response* revealed that the increase in MEP amplitude was not significantly different across days ($F_{4,80}$=1.7, P=0.1).

Following the second block of anodal tDCS, MEP amplitudes were reduced, consistent with a normal *homeostatic* response, on days 0, and 14 (raw data; post hoc between vs. 10 min; P<0.001). However, there was no significant reduction in MEP amplitudes on days 2, 4, and 6 (raw data; post hoc between vs. 10 min; day 2: P=0.07, day 4: P=0.7, day 6 P=0.5; Figure 4.3). These findings were supported by analysis of the ratio data for the *homeostatic* response which was different across days ($F_{4,80}$=4.0, P=0.005). Compared with days 0 and 14, the ratio was smaller on day 2 (P=0.027), and day 4 (P=0.022) with a similar tendency observed on day 6 (P=0.076). On day 4, the MEP amplitude was *increased* following the second block of anodal tDCS (Figure 4.3). The temporal profile

for the development of pain relative to changes in *homeostatic* response is presented in Figure 4.4.

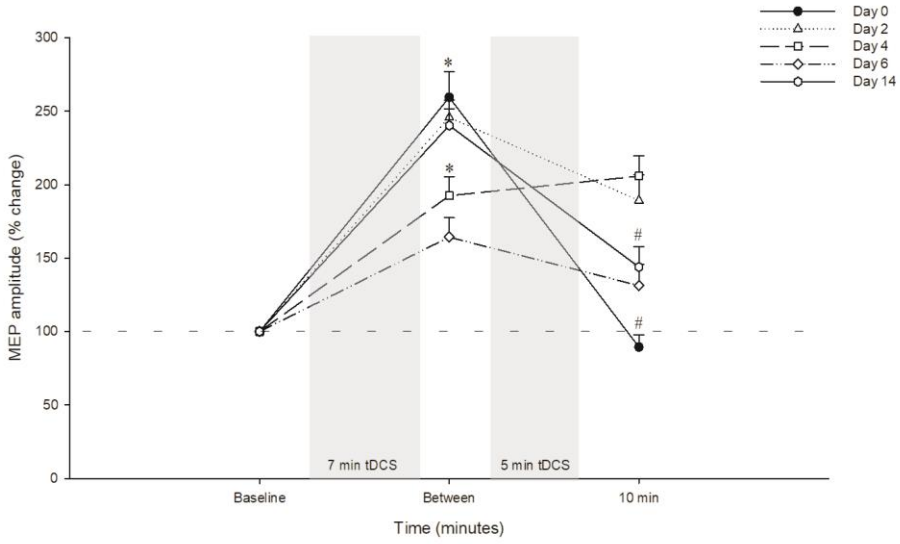


Figure 4.3. *Mean + standard error (N=21) for motor evoked potential amplitude normalised to baseline after the first ('between'), and second block of tDCS ('10-min)' on days 0, 2, 4, 6, and 14. *Significant increase in MEP amplitude following the first block of tDCS (P<0.004) or #significant decrease in MEP amplitude following the second block of tDCS (P<0.001).*

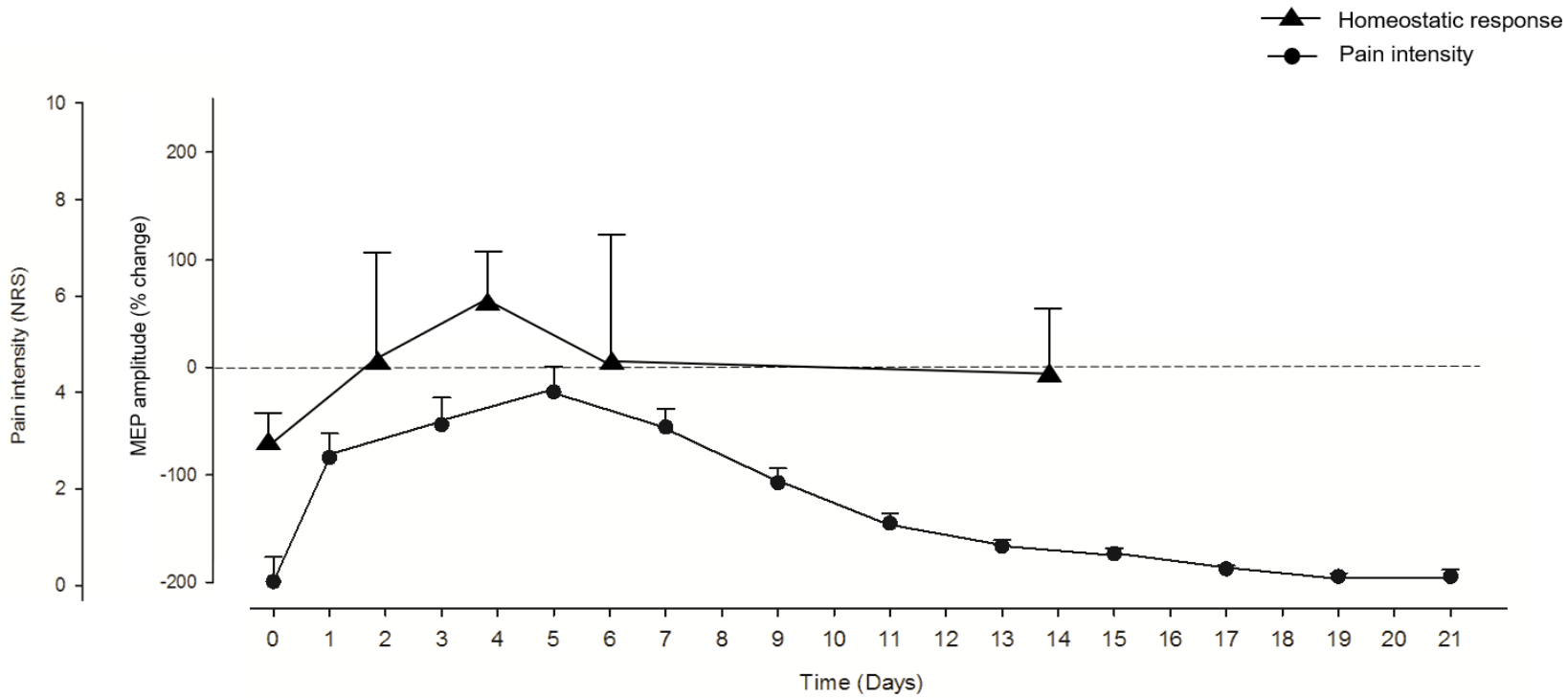


Figure 4.4. *Mean (+ SEM, N=21) pain scores (closed circles) and the homeostatic response (percent change of the MEP amplitude after the second block of tDCS relative to the MEP amplitude immediately after the first block of tDCS; closed triangles) demonstrating the temporal profile of the change in homeostatic regulation (values < 0 % represent a normal homeostatic response) relative to the development of NGF-induced sustained muscle pain.*

4.5 Discussion

This study is the first longitudinal investigation of homeostatic plasticity in the transition to sustained musculoskeletal pain. We demonstrate that M1 homeostatic plasticity is altered in response to the development and resolution of sustained muscle pain. Specifically, the homeostatic response was disrupted after two days of progressively developing muscle pain, with the greatest impairment observed at day 4. These unique findings have relevance for our understanding of the maladaptive plasticity hypothesis in pain which has focussed almost exclusively on synaptic plasticity mechanisms. Altered homeostatic control could plausibly explain the aberrant synaptic plasticity reported in chronic pain and may contribute to the pathogenesis of this condition, providing new avenues for understanding and treatment.

4.5.1 Homeostatic plasticity

Early studies investigating homeostatic plasticity occurred in slice preparations and in animal models (Bear, 2003; Bienenstock et al., 1982; Turrigiano, 2007; Turrigiano, 1999; Turrigiano & Nelson, 2004). However, with the advent of non-invasive brain stimulation, a growing body of work has examined this mechanism in the intact human cortex (for review see (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015)). The induction and assessment of homeostatic plasticity is typically achieved using non-invasive brain stimulation to ‘prime’ the response to a subsequent period of stimulation. In the primary

motor cortex, LTP and LTD-like effects can be indexed using TMS. For example, a single block of anodal tDCS can induce an increase in the motor evoked potential amplitude to TMS and this response is thought to reflect the engagement of LTP-like processes (Fricke et al., 2011). However, when preceded at short interval by a second block of anodal tDCS, this effect is reversed, and a reduction in motor evoked potential amplitude (LTD-like effect) is observed (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). These effects are interpreted to reflect homeostatic plasticity such that a period of high LTP formation (excitation) causes the synaptic threshold to favour the induction of LTD. Although a range of non-invasive brain stimulation techniques have been used to examine homeostatic plasticity in the human M1 (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015; Ziemann & Siebner, 2008), only one study has examined the reliability of the homeostatic response over time (Thapa & Schabrun, 2018). That study demonstrated that two blocks of anodal tDCS can reliably induce M1 homeostatic plasticity at intervals of 48 hours, 7 days, and 2 weeks in healthy individuals, supporting the use of this measure in the present study.

4.5.2 Homeostatic plasticity and pain

A wide range of neuronal inputs are known to result in the induction of LTP- and LTD-like synaptic plasticity (Classen et al., 2004; Stefan et al., 2000). Despite this, the impact of sustained periods of pain on homeostatic mechanisms that regulate synaptic plasticity is

unknown. Preliminary studies in animal models of central pain syndrome (Wang & Thompson, 2008) and neuropathic pain (Xiong et al., 2017) suggest a link between pain-induced hyperalgesia and altered homeostatic plasticity. In humans, studies of pain and homeostatic plasticity are restricted to patient populations with migraine (Antal et al., 2008b; Brighina et al., 2011; Brighina et al., 2005; Cosentino et al., 2014b) and chronic low back pain (Thapa et al., 2018a). These studies report altered M1 homeostatic plasticity that is hypothesised to contribute to excessive cortical excitability, enlarged cortical representations, and symptoms in these conditions. Notably, cyclic impairments in homeostatic control are associated with the initiation, continuation, and termination of pain in migraine patients (Antal et al., 2008b; Cosentino et al., 2014a; Cosentino et al., 2014b). However, where in the transition from acute to chronic pain altered homeostatic plasticity develops has not been investigated.

The current study is the first to examine whether sustained pain impacts the M1 homeostatic response. Repeated intra-muscular injection of NGF sensitises muscle nociceptors and dorsal horn neurons (Hoheisel et al., 2007) resulting in pain and dysfunction that mimic symptoms of chronic musculoskeletal pain conditions (Andersen et al., 2008). For example, injection of NGF induces comparable pain, hyperalgesia, and functional limitation to patients with chronic lateral elbow pain of ~26 weeks duration (Bergin et al., 2015). Using this model, the present data provide the first evidence that

several days of sustained pain is sufficient to alter the M1 homeostatic response. Consistent with studies in chronic migraine, the temporal profile of the altered homeostatic response mimicked the trajectory of pain development. Specifically, two days of sustained muscle pain altered the M1 homeostatic response, with the greatest disturbance in homeostatic control observed around the time of greatest pain severity. As pain resolved, so too did the alteration in homeostatic plasticity, returning to normal at day 14 (see Figure 4.4).

The functional relevance of altered homeostatic plasticity in response to several days of sustained muscle pain requires further investigation. One possibility is that altered homeostatic plasticity in the early stages of pain represents an adaptive response that prevents memory encoding of pain-driven synaptic patterns of activity. Evidence from human and animal studies suggest that high levels of LTP, as would be expected if homeostatic mechanisms fail to bias synaptic thresholds toward LTD, impairs subsequent learning (Kang et al., 2011; Rioult-Pedotti et al., 2000). For example, the learning of a motor skill in humans results in high LTP formation that has been shown to interfere with the learning of subsequent motor skills (Shadmehr & Brashers-Krug, 1997). Similarly, spatial learning is impaired following high levels of hippocampal LTP in animals (Moser et al., 1998). Some support for this hypothesis can be drawn from studies that report impaired motor learning in people with acute (Sterling et al., 2001), and chronic pain (Boudreau et al., 2010; Kang et al., 2011). Alternatively, it is tempting to speculate that

altered homeostatic plasticity represents an impairment that if maintained over weeks to months (i.e. when pain does not resolve as expected) allows consolidation of maladaptive patterns of synaptic plasticity that underpin sensorimotor symptoms and dysfunction in clinical conditions. Indeed, studies in writer's cramp suggest that prolonged periods of afferent input in the absence of effective homeostatic control lead to excessive synaptic strengthening that consolidates unwanted movement patterns (Kang et al., 2011; Quartarone & Pisani, 2011). In the context of chronic musculoskeletal pain, movement dysfunction has been hypothesised to contribute to chronicity of symptoms by altering the load on surrounding tissues, presumably resulting in a prolonged alteration of afferent input (Hodges, 2011; Hodges & Tucker, 2011).

In the present study, homeostatic plasticity was examined using an excitatory priming protocol only. This approach was selected as previous studies in chronic pain have reported impaired M1 homeostatic plasticity characterised by a failure to reduce the MEP amplitude (slide the threshold towards LTD-like effects) following two blocks of excitatory anodal tDCS (Antal et al., 2008b; Kang et al., 2011). Further research is required to understand the impact of sustained pain on homeostatic mechanisms induced using inhibitory priming protocols. In addition, the administration of the double tDCS protocol to elicit a homeostatic response and the implementation of TMS to assess this response was conducted by the same assessor from a previous test retest reliability study including these

techniques but involving the hand muscle (Thapa & Schabrun, 2018). Future studies will need to establish intra- and inter-session reliability of the operator with assistance from individualised T1 scans and Brainsight to extend current findings whilst taking into account the capacity for plasticity when targeting painful and non-painful muscle groups (Chen et al., 1998). Further, while the implementation of the double tDCS protocol is in line with previous studies, the use of 5 x 7 cm^2 electrodes may have resulted in current spread to brain areas close to the M1. Use of high definition tDCS in future studies can help localise tDCS effects and homeostatic regulation to specific brain regions. Lastly, the impact of sustained pain on homeostatic plasticity in other brain regions known to play a key role in pain perception (i.e. primary somatosensory cortex, dorsolateral prefrontal cortex) or within intracortical facilitatory and inhibitory networks was not investigated in the current study. Further work is needed to comprehensively disentangle the influence of sustained pain on homeostatic plasticity in humans.

4.6 Conclusion

This study provides unique insight into the influence of progressively developing, sustained muscle pain on homeostatic plasticity in the human M1. Impaired homeostatic plasticity developed in parallel with the pain trajectory – manifesting after two days of sustained pain and returning toward baseline as pain resolved at day 14. Altered homeostatic control could plausibly explain the aberrant synaptic plasticity reported in

chronic pain and may contribute to the pathogenesis of this condition, providing new insight into the maladaptive plasticity hypothesis in chronic pain.

Chapter 5: General discussion

The primary aim of the thesis was to explore the effect of pain, using an experimental pain model and a clinical chronic musculoskeletal pain population, on homeostatic plasticity in the human primary motor cortex. This chapter provides a discussion of findings from all three studies, with novel insight into homeostatic plasticity in pain and directions for future research. Clinical implications, and limitations are also discussed.

5.1. Contribution of the thesis to the body of evidence

This thesis provides novel and original data on homeostatic plasticity, and the influence of pain on homeostatic regulation, in the human primary motor cortex (M1). Although mechanisms that underpin neural change (i.e. synaptic plasticity) have been well characterised in healthy and clinical populations (Bear & Malenka, 1994; Citri & Malenka, 2008), homeostatic mechanisms that underpin neural stability have received limited attention (Karabanov et al., 2015). In particular, how pain influences homeostatic plasticity in the motor cortex, and whether disruption of homeostatic regulation contributes to the clinical and neurophysiological manifestations of clinical pain syndromes is unknown. Understanding both sides of the neuroplasticity 'coin' (i.e., change and stability) is essential to advance our knowledge of the healthy human brain and of disease mechanisms underpinning a range of pathological conditions.

Non-invasive brain stimulation (NIBS) techniques have been used to induce and assess homeostatic plasticity in the human brain (Karabanov et al., 2015; Ziemann et al., 2008). Here, NIBS techniques are applied using a priming-test paradigm to elicit a homeostatic response. For example, successive application of anodal tDCS has been demonstrated to elicit a homeostatic response in the human M1, such that LTP-like plasticity (increased MEP amplitude) induced by a single block of anodal tDCS is reversed toward inhibition (decreased MEP amplitude) when preceded by an additional block of anodal tDCS (Fricke

et al., 2011). Similarly, homeostatic plasticity has been observed as enhanced LTP-like plasticity following excitatory rTMS when primed by cathodal tDCS (Lang et al., 2004). Despite the use of these protocols to investigate homeostatic plasticity in the healthy human brain in 26 previous studies, the reliability of these techniques has not been established. To provide a foundation for the use of these techniques in longitudinal study designs and to advance an essential metric of homeostatic measurement in the human M1, study one of this thesis investigated the reliability of the homeostatic response induced by anodal tDCS in the M1 of healthy humans at intervals of 48 hours, 7 days, and 2 weeks. This study demonstrated moderate-to-good test-retest reliability for the induction (using anodal tDCS) and assessment (using single-pulse transcranial magnetic stimulation) of homeostatic plasticity in the human M1 over a two-week period.

Although a number of studies have explored homeostatic plasticity in the healthy human brain, studies exploring homeostatic plasticity in individuals with pathology are restricted to seven studies in neurological patients with writer's cramp, and chronic migraine (Karabanov et al., 2015). The paucity of research notwithstanding, these studies suggest impaired homeostatic plasticity is associated with high M1 excitability, altered cortical reorganisation, increased pain perception, and sensorimotor dysfunction (Antal et al., 2008b; Cosentino et al., 2014b; Kang et al., 2011; Quartarone et al., 2005). As similar clinical and neurophysiological manifestations are reported in individuals with chronic

musculoskeletal pain (Apkarian, 2011; Apkarian et al., 2009; Baliki et al., 2011; Elgueta-Cancino et al., 2017; Schabrun et al., 2016; Schabrun et al., 2017b; Tsao et al., 2011b; Tsao et al., 2008; Tsao et al., 2011c), it is plausible that homeostatic plasticity may also be impaired in these individuals. To explore this hypothesis, study two compared homeostatic regulation in the M1 of people with non-specific chronic low back pain (cLBP) and age- and sex-matched healthy, pain-free, individuals. This study demonstrated impaired homeostatic plasticity in the M1 of individuals with non-specific cLBP.

The findings of study two provided unique insight into homeostatic plasticity in people with non-specific cLBP. However, these data raise a critical question: where in the transition from acute to chronic pain does impaired homeostatic plasticity develop? As previous studies on homeostatic plasticity in pathological populations have been cross-sectional in nature, no longitudinal data exist to inform this question. With this knowledge gap in mind, study three examined homeostatic plasticity in the M1 as muscle pain, induced using an experimental pain model, developed, peaked, and resolved over 14 days. This study provided the first evidence of altered M1 homeostatic plasticity, that developed in response to several days of sustained muscle pain and returned toward pre-pain values as pain resolved.

In summary, each of these studies provides an original contribution to the available body of evidence on homeostatic plasticity in the human M1, and in particular, homeostatic regulation in the presence of musculoskeletal pain. The following section provides a discussion of the major findings arising from the studies presented in this thesis.

5.2. The induction and assessment of homeostatic plasticity in humans

The basic mechanisms underpinning homeostatic plasticity were initially identified *in vitro* (Bienenstock et al., 1982; Cooper et al., 1979; Turrigiano, 2012; Turrigiano, 1999, 2008). In slice preparations, homeostatic plasticity was investigated using high and low frequency tetanic stimulation applied in a priming-test paradigm (Abbott & Nelson, 2000; Abraham, 2008; Abraham & Bear, 1996; Turrigiano, 2004; Turrigiano, 1999; Turrigiano & Nelson, 2000, 2004). Here, induction of long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy was dependent on the activation history of a neural circuit, such that LTP or LTD was induced in the direction opposite to that observed following priming stimulation applied alone.

Based on the principles of homeostatic plasticity identified in slice preparations, non-invasive brain stimulation (NIBS) paradigms have been developed to allow the investigation of homeostatic plasticity in humans (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). Techniques such as transcranial magnetic stimulation (TMS),

transcranial direct current stimulation (tDCS), and paired associative stimulation (PAS) have been used to induce and assess homeostatic plasticity in the human brain (Bliem et al., 2008; Fricke et al., 2011; Lang et al., 2004; Siebner et al., 2004). Excitatory or inhibitory forms of TMS, tDCS, and PAS are applied in priming-test paradigms to elicit homeostatic plasticity characterised as LTP -or -LTD-like changes opposite to those observed with priming stimulation applied alone.

As TMS, tDCS, and PAS have been shown to be appropriate techniques to investigate homeostatic plasticity in humans, 26 studies (see Table 1.2, and 1.3 in chapter one) have used these techniques to induce and assess homeostatic plasticity in the human brain (Karabanov et al., 2015). Although the majority of these studies have demonstrated a homeostatic response following their chosen priming-test paradigm, only tDCS has been systematically explored to determine the appropriate stimulation duration and interval between the priming-and -test tDCS protocols to induce homeostatic plasticity in the human M1 (Fricke et al., 2011). Importantly, there has been no investigation of reliability using any form of NIBS in the context of homeostatic plasticity (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). This is essential if longitudinal studies are to be carried out that comprehensively investigate homeostatic plasticity in humans.

Study one addressed the lack of reliability studies in the field of homeostatic plasticity. Using two blocks of anodal tDCS to induce a homeostatic response in M1, study one demonstrated moderate-to-good test-retest reliability in 10 healthy individuals across intervals of 48 hours, 7 days, and 2 weeks (Thapa & Schabrun, 2018). These findings are the first to establish the reliability of the homeostatic response over two weeks, and provide a foundation for the longitudinal investigation of homeostatic plasticity in the human M1. However, as homeostatic plasticity was investigated only in one direction (i.e. response to excitatory stimulation), further investigation using inhibitory forms of tDCS is needed to extend these findings. In addition, future studies should seek to investigate the reliability of homeostatic plasticity elicited using other forms of NIBS techniques (i.e., rTMS, and PAS).

Notably, moderate-to-good test-retest reliability in study one indicates homeostatic plasticity elicited using the double tDCS protocol has a certain degree of variability. Indeed, all three studies in this thesis demonstrated some level of inter-individual variability in homeostatic plasticity following the double tDCS protocol (Figure 5.1A and B). For instance, approximately 90 % of participants (n = 10) demonstrated a normal homeostatic response following the double tDCS protocol in study one (Figure 5.2), while approximately 72 % (n = 25) of the total sample demonstrated homeostatic regulation following the double tDCS protocol in study two (Figure 5.3). Similar findings were

observed in study three, where 76 % of all participants (n= 21) responded to the double tDCS protocol (Figure 5.3). Together, these data demonstrate variability in homeostatic plasticity elicited using the double tDCS protocol.

The inter-individual variability in homeostatic response following the double tDCS protocol may be explained by the low number of responders and non-responders previously reported in tDCS studies (Datta et al., 2012; Horvath et al., 2015; Li et al., 2015). On average, studies report less than 64 % of the total sample respond to either anodal or cathodal tDCS applied alone (Chew et al., 2015; Horvath et al., 2015; Li et al., 2015; Lopez-Alonso et al., 2015). This indicates response to tDCS applied alone is considerably low, and such reports may in-turn explain variability in homeostatic regulation following the double tDCS protocol in chapter two, three, and four. Further research exploring the implications of inter-individual variability in homeostatic regulation is warranted in healthy and clinical populations.

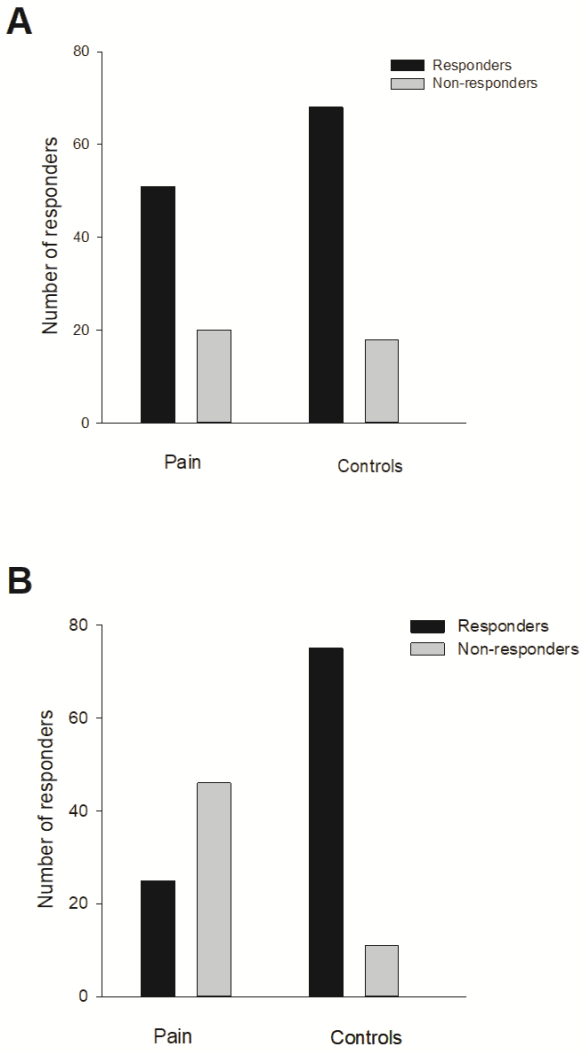


Figure 5.1. *Responders and non-responders to the first (A) and second (B) block of anodal tDCS recorded at time-points 'between' and '10-min' follow-up, respectively. The 'pain' group represents data collected from individuals with non-specific chronic low back pain in study two, and data obtained on the day of highest pain intensity (Day 4) in study three. The 'control' group represents data obtained from healthy, pain-free, individuals in study one, study two, and study three on Day 0. Responders to the first block of anodal tDCS were defined as individuals who demonstrated an increase in MEP amplitude, while responders to the second block of anodal tDCS were defined as individuals who demonstrated a decrease in MEP amplitude (homeostatic response). Individuals who contradicted the definition of responders to the first and second block anodal tDCS were classified as non-responders.*

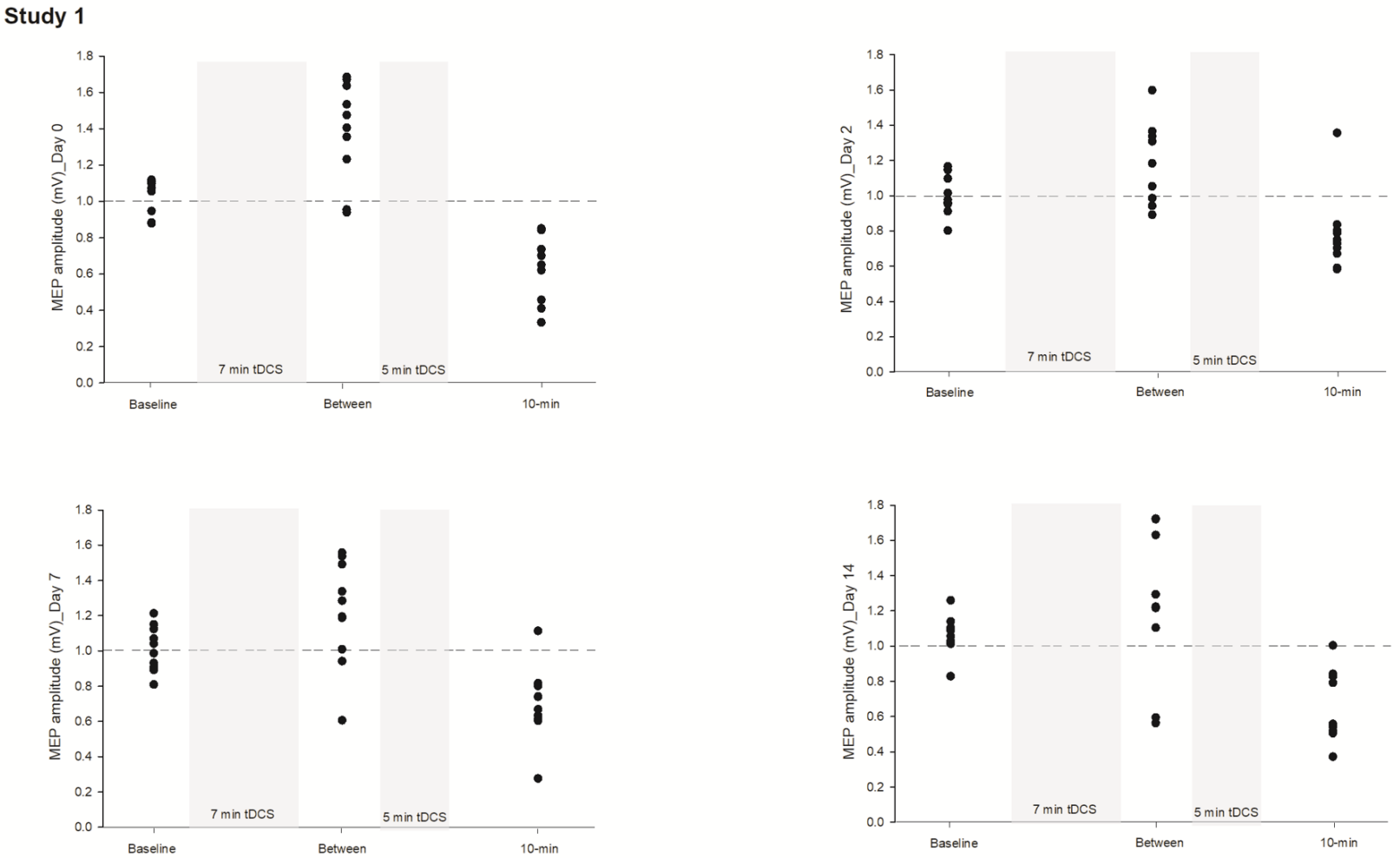


Figure 5.2. *Variability in synaptic and homeostatic plasticity response following the first ('between') and second block ('10-min') of anodal tDCS, respectively. Presented data were obtained from healthy individuals on days 0, 2, 7, and 14 in study one.*

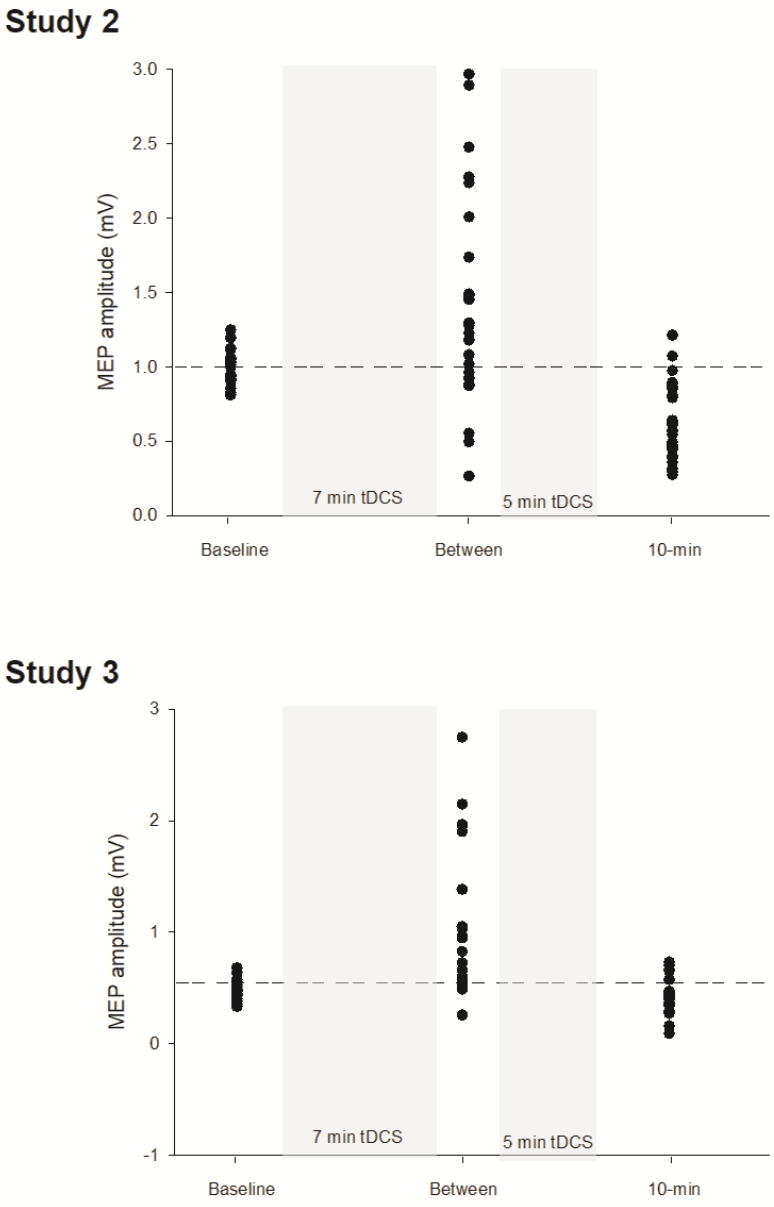


Figure 5.3. *Variability in synaptic and homeostatic plasticity response following the first ('between') and second block ('10-min') of anodal tDCS in study two (pain-free healthy controls only) and study three (day zero only). The time-point 'between' in study two represents the 0-min time-point following the single block of 7-min tDCS.*

5.3. Homeostatic regulation and musculoskeletal pain

Traditionally, musculoskeletal pain was thought to fit a structural-pathology model where pain was correlated linearly with tissue integrity, and vice-versa (Chen, 2011; Coderre et al., 1993; Melzack & Wall, 1965; Moseley, 2007; Perl, 2007). An essential feature of this model was the idea that as tissue healed, pain would subside. In the case of chronic musculoskeletal pain, where credible explanation for pain perception was lacking (i.e. when there was no evidence of structural pathology or tissue damage), or when pain had persisted beyond accepted tissue healing times, the condition was classified as psychiatric (Clarke & Iphofen, 2005; Glenton, 2003; Newton et al., 2013; Richardson, 2005; Werner & Malterud, 2003). However, over the last few decades a dramatic increase in our understanding of synaptic plasticity within the central nervous system, and its relationship to pain perception, has challenged the traditional view of chronic musculoskeletal pain as a psychiatric condition (Chen, 2011; Melzack & Wall, 1965; Moseley, 2007; Perl, 2007).

It is now well accepted that pain does not always equate to or involve tissue damage (Moseley, 2003; Moseley, 2007; Moseley & Flor, 2012). The clearest example of dissociation between pain and tissue damage can be observed in individuals with chronic osteoarthritis (OA) (Bedson & Croft, 2008; Finan et al., 2013; Lluch et al., 2014). Chronic OA is a well-established peripheral musculoskeletal condition characterised by progressive cartilage loss, ligament derangement, muscular impairments, and synovial inflammation

(Braun & Gold, 2012; Glyn-Jones et al., 2015; Hunter, 2011; Suri et al., 2012). However, despite clear peripheral changes, studies report a weak relationship between pain intensity and radiographic abnormalities (Bedson & Croft, 2008; Finan et al., 2013; Neogi et al., 2015). For example, chronic knee OA patients with less pronounced radiographic abnormalities report higher levels of pain intensity when compared to those with moderate to severe radiographic abnormalities (Finan et al., 2013). Similarly, individuals with cLBP report high levels of pain and disability despite radiographic abnormalities akin to those of healthy, asymptomatic persons (Chou et al., 2009; Chou et al., 2011; Jarvik et al., 2001; Jensen et al., 1994; van Tulder et al., 1997). These findings suggest that pain is not always associated with tissue pathology. Maladaptive changes to synaptic plasticity within the central nervous system have instead been suggested to explain this incongruence between pain and tissue pathology (Finan et al., 2013; Lluch et al., 2014; Masse-Alarie & Schneider, 2016; Neogi et al., 2015).

There has been some investigation of maladaptive synaptic plasticity within the central nervous system in individuals with musculoskeletal pain (Baliki et al., 2011; Cauda et al., 2014; Chang et al., 2017; Flor et al., 1997; Lloyd et al., 2008; Strutton et al., 2003; Strutton et al., 2005; Tsao et al., 2011b; Tsao et al., 2010; Tsao et al., 2008). These studies suggest maladaptive changes to synaptic plasticity characterised by cortical reorganisation, and altered brain morphometry are present in subjects with musculoskeletal pain conditions.

For example, a 2.5 cm shift in the somatotopic representation of back muscles in the primary somatosensory cortex (S1) is interpreted to indicate maladaptive synaptic plasticity within the central nervous system in individuals with low back pain (Flor et al., 1997; Lloyd et al., 2008). These findings are extended by decreased S1, brainstem, and prefrontal gray matter volume shown to correlate with symptom chronicity in cLBP (Apkarian et al., 2004; Schmidt-Wilcke et al., 2006). Similar findings have been reported in individuals with carpel tunnel syndrome, where loss in spatially discrete representations of digits two and three in the contralateral S1 (Maeda et al., 2013; Napadow et al., 2006; Tinazzi et al., 1998) and decreased S1 gray matter density (Maeda et al., 2013) was shown to correlate with reduced median nerve conduction velocity. Somatotopic reorganisation in the S1 and reduced median nerve conduction velocity have been shown to have a role in pain perception in individuals with carpel tunnel syndrome (Maeda et al., 2013; Tecchio et al., 2002). Together, these findings support the maladaptive synaptic plasticity hypothesis in musculoskeletal pain, and suggest maladaptive changes to synaptic plasticity within the central nervous system may contribute to pain perception, and symptom chronicity in individuals with musculoskeletal pain conditions. Despite this, the mechanisms that underpin maladaptive synaptic plasticity and the link to the development and persistence of musculoskeletal pain are unknown.

Synaptic plasticity provides a mechanism for learning and change in the human brain through the bidirectional modulation of synaptic strength, such that a synapse can undergo both strengthening (long-term potentiation; LTP) and weakening (long-term depression; LTD) (Joseph, 2013). However, synaptic plasticity relies on a positive feedback loop that left unrestrained, would lead to excessive synaptic strengthening or weakening and unstable neural function. In the healthy brain, homeostatic plasticity ensures synaptic activity is maintained within a functional range by shifting the threshold for LTP and LTD based on activity in the postsynaptic neuron (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). For example, high ongoing postsynaptic activity will lead to reduced thresholds for LTD, and increased thresholds for LTP, while low ongoing postsynaptic activity will lead to reduced thresholds of LTP, and increased thresholds for LTD (Bienenstock et al., 1982). In this way, homeostatic plasticity mechanisms are argued to prevent excessive synaptic strengthening and weakening in the human brain when exposed to extended periods of high LTP or LTD activity.

Given that synaptic plasticity must be carefully controlled, a disturbance in homeostatic plasticity could plausibly play a role in maladaptive changes to synaptic plasticity observed in individuals with musculoskeletal pain. Indeed, the role of homeostatic plasticity during musculoskeletal pain is supported by observations of impaired homeostatic regulation in writer's cramp and chronic migraine, with authors hypothesising that this impairment

could contribute to observations of altered synaptic plasticity and clinical symptoms. For example, when exposed to high levels of post-synaptic activity (indexed as an increase in MEP amplitude to TMS) induced using a tDCS or rTMS priming-test paradigm, individuals with writer's cramp and chronic migraine did not display the reduction in excitability (indexed as a decrease in MEP amplitude to TMS) typically observed in healthy individuals (Antal et al., 2008b; Cosentino et al., 2014b; Kang et al., 2011; Quartarone et al., 2005). These observations were later correlated with symptom severity, suggesting an association between homeostatic impairment and uncontrolled muscle activity in individuals with writer's cramp (Quartarone & Pisani, 2011; Quartarone et al., 2005), and headache recurrence in chronic migraineurs (Brighina et al., 2011; Brighina et al., 2005; Brighina et al., 2010; Cosentino et al., 2014b).

While impaired homeostatic plasticity in writer's cramp and chronic migraine support the potential relevance of homeostatic plasticity to musculoskeletal pain and to contemporary theories of pain persistence that centre on maladaptive synaptic plasticity (Moseley & Flor, 2012), these findings are not without limitations. A major limitation is that impaired homeostatic plasticity has been observed only in two studies in individuals with writer's cramp and in seven studies in chronic migraineurs, with sample sizes ranging from eight to 14 participants in each study. Further, the absence of a sample size calculation in any previous study could indicate Type I error, where homeostatic impairment observed in the

study population may not truly exist in the wider patient population (Cohen, 1969; Gogtay, 2010; Hackshaw, 2008; Kadam & Bhalerao, 2010; Sedgwick, 2014). To address these limitations, studies included in this thesis explored homeostatic regulation during musculoskeletal pain in samples informed by appropriate sample size calculations. These calculations included conservative effect size estimates of ≤ 0.4 to quantify the size of the homeostatic response in healthy individuals and those with musculoskeletal pain (Ialongo, 2016; Nakagawa & Cuthill, 2007; Zakzanis, 2001), an alpha of 0.05 to avoid false positives (Devane et al., 2004; Gogtay, 2010; Karlsson et al., 2003), and a power of 0.8 to determine a homeostatic response when one truly exists (Cohen, 1992; Jones et al., 2003; Lieber, 1990).

Data arising from this thesis show that homeostatic plasticity was impaired in 50 individuals suffering from non-specific cLBP (mean ± standard deviation for musculoskeletal pain duration: 12 ± 14 years) when compared with 25 age-and -sex matched healthy, pain-free, controls (Thapa et al., 2018b). This is characterised by a failure to reduce excessive increases in M1 excitability (indexed using MEP amplitudes) in individuals with non-specific cLBP following the double tDCS protocol. Study three extends these findings using an identical double tDCS protocol that Study 1 showed to be reliable over 48 hours, 7 days, and 2 weeks (Thapa & Schabrun, 2018). Here, homeostatic plasticity was impaired in the very early stage of sustained pain (within 2 days of pain

onset). One interpretation of these data is that impaired homeostatic plasticity arises early in the transition to sustained pain, and in a proportion of people does not resolve, contributing to the development of chronic musculoskeletal pain. This interpretation is supported by impaired homeostatic regulation in chronic migraineurs, where failure to regulate high levels of LTP-like plasticity is hypothesised to lead to a build-up of cortical excitability, facilitating recurrence of migraine attacks (Cosentino et al., 2014b). Therefore, it is possible that failure to resolve homeostatic impairment during the early stage of musculoskeletal pain may lead to increased levels of LTP-like plasticity that facilitate symptom recurrence, and consequently symptom persistence. Further studies are needed to test these hypotheses.

Alternatively, change in homeostatic regulation during the early stages of pain onset could imply an adaptive process where homeostatic regulation accommodates to pain onset to prevent pain-driven changes to synaptic plasticity. This hypothesis is in line with studies that have demonstrated high levels of M1 excitability prevent subsequent motor learning (Amadi et al., 2015; Jung & Ziemann, 2009; Ziemann et al., 2004). For example, repeated fast thumb abduction that increases M1 excitability was shown to occlude the effects of a succeeding excitatory PAS protocol to the M1 (Elahi et al., 2014; Rosenkranz et al., 2007). Similarly, increase in M1 excitability following task performance was occluded when preceded by anodal tDCS (Amadi et al., 2015). Together, these studies suggest homeostatic

plasticity fluctuates in response to afferent input, and altered homeostatic regulation during pain onset may represent an adaptive process that aims to prevent pain-driven changes to synaptic activity.

A critical observation in Study 2 was that, impairment in homeostatic plasticity was not restricted to the cortical representations of the muscles clinically affected by low back pain but was observed in the cortical representations of the 'unaffected' hand muscles. However, in study three, impaired homeostatic plasticity was observed in the cortical representation of the painful muscle. Although not directly measured, these observations could suggest that impaired homeostatic plasticity is global when pain is chronic, but localised when pain is acute. Consistent with this hypothesis, previous literature in chronic musculoskeletal pain has shown widespread changes to brain areas outside the region of pain (Apkarian, 2011; Apkarian et al., 2009; Apkarian et al., 2011; May, 2008), while acute pain has been shown to involve brain regions corresponding only to the painful muscle (Schabrun et al., 2015a; Schabrun et al., 2013). In addition, previous studies in pathological populations that share clinical symptoms of pain and sensorimotor dysfunction demonstrate impaired homeostatic plasticity in cortical areas associated with and without pain (Antal et al., 2008b; Brighina et al., 2011; Brighina et al., 2005; Quartarone et al., 2008). Together, these data suggest widespread and local changes to brain areas following chronic or acute pain may explain the global and local impairment in homeostatic

regulation observed in individuals with non-specific cLBP in study two, and in individuals experiencing experimentally induced sustained muscle pain in study three. Future studies should seek to directly compare homeostatic plasticity during the acute and chronic stage of pain in cortical areas corresponding to the affected and unaffected muscle using crossover trials.

Notably, pain intensity was not correlated with the magnitude of homeostatic impairment in the M1 of individuals with non-specific cLBP. One explanation is that pain intensity is a subjective measure that depends on a number of factors including threat value, and previous experience (Ablin & Buskila, 2015; Finnern et al., 2018; Garg et al., 2012; Gorczyca et al., 2013; Keefe et al., 2004; Okifuji & Ackerlind, 2007; Wang et al., 2009). Therefore, it is perhaps not surprising that objective physiological measures of homeostatic plasticity did not correlate with subjective measures of pain intensity in study two. More holistic measures of pain intensity such as the McGill Pain Questionnaire may provide a better understanding of the relationship between homeostatic regulation and pain in future (Hawker et al., 2011; Melzack, 2005; Waldman, 2009).

Alternatively, there is increasing evidence that the relationship between pain and tissue damage becomes less clear when pain is chronic (Bedson & Croft, 2008; Boersma & Linton, 2005; Finan et al., 2013; Finnern et al., 2018; Lluch et al., 2014). This is explained

by numerous factors such as treatment and medication history (Ernstzen et al., 2017; Garg et al., 2012; Hunter, 2001), modifiable and non-modifiable lifestyle factors (Docking et al., 2011; Elliott et al., 1999; van Hecke et al., 2013), pain perception (Crofford, 2015; Jensen, 2010; Wijma et al., 2016), and emotional well-being (Gatchel et al., 2007; Gorczyca et al., 2013; Innes, 2005; Keefe et al., 2004) which have been shown to explain the chronic pain experience. Therefore, it is possible that the relationship between homeostatic plasticity and pain is clearer during the early stages of pain development (Figure 4.4 in study three) as opposed to when pain in chronic. Further studies are needed to explore homeostatic plasticity during the different stages of pain development and persistence in individuals with musculoskeletal disorders.

Thus, taken together, the novel findings from this thesis suggests that two days of sustained pain is sufficient to alter homeostatic regulation, that this alteration worsens as pain is sustained, returns to normal when pain resolves, and is impaired relative to healthy controls when pain has persisted for more than 6 months. These findings expand our understanding of the maladaptive synaptic plasticity hypothesis in musculoskeletal pain and make an important contribution to the field by providing a foundation for comprehensive longitudinal investigation of homeostatic plasticity in healthy and clinical populations.

5.4. Clinical implications

The rapid increase in therapies designed to target maladaptive synaptic plasticity in chronic musculoskeletal pain make the present findings highly relevant (Moseley & Flor, 2012; O'Connell et al., 2010; Pelletier et al., 2015). These findings suggest that impaired homeostatic plasticity could be important in the development and persistence of musculoskeletal pain, and if relevance of this mechanism to clinical outcome is confirmed in future longitudinal trials, homeostatic plasticity could be a viable treatment target in the future.

At present, relatively few therapies have been designed to directly target homeostatic plasticity. For example, patterned peripheral electrical stimulation applied to reduce cortical excitability (induce synaptic weakening; LTD) in writer's cramp has been shown to improve cortical organisation and reduce symptoms (Schabrun et al., 2009a). Similarly, priming of M1 using high-frequency repetitive transcranial magnetic stimulation (rTMS; 10 Hz) prior to a low-frequency rTMS intervention (1 Hz) normalises cortical excitability in people with migraine (Brighina et al., 2010), and priming the M1 to induce synaptic weakening using peripheral electrical stimulation concurrent with an excitatory non-invasive brain stimulation intervention in cLBP improves cortical organisation and pain beyond that of either intervention applied alone (Schabrun et al., 2017a). Although further work is needed, these findings suggest that priming the cortex to alter the threshold for

synaptic plasticity (i.e., LTP – LTD formation) prior to a second treatment may be a useful approach that can promote normal homeostatic plasticity in pain. Potential therapies to target homeostatic plasticity are an area for future research that could help address the urgent need for more effective treatments in chronic musculoskeletal pain conditions.

5.5. Limitations

The limitations of individual studies have been mentioned and discussed in chapters two, three, and four. Therefore, the following section discusses limitations associated with the overarching framework of this thesis.

First, work presented in this thesis is limited to homeostatic plasticity elicited in one direction only. This approach was selected as previous studies exploring homeostatic plasticity in clinical populations have demonstrated failure to reduce high levels of cortical excitability (Antal et al., 2008b; Cosentino et al., 2014b; Kang et al., 2011; Quartarone et al., 2005). However, as homeostatic plasticity is bidirectional, and dependent upon the activation history of a neural circuit, it is possible that similar impairments exist following high levels of cortical inhibition (Karabanov et al., 2015; Muller-Dahlhaus & Ziemann, 2015). Future studies should extend the work presented in this thesis using inhibitory NIBS techniques.

Second, findings of this thesis are limited to M1. As previous studies have demonstrated impaired homeostatic plasticity in cortical regions outside the M1 (Bocci et al., 2014; Brighina et al., 2002; Hamada et al., 2008; Popa et al., 2013; Potter-Nerger et al., 2009; Ragert et al., 2009), and musculoskeletal pain has been shown to involve widespread changes across a variety of brain regions (Apkarian, 2011; Apkarian et al., 2009; Apkarian et al., 2011; Baliki et al., 2011; May, 2008), it is possible that impaired homeostatic plasticity may be generalised throughout the cortex. Future studies are needed to explore homeostatic plasticity locally and globally within and between, different cortical regions in people with musculoskeletal pain.

Third, the work presented in this thesis is limited to one clinical musculoskeletal pain condition i.e., non-specific cLBP. A growing body of evidence has demonstrated altered synaptic plasticity characterised by increased pain perception, and motor dysfunction in a variety of chronic musculoskeletal pain conditions including chronic osteoarthritis (Fingleton et al., 2015; Kittelson et al., 2014; Lluch et al., 2014; Shanahan et al., 2015), patellofemoral pain (Jensen et al., 2008; Te et al., 2017), and chronic neck pain (Curatolo et al., 2001; Freeman et al., 2009; Sterling et al., 2004; Van Oosterwijck et al., 2013). It is therefore possible that homeostatic plasticity is impaired in other chronic musculoskeletal pain conditions and this requires on-going investigation in other studies.

Fourth, spinal mechanisms known to be altered during musculoskeletal pain have not been accounted for in this thesis. As previous studies have demonstrated altered spinal excitability contributes to the pathophysiology and clinical presentations seen in musculoskeletal pain (Banic et al., 2004; Curatolo et al., 2004; Curatolo et al., 2001; D'Mello & Dickenson, 2008), it is possible that spinal mechanisms may have contributed to impaired homeostatic plasticity in study two and study three. The role spinal mechanisms play during homeostatic regulation in individuals with musculoskeletal pain requires further exploration through neurophysiological measures of spinal excitability.

Fifth, while altered homeostatic plasticity in study two and study three suggest pain when acute, sustained or chronic influence homeostatic regulation differently, no direct comparison of homeostatic regulation in cortical areas corresponding to the affected and unaffected muscles limits these conclusions. Future studies should look to compare homeostatic plasticity in cortical areas corresponding to the affected and unaffected muscles within the same individual to extend findings presented herein.

Finally, the investigator was not blinded to group allocation during data collection and analyses. Investigator blinding reduces the risk of bias, and improves internal validity (Day & Altman, 2000; Page & Persch, 2013). Therefore, to improve data accuracy and to extend the work presented in this thesis, future studies should implement investigator blinding

during data collection and analysis (Eldridge et al., 2008; Emanuel et al., 2000; Page & Persch, 2013).

5.6. Conclusion

This thesis provides the first evidence for the reliability of homeostatic plasticity in healthy individuals across two weeks, and for altered homeostatic plasticity in response to pain, using a chronic musculoskeletal pain population and an experimental pain model. Impaired homeostatic plasticity in individuals with non-specific cLBP may reflect an inability to counter excessive increases in M1 excitability, while altered homeostatic responses following experimentally induced sustained muscle pain in otherwise healthy individuals suggest homeostatic plasticity is disturbed after two days of pain, although whether this is an adaptive or a maladaptive mechanism remains unclear. However, these data have relevance for understanding the maladaptive synaptic plasticity hypothesis previously reported in musculoskeletal pain conditions. Together, these studies provide novel data and extend the field of homeostatic plasticity during musculoskeletal pain. However, further work using longitudinal study designs in clinical populations is required to advance the work presented herein. Future studies should also examine homeostatic plasticity using similar and / or other NIBS techniques to extend work presented in this thesis. Lastly, if shown to underpin development and persistence of musculoskeletal pain, studies could test

novel therapeutic approaches that target homeostatic, rather than synaptic plasticity mechanisms, to improve treatment efficacy, and patient responsiveness to therapy.

References

Abbott, L. F., & Nelson, S. B. (2000). Synaptic plasticity: taming the beast. *Nat Neurosci, 3 Suppl,* 1178-1183. doi:10.1038/81453

Abbruzzese, G., Marchese, R., Buccolieri, A., Gasparetto, B., & Trompetto, C. (2001). Abnormalities of sensorimotor integration in focal dystonia: a transcranial magnetic stimulation study. *Brain, 124*(Pt 3), 537-545. doi:10.1093/brain/124.3.537

Ablin, J. N., & Buskila, D. (2015). "Real-life" treatment of chronic pain: Targets and goals. *Best Practice & Research Clinical Rheumatology, 29*(1), 111-119. doi:10.1016/j.berh.2015.04.020

Abraham, W. C. (2008). Metaplasticity: tuning synapses and networks for plasticity. *Nat Rev Neurosci, 9*(5), 387. doi:10.1038/nrn2356

Abraham, W. C., & Bear, M. F. (1996). Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci, 19*(4), 126-130. doi:10.1016/S0166-2236(96)80018-X

Abraham, W. C., Mason-Parker, S. E., Bear, M. F., Webb, S., & Tate, W. P. (2001). Heterosynaptic metaplasticity in the hippocampus in vivo: a BCM-like modifiable threshold for LTP. *Proc Natl Acad Sci U S A, 98*(19), 10924-10929. doi:10.1073/pnas.181342098

Abu-Saad Huijer, H. (2010). Chronic pain: a review. *J. Med. Liban., 58*(1), 21-27.

Akobeng, A. K. (2008). Assessing the validity of clinical trials. *J Pediatr Gastroenterol Nutr, 47*(3), 277-282. doi:10.1097/MPG.0b013e31816c749f

Alberini, C. M. (2009). Transcription factors in long-term memory and synaptic plasticity. *Physiol Rev, 89*(1), 121-145. doi:10.1152/physrev.00017.2008

Amadi, U., Allman, C., Johansen-Berg, H., & Stagg, C. J. (2015). The Homeostatic Interaction Between Anodal Transcranial Direct Current Stimulation and Motor Learning in Humans is Related to GABAA Activity. *Brain Stimul, 8*(5), 898-905. doi:10.1016/j.brs.2015.04.010

Andersen, H., Arendt-Nielsen, L., Svensson, P., Danneskiold-Samsøe, B., & Graven-Nielsen, T. (2008). Spatial and temporal aspects of muscle hyperalgesia induced by nerve growth factor in humans. *Experimental Brain Research, 191*(3), 371-382. doi:10.1007/s00221-008-1531-5

Andresen, T., Nilsson, M., Nielsen, A. K., Lassen, D., Arendt-Nielsen, L., & Drewes, A. M. (2014). Intradermal Injection with Nerve Growth Factor: A Reproducible Model to Induce Experimental Allodynia and Hyperalgesia. *Pain Pract*. doi:10.1111/papr.12267

Antal, A., Boros, K., Poreisz, C., Chaieb, L., Terney, D., & Paulus, W. (2008a). Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. *Brain Stimul, 1*(2), 97-105. doi:10.1016/j.brs.2007.10.001

Antal, A., Kincses, T. Z., Nitsche, M. A., Bartfai, O., & Paulus, W. (2004). Excitability changes induced in the human primary visual cortex by transcranial direct current stimulation: direct electrophysiological evidence. *Invest Ophthalmol Vis Sci, 45*(2), 702-707. doi:10.1167/iovs.03-0688

Antal, A., Lang, N., Boros, K., Nitsche, M., Siebner, H. R., & Paulus, W. (2008b). Homeostatic metaplasticity of the motor cortex is altered during headache-free intervals in migraine with aura. *Cereb Cortex, 18*(11), 2701-2705. doi:10.1093/cercor/bhn032

Antal, A., Terney, D., Poreisz, C., & Paulus, W. (2007). Towards unravelling task-related modulations of neuroplastic changes induced in the human motor cortex. *Eur J Neurosci, 26*(9), 2687-2691. doi:10.1111/j.1460-9568.2007.05896.x

Apkarian, A. V. (2011). The brain in chronic pain: clinical implications. *Pain Manag, 1*(6), 577-586. doi:10.2217/pmt.11.53

Apkarian, A. V., Baliki, M. N., & Geha, P. Y. (2009). Towards a theory of chronic pain. *Prog Neurobiol, 87*(2), 81-97. doi:10.1016/j.pneurobio.2008.09.018

Apkarian, A. V., Hashmi, J. A., & Baliki, M. N. (2011). Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. *Pain, 152*(3 Suppl), S49-64. doi:10.1016/j.pain.2010.11.010

Apkarian, A. V., Sosa, Y., Sonty, S., Levy, R. M., Harden, R. N., Parrish, T. B., et al. (2004). Chronic Back Pain Is Associated with Decreased Prefrontal and Thalamic Gray Matter Density. *The Journal of Neuroscience, 24*(46), 10410-10415. doi:10.1523/jneurosci.2541-04.2004

Arai, N., Muller-Dahlhaus, F., Murakami, T., Bliem, B., Lu, M. K., Ugawa, Y., et al. (2011). State-dependent and timing-dependent bidirectional associative plasticity in the

human SMA-M1 network. *J Neurosci, 31*(43), 15376-15383. doi:10.1523/JNEUROSCI.2271-11.2011

Atwood, H. L., & Wojtowicz, J. M. (1999). Silent synapses in neural plasticity: current evidence. *Learn Mem, 6*(6), 542-571.

Balague, F., Mannion, A. F., Pellise, F., & Cedraschi, C. (2012). Non-specific low back pain. *Lancet, 379*(9814), 482-491. doi:10.1016/S0140-6736(11)60610-7

Baliki, M. N., Geha, P. Y., Apkarian, A. V., & Chialvo, D. R. (2008). Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. *J Neurosci, 28*(6), 1398-1403. doi:10.1523/jneurosci.4123-07.2008

Baliki, M. N., Schnitzer, T. J., Bauer, W. R., & Apkarian, A. V. (2011). Brain morphological signatures for chronic pain. *PLoS One, 6*(10), e26010. doi:10.1371/journal.pone.0026010

Banic, B., Petersen-Felix, S., Andersen, O. K., Radanov, B. P., Villiger, P. M., Arendt-Nielsen, L., et al. (2004). Evidence for spinal cord hypersensitivity in chronic pain after whiplash injury and in fibromyalgia. *Pain, 107*(1-2), 7-15. doi:10.1016/j.pain.2003.05.001

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet, 1*(8437), 1106-1107. doi:10.1016/S0140-6736(85)92413-4

Bashir, S., Perez, J. M., Horvath, J. C., & Pascual-Leone, A. (2013). Differentiation of motor cortical representation of hand muscles by navigated mapping of optimal TMS

current directions in healthy subjects. *J Clin Neurophysiol, 30*(4), 390-395. doi:10.1097/WNP.0b013e31829dda6b

Bastani, A., & Jaberzadeh, S. (2012). A higher number of TMS-elicited MEP from a combined hotspot improves intra- and inter-session reliability of the upper limb muscles in healthy individuals. *PLoS One, 7*(10), e47582. doi:10.1371/journal.pone.0047582

Bastani, A., & Jaberzadeh, S. (2013). a-tDCS differential modulation of corticospinal excitability: the effects of electrode size. *Brain Stimul, 6*(6), 932-937. doi:10.1016/j.brs.2013.04.005

Bear, M. F. (2003). Bidirectional synaptic plasticity: from theory to reality. *Philos Trans R Soc Lond B Biol Sci, 358*(1432), 649-655. doi:10.1098/rstb.2002.1255

Bear, M. F., & Malenka, R. C. (1994). Synaptic plasticity: LTP and LTD. *Curr. Opin. Neurobiol., 4*(3), 389-399. doi:10.1016/0959-4388(94)90101-5

Bedson, J., & Croft, P. R. (2008). The discordance between clinical and radiographic knee osteoarthritis: a systematic search and summary of the literature. *BMC musculoskeletal disorders, 9*, 116. doi:10.1186/1471-2474-9-116

Bergin, M. J., Hirata, R., Mista, C., Christensen, S. W., Tucker, K., Vicenzino, B., et al. (2015). Movement Evoked Pain and Mechanical Hyperalgesia after Intramuscular Injection of Nerve Growth Factor: A Model of Sustained Elbow Pain. *Pain Med, 16*(11), 2180-2191. doi:10.1111/pme.12824

Bi, G.-q., & Poo, M.-m. (1998). Synaptic Modifications in Cultured Hippocampal Neurons: Dependence on Spike Timing, Synaptic Strength, and Postsynaptic Cell Type. *The Journal of Neuroscience, 18*(24), 10464-10472.

Bi, G., & Poo, M. (2001). Synaptic modification by correlated activity: Hebb's postulate revisited. *Annu Rev Neurosci, 24*, 139-166. doi:10.1146/annurev.neuro.24.1.139

Biabani, M., Aminitehrani, M., Zoghi, M., Farrell, M., Egan, G., & Jaberzadeh, S. (2017). The effects of transcranial direct current stimulation on short-interval intracortical inhibition and intracortical facilitation: a systematic review and meta-analysis. *Rev Neurosci*. doi:10.1515/revneuro-2017-0023

Bienenstock, E. L., Cooper, L. N., & Munro, P. W. (1982). Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *J Neurosci, 2*(1), 32-48.

Bikson, M., Datta, A., Rahman, A., & Scaturro, J. (2010). Electrode montages for tDCS and weak transcranial electrical stimulation: Role of "return" electrode's position and size. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology, 121*(12), 1976-1978. doi:10.1016/j.clinph.2010.05.020

Biurrun Manresa, J. A., Neziri, A. Y., Curatolo, M., Arendt-Nielsen, L., & Andersen, O. K. (2013). Reflex receptive fields are enlarged in patients with musculoskeletal low back and neck pain. *Pain, 154*(8), 1318-1324. doi:10.1016/j.pain.2013.04.013

Bliem, B., Muller-Dahlhaus, J. F., Dinse, H. R., & Ziemann, U. (2008). Homeostatic metaplasticity in the human somatosensory cortex. *J Cogn Neurosci, 20*(8), 1517-1528. doi:10.1162/jocn.2008.20106

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature, 361*(6407), 31-39. doi:10.1038/361031a0

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol, 232*(2), 331-356.

Bliss, T. V. P., & Cooke, S. F. (2011). Long-term potentiation and long-term depression: a clinical perspective. *Clinics, 66*(Suppl 1), 3-17. doi:10.1590/S1807-59322011001300002

Bocci, T., Caleo, M., Tognazzi, S., Francini, N., Briscese, L., Maffei, L., et al. (2014). Evidence for metaplasticity in the human visual cortex. *J Neural Transm, 121*(3), 221-231. doi:10.1007/s00702-013-1104-z

Boersma, K., & Linton, S. J. (2005). How does persistent pain develop? An analysis of the relationship between psychological variables, pain and function across stages of chronicity. *Behav. Res. Ther., 43*(11), 1495-1507. doi:10.1016/j.brat.2004.11.006

Bosch, M., & Hayashi, Y. (2012). Structural plasticity of dendritic spines. *Curr. Opin. Neurobiol., 22*(3), 383-388. doi:10.1016/j.conb.2011.09.002

Boudreau, S. A., Farina, D., & Falla, D. (2010). The role of motor learning and neuroplasticity in designing rehabilitation approaches for musculoskeletal pain disorders. *Man. Ther., 15*(5), 410-414. doi:10.1016/j.math.2010.05.008

Brasil-Neto, J. P., Cohen, L. G., Panizza, M., Nilsson, J., Roth, B. J., & Hallett, M. (1992). Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. *J Clin Neurophysiol, 9*(1), 132-136. doi:10.1097/00004691-199201000-00014

Braun, H. J., & Gold, G. E. (2012). Diagnosis of Osteoarthritis: Imaging. *Bone, 51*(2), 278-288. doi:10.1016/j.bone.2011.11.019

Brighina, F., Cosentino, G., Vigneri, S., Talamanca, S., Palermo, A., Giglia, G., et al. (2011). Abnormal facilitatory mechanisms in motor cortex of migraine with aura. *Eur J Pain, 15*(9), 928-935. doi:10.1016/j.ejpain.2011.03.012

Brighina, F., Giglia, G., Scalia, S., Francolini, M., Palermo, A., & Fierro, B. (2005). Facilitatory effects of 1 Hz rTMS in motor cortex of patients affected by migraine with aura. *Exp Brain Res, 161*(1), 34-38. doi:10.1007/s00221-004-2042-7

Brighina, F., Palermo, A., Daniele, O., Aloisio, A., & Fierro, B. (2010). High-frequency transcranial magnetic stimulation on motor cortex of patients affected by migraine with aura: a way to restore normal cortical excitability? *Cephalalgia, 30*(1), 46-52. doi:10.1111/j.1468-2982.2009.01870.x

Brighina, F., Piazza, A., Daniele, O., & Fierro, B. (2002). Modulation of visual cortical excitability in migraine with aura: effects of 1 Hz repetitive transcranial magnetic stimulation. *Exp Brain Res, 145*(2), 177-181. doi:10.1007/s00221-002-1096-7

Brookhart, M. A., Stürmer, T., Glynn, R. J., Rassen, J., & Schneeweiss, S. (2010). Confounding control in healthcare database research: challenges and potential approaches. *Med. Care, 48*(6 0), S114-S120. doi:10.1097/MLR.0b013e3181dbebe3

Brown, T. H., Zhao, Y., & Leung, V. (2009). Hebbian Plasticity *Encyclopedia of Neuroscience* (pp. 1049-1056). Oxford: Academic Press.

Bryan, K., Robbin, G., & Terry, E. R. (2003). Brain Plasticity and Behavior. *Curr. Dir. Psychol. Sci., 12*(1), 1-5. doi:10.1111/1467-8721.01210

Buchs, P. A., & Muller, D. (1996). Induction of long-term potentiation is associated with major ultrastructural changes of activated synapses. *Proc Natl Acad Sci U S A, 93*(15), 8040-8045.

Burns, E., Chipchase, L. S., & Schabrun, S. M. (2016). Primary sensory and motor cortex function in response to acute muscle pain: A systematic review and meta-analysis. *Eur J Pain, 20*(8), 1203-1213. doi:10.1002/ejp.859

Bütefisch, C. M., Davis, B. C., Wise, S. P., Sawaki, L., Kopylev, L., Classen, J., et al. (2000). Mechanisms of use-dependent plasticity in the human motor cortex. *Proceedings of the National Academy of Sciences, 97*(7), 3661. doi:10.1073/pnas.97.7.3661

Cajal, R. y. (1928). *Degeneration and regeneration of the nervous system* (Vol. 2). New York, USA: Haffner Publishing Co.

Carasatorre, M., & Ramirez-Amaya, V. (2013). Network, cellular, and molecular mechanisms underlying long-term memory formation. *Curr Top Behav Neurosci, 15*, 73-115. doi:10.1007/7854_2012_229

Carew, T. J., & Sahley, C. L. (1986). Invertebrate learning and memory: from behavior to molecules. *Annu Rev Neurosci, 9*, 435-487. doi:10.1146/annurev.ne.09.030186.002251

Carson, R. G., & Kennedy, N. C. (2013). Modulation of human corticospinal excitability by paired associative stimulation. *Front Hum Neurosci, 7*, 823. doi:10.3389/fnhum.2013.00823

Carvalho, S., Boggio, P. S., Goncalves, O. F., Vigario, A. R., Faria, M., Silva, S., et al. (2015). Transcranial direct current stimulation based metaplasticity protocols in working memory. *Brain Stimul, 8*(2), 289-294. doi:10.1016/j.brs.2014.11.011

Catterall, W. A., Leal, K., & Nanou, E. (2013). Calcium channels and short-term synaptic plasticity. *J Biol Chem, 288*(15), 10742-10749. doi:10.1074/jbc.R112.411645

Cauda, F., Palermo, S., Costa, T., Torta, R., Duca, S., Vercelli, U., et al. (2014). Gray matter alterations in chronic pain: A network-oriented meta-analytic approach. *NeuroImage : Clinical, 4*, 676-686. doi:10.1016/j.nicl.2014.04.007

Cavaleri, R., Schabrun, S. M., & Chipchase, L. S. (2017). The number of stimuli required to reliably assess corticomotor excitability and primary motor cortical representations

using transcranial magnetic stimulation (TMS): a systematic review and meta-analysis. *Syst Rev, 6*(1), 48. doi:10.1186/s13643-017-0440-8

Caytak, H., Shapiro, D., Borisenko, A., & Bolic, M. (2015). Outside access: advances in tDCS could provide a mainstream clinical tool for noninvasive neuromodulation. *IEEE pulse, 6*(2), 21-24. doi:10.1109/MPUL.2014.2386551

Chang, W.-J., O'Connell, N. E., Beckenkamp, P. R., Alhassani, G., Liston, M. B., & Schabrun, S. M. (2017). Altered Primary Motor Cortex Structure, Organisation and Function in Chronic Pain: a Systematic Review and Meta-Analysis. *The Journal of Pain*. doi:10.1016/j.jpain.2017.10.007

Chang, W. H., Fried, P. J., Saxena, S., Jannati, A., Gomes-Osman, J., Kim, Y.-H., et al. (2016). Optimal Number of Pulses as Outcome Measures of Neuronavigated Transcranial Magnetic Stimulation. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology, 127*(8), 2892-2897. doi:10.1016/j.clinph.2016.04.001

Chen, J. (2011). History of pain theories. *Neurosci. Bull., 27*(5), 343-350. doi:10.1007/s12264-011-0139-0

Chen, L. U., & Maghsoodi, B. (2007). Synaptic Trafficking of AMPA Receptors *Protein Trafficking in Neurons* (pp. 175-201). Burlington: Academic Press.

Chen, R., Tam, A., Butefisch, C., Corwell, B., Ziemann, U., Rothwell, J. C., et al. (1998). Intracortical inhibition and facilitation in different representations of the human motor cortex. *J Neurophysiol, 80*(6), 2870-2881. doi:10.1152/jn.1998.80.6.2870

Chew, T., Ho, K. A., & Loo, C. K. (2015). Inter- and Intra-individual Variability in Response to Transcranial Direct Current Stimulation (tDCS) at Varying Current Intensities. *Brain Stimul, 8*(6), 1130-1137. doi:10.1016/j.brs.2015.07.031

Chou, R., Fu, R., Carrino, J. A., & Deyo, R. A. (2009). Imaging strategies for low-back pain: systematic review and meta-analysis. *Lancet, 373*(9662), 463-472. doi:10.1016/s0140-6736(09)60172-0

Chou, R., Qaseem, A., Owens, D. K., & Shekelle, P. (2011). Diagnostic imaging for low back pain: advice for high-value health care from the American College of Physicians. *Ann Intern Med, 154*(3), 181-189. doi:10.7326/0003-4819-154-3-201102010-00008

Christie, A., Fling, B., Crews, R. T., Mulwitz, L. A., & Kamen, G. (2007). Reliability of motor-evoked potentials in the ADM muscle of older adults. *J Neurosci Methods, 164*(2), 320-324. doi:10.1016/j.jneumeth.2007.05.011

Christova, M., Rafolt, D., & Gallasch, E. (2015). Cumulative effects of anodal and priming cathodal tDCS on pegboard test performance and motor cortical excitability. *Behav Brain Res, 287*, 27-33. doi:10.1016/j.bbr.2015.03.028

Citri, A., & Malenka, R. C. (2008). Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology, 33*(1), 18-41. doi:10.1038/sj.npp.1301559

Clarke, K. A., & Iphofen, R. (2005). Believing the patient with chronic pain: a review of the literature. *Br J Nurs, 14*(9), 490-493. doi:10.12968/bjon.2005.14.9.18073

Classen, J., Wolters, A., Stefan, K., Wycislo, M., Sandbrink, F., Schmidt, A., et al. (2004). Paired associative stimulation. *Suppl Clin Neurophysiol, 57,* 563-569. doi:10.1016/S1567-424X(09)70395-2

Cleophas, T. J., & Zwinderman, A. H. (2007). Clinical trials: how to assess confounding and why so. *Curr. Clin. Pharmacol., 2*(2), 129-133. doi:10.2174/157488407780598162

Coderre, T. J., Katz, J., Vaccarino, A. L., & Melzack, R. (1993). Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. *Pain, 52*(3), 259-285. doi:10.1016/0304-3959(93)90161-H

Cohen, J. (1969). *Statistical power analysis for the behavioural sciences.*: New York: Academic Press.

Cohen, J. (1992). A power primer. *Psychol. Bull., 112*(1), 155-159.

Cohen, S., & Greenberg, M. E. (2008). Communication Between the Synapse and the Nucleus in Neuronal Development, Plasticity, and Disease. *Annu. Rev. Cell Dev. Biol., 24*(1), 183-209. doi:10.1146/annurev.cellbio.24.110707.175235

Contractor, A., & Heinemann, S. F. (2002). Glutamate receptor trafficking in synaptic plasticity. *Sci. STKE, 2002*(156), re14. doi:10.1126/stke.2002.156.re14

Cooper, L. N., & Bear, M. F. (2012). The BCM theory of synapse modification at 30: interaction of theory with experiment. *Nat Rev Neurosci, 13*(11), 798-810. doi:10.1038/nrn3353

Cooper, L. N., Liberman, F., & Oja, E. (1979). A theory for the acquisition and loss of neuron specificity in visual cortex. *Biol Cybern, 33*(1), 9-28.

Cosentino, G., Brighina, F., Talamanca, S., Paladino, P., Vigneri, S., Baschi, R., et al. (2014a). Reduced threshold for inhibitory homeostatic responses in migraine motor cortex? A tDCS/TMS study. *Headache, 54*(4), 663-674. doi:10.1111/head.12249

Cosentino, G., Fierro, B., Vigneri, S., Talamanca, S., Paladino, P., Baschi, R., et al. (2014b). Cyclical changes of cortical excitability and metaplasticity in migraine: evidence from a repetitive transcranial magnetic stimulation study. *Pain, 155*(6), 1070-1078. doi:10.1016/j.pain.2014.02.024

Creutzfeldt, O. D., Fromm, G. H., & Kapp, H. (1962). Influence of transcortical d-c currents on cortical neuronal activity. *Exp Neurol, 5*, 436-452. doi:10.1016/0014-4886(62)90056-0

Crofford, L. J. (2015). Psychological aspects of chronic musculoskeletal pain. *Best Practice & Research Clinical Rheumatology, 29*(1), 147-155. doi:10.1016/j.berh.2015.04.027

Cuevas, J. (2007). The Action Potential *xPharm: The Comprehensive Pharmacology Reference* (pp. 1-5). New York: Elsevier.

Curatolo, M., Arendt-Nielsen, L., & Petersen-Felix, S. (2004). Evidence, Mechanisms, and Clinical Implications of Central Hypersensitivity in Chronic Pain After Whiplash Injury. *The Clinical Journal of Pain, 20*(6), 469-476. doi:10.1097/00002508-200411000-00013

Curatolo, M., Petersen-Felix, S., Arendt-Nielsen, L., Giani, C., Zbinden, A. M., & Radanov, B. P. (2001). Central hypersensitivity in chronic pain after whiplash injury. *Clin J Pain, 17*(4), 306-315.

D'Mello, R., & Dickenson, A. H. (2008). Spinal cord mechanisms of pain. *BJA: British Journal of Anaesthesia, 101*(1), 8-16. doi:10.1093/bja/aen088

Daoudal, G., & Debanne, D. (2003). Long-term plasticity of intrinsic excitability: learning rules and mechanisms. *Learn Mem, 10*(6), 456-465. doi:10.1101/lm.64103

DaSilva, A. F., Volz, M. S., Bikson, M., & Fregni, F. (2011). Electrode positioning and montage in transcranial direct current stimulation. *J Vis Exp*(51). doi:10.3791/2744

Datta, A., Bikson, M., & Fregni, F. (2010). Transcranial direct current stimulation in patients with skull defects and skull plates: high-resolution computational FEM study of factors altering cortical current flow. *Neuroimage, 52*(4), 1268-1278. doi:10.1016/j.neuroimage.2010.04.252

Datta, A., Truong, D., Minhas, P., Parra, L. C., & Bikson, M. (2012). Inter-Individual Variation during Transcranial Direct Current Stimulation and Normalization of Dose Using MRI-Derived Computational Models. *Front Psychiatry, 3*, 91. doi:10.3389/fpsyt.2012.00091

Day, S. J., & Altman, D. G. (2000). Blinding in clinical trials and other studies. *BMJ : British Medical Journal, 321*(7259), 504-504. doi:10.1136/bmj.321.7259.504

Debanne, D., Gahwiler, B. H., & Thompson, S. M. (1998). Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. *J Physiol, 507 (Pt 1)*, 237-247.

Delvendahl, I., Jung, N. H., Mainberger, F., Kuhnke, N. G., Cronjaeger, M., & Mall, V. (2010). Occlusion of bidirectional plasticity by preceding low-frequency stimulation in the human motor cortex. *Clin Neurophysiol, 121*(4), 594-602. doi:10.1016/j.clinph.2009.09.034

Desai, N. S., Cudmore, R. H., Nelson, S. B., & Turrigiano, G. G. (2002). Critical periods for experience-dependent synaptic scaling in visual cortex. *Nat Neurosci, 5*(8), 783-789. doi:10.1038/nn878

Devane, D., Begley, C. M., & Clarke, M. (2004). How many do I need? Basic principles of sample size estimation. *J Adv Nurs, 47*(3), 297-302. doi:10.1111/j.1365-2648.2004.03093.x

Di Lazzaro, V. (2013). Biological effects of non-invasive brain stimulation. *Handb Clin Neurol, 116*, 367-374. doi:10.1016/B978-0-444-53497-2.00030-9

Di Lazzaro, V., Oliviero, A., Mazzone, P., Insola, A., Pilato, F., Saturno, E., et al. (2001). Comparison of descending volleys evoked by monophasic and biphasic magnetic

stimulation of the motor cortex in conscious humans. *Exp Brain Res, 141*(1), 121-127. doi:10.1007/s002210100863

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Mazzone, P., et al. (2004). The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clin Neurophysiol, 115*(2), 255-266. doi:10.1016/j.clinph.2003.10.009

Di Lazzaro, V., Oliviero, A., Profice, P., Pennisi, M. A., Pilato, F., Zito, G., et al. (2003). Ketamine increases human motor cortex excitability to transcranial magnetic stimulation. *J Physiol, 547*(Pt 2), 485-496. doi:10.1113/jphysiol.2002.030486

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Oliviero, A., Mazzone, P., et al. (2008). Low-frequency repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. *J Physiol, 586*(Pt 18), 4481-4487. doi:10.1113/jphysiol.2008.159558

Di Lazzaro, V., Profice, P., Pilato, F., Dileone, M., Oliviero, A., & Ziemann, U. (2010). The effects of motor cortex rTMS on corticospinal descending activity. *Clin Neurophysiol, 121*(4), 464-473. doi:10.1016/j.clinph.2009.11.007

Di Lazzaro, V., Rothwell, J., & Capogna, M. (2017). Noninvasive Stimulation of the Human Brain: Activation of Multiple Cortical Circuits. *Neuroscientist*, 1073858417717660. doi:10.1177/1073858417717660

Dileone, M., Profice, P., Pilato, F., Alfieri, P., Cesarini, L., Mercuri, E., et al. (2010). Enhanced human brain associative plasticity in Costello syndrome. *J Physiol, 588*(Pt 18), 3445-3456. doi:10.1113/jphysiol.2010.191072

Docking, R. E., Fleming, J., Brayne, C., Zhao, J., Macfarlane, G. J., & Jones, G. T. (2011). Epidemiology of back pain in older adults: prevalence and risk factors for back pain onset. *Rheumatology (Oxford), 50*(9), 1645-1653. doi:10.1093/rheumatology/ker175

Doeltgen, S. H., & Ridding, M. C. (2011). Modulation of cortical motor networks following primed theta burst transcranial magnetic stimulation. *Exp Brain Res, 215*(3-4), 199-206. doi:10.1007/s00221-011-2886-6

Doeltgen, S. H., Ridding, M. C., O'Beirne, G. A., Dalrymple-Alford, J., & Huckabee, M. L. (2009). Test-retest reliability of motor evoked potentials (MEPs) at the submental muscle group during volitional swallowing. *J Neurosci Methods, 178*(1), 134-137. doi:10.1016/j.jneumeth.2008.12.005

Doyon, J., & Benali, H. (2005). Reorganization and plasticity in the adult brain during learning of motor skills. *Curr Opin Neurobiol, 15*(2), 161-167. doi:10.1016/j.conb.2005.03.004

Dupret, D., Revest, J.-M., Koehl, M., Ichas, F., De Giorgi, F., Costet, P., et al. (2008). Spatial Relational Memory Requires Hippocampal Adult Neurogenesis. *PLOS ONE, 3*(4), e1959. doi:10.1371/journal.pone.0001959

Elahi, B., Hutchison, W. D., Daskalakis, Z. J., Gunraj, C., & Chen, R. (2014). Dose-response curve of associative plasticity in human motor cortex and interactions with motor practice. *Journal of Neurophysiology, 111*(3), 594-601. doi:10.1152/jn.00920.2012

Eldridge, S., Ashby, D., Bennett, C., Wakelin, M., & Feder, G. (2008). Internal and external validity of cluster randomised trials: systematic review of recent trials. *Bmj, 336*(7649), 876-880. doi:10.1136/bmj.39517.495764.25

Elgueta-Cancino, E., Schabrun, S., & Hodges, P. (2017). Is the Organisation of the Primary Motor Cortex in Low Back Pain Related to Pain, Movement and/or Sensation? *Clin J Pain*. doi:10.1097/ajp.0000000000000535

Elliott, A. M., Smith, B. H., Penny, K. I., Smith, W. C., & Chambers, W. A. (1999). The epidemiology of chronic pain in the community. *Lancet, 354*(9186), 1248-1252.

Emanuel, E. J., Wendler, D., & Grady, C. (2000). What makes clinical research ethical? *Jama, 283*(20), 2701-2711. doi:10.1001/jama.283.20.2701

Engert, F., & Bonhoeffer, T. (1999a). Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature, 399*(6731), 66-70. doi:10.1038/19978

Engert, F., & Bonhoeffer, T. (1999b). Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature, 399*, 66. doi:10.1038/19978

Ernstzen, D. V., Louw, Q. A., & Hillier, S. L. (2017). Clinical practice guidelines for the management of chronic musculoskeletal pain in primary healthcare: a systematic review. *Implementation Science : IS, 12*, 1. doi:10.1186/s13012-016-0533-0

Faber, J., & Fonseca, L. M. (2014). How sample size influences research outcomes. *Dental Press J. Orthod., 19*(4), 27-29. doi:10.1590/2176-9451.19.4.027-029.ebo

Fathi, D., Ueki, Y., Mima, T., Koganemaru, S., Nagamine, T., Tawfik, A., et al. (2010). Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. *Clin Neurophysiol, 121*(1), 90-93. doi:10.1016/j.clinph.2009.07.048

Feher, J. (2017). 3.3 - Propagation of the Action Potential *Quantitative Human Physiology (Second Edition)* (pp. 280-288). Boston: Academic Press.

Feldman, D. E. (2009). Synaptic Mechanisms for Plasticity in Neocortex. *Annu. Rev. Neurosci., 32*(1), 33-55. doi:10.1146/annurev.neuro.051508.135516

Fernandes, D., & Carvalho, A. L. (2016). Mechanisms of homeostatic plasticity in the excitatory synapse. *J Neurochem, 139*(6), 973-996. doi:10.1111/jnc.13687

Finan, P. H., Buenaver, L. F., Bounds, S. C., Hussain, S., Park, R. J., Haque, U. J., et al. (2013). Discordance between pain and radiographic severity in knee osteoarthritis: findings from quantitative sensory testing of central sensitization. *Arthritis Rheum, 65*(2), 363-372. doi:10.1002/art.34646

Fingleton, C., Smart, K., Moloney, N., Fullen, B. M., & Doody, C. (2015). Pain sensitization in people with knee osteoarthritis: a systematic review and meta-analysis. *Osteoarthritis and Cartilage, 23*(7), 1043-1056. doi:10.1016/j.joca.2015.02.163

Finnern, M. M., Kleinbohl, D., Flor, H., Benrath, J., & Holzl, R. (2018). Deconstructing chronicity of musculoskeletal pain: intensity-duration relations, minimal dimensions and clusters of chronicity. *Scand J Pain, 18*(3), 363-377. doi:10.1515/sjpain-2018-0021

Fitzgerald, P. B., Fountain, S., & Daskalakis, Z. J. (2006). A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. *Clin Neurophysiol, 117*(12), 2584-2596. doi:10.1016/j.clinph.2006.06.712

Flor, H. (2002). Painful memories: Can we train chronic pain patients to 'forget' their pain? *EMBO Reports, 3*(4), 288-291. doi:10.1093/embo-reports/kvf080

Flor, H. (2003). Cortical reorganisation and chronic pain: implications for rehabilitation. *J Rehabil Med*(41 Suppl), 66-72. doi:10.1080/16501960310010179

Flor, H. (2008). Maladaptive plasticity, memory for pain and phantom limb pain: review and suggestions for new therapies. *Expert Rev Neurother, 8*(5), 809-818. doi:10.1586/14737175.8.5.809

Flor, H., Braun, C., Elbert, T., & Birbaumer, N. (1997). Extensive reorganization of primary somatosensory cortex in chronic back pain patients. *Neurosci Lett, 224*(1), 5-8.

Fox, K., & Stryker, M. (2017). Integrating Hebbian and homeostatic plasticity: introduction. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372*(1715), 20160413. doi:10.1098/rstb.2016.0413

Frank, C. A. (2014). Homeostatic plasticity at the Drosophila neuromuscular junction. *Neuropharmacology, 78*, 63-74. doi:10.1016/j.neuropharm.2013.06.015

Frank, C. A., Kennedy, M. J., Goold, C. P., Marek, K. W., & Davis, G. W. (2006). Mechanisms underlying the rapid induction and sustained expression of synaptic homeostasis. *Neuron, 52*(4), 663-677. doi:10.1016/j.neuron.2006.09.029

Freeman, M. D., Nystrom, A., & Centeno, C. (2009). Chronic whiplash and central sensitization; an evaluation of the role of a myofascial trigger points in pain modulation. *Journal of brachial plexus and peripheral nerve injury, 4*, 2. doi:10.1186/1749-7221-4-2

Fregni, F., & Pascual-Leone, A. (2007). Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. *Nat. Clin. Pract. Neurol., 3*(7), 383-393. doi:10.1038/ncpneuro0530

Fricke, K., Seeber, A. A., Thirugnanasambandam, N., Paulus, W., Nitsche, M. A., & Rothwell, J. C. (2011). Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. *J Neurophysiol, 105*(3), 1141-1149. doi:10.1152/jn.00608.2009

Fröhlich, F. (2016). Synaptic Plasticity *Network Neuroscience* (pp. 47-58). San Diego: Academic Press.

Fujiyama, H., Hinder, M. R., Barzideh, A., Van de Vijver, C., Badache, A. C., Manrique, C. M., et al. (2017). Preconditioning tDCS facilitates subsequent tDCS effect on skill acquisition in older adults. *Neurobiol Aging, 51*, 31-42. doi:10.1016/j.neurobiolaging.2016.11.012

Gainey, M. A., Hurvitz-Wolff, J. R., Lambo, M. E., & Turrigiano, G. G. (2009). Synaptic Scaling Requires the GluR2 Subunit of the AMPA Receptor. *The Journal of Neuroscience, 29*(20), 6479-6489. doi:10.1523/jneurosci.3753-08.2009

Gallea, C., Herath, P., Voon, V., Lerner, A., Ostuni, J., Saad, Z., et al. (2017). Loss of inhibition in sensorimotor networks in focal hand dystonia. *NeuroImage: Clinical, 17*(Supplement C), 90-97. doi:10.1016/j.nicl.2017.10.011

Garg, R., Joshi, S., Mishra, S., & Bhatnagar, S. (2012). Evidence Based Practice of Chronic Pain. *Indian J. Palliat. Care, 18*(3), 155-161. doi:10.4103/0973-1075.105684

Gatchel, R. J., Peng, Y. B., Peters, M. L., Fuchs, P. N., & Turk, D. C. (2007). The biopsychosocial approach to chronic pain: scientific advances and future directions. *Psychol. Bull., 133*(4), 581-624. doi:10.1037/0033-2909.133.4.581

Giesbrecht, R. J. S., & Battié, M. C. (2005). A Comparison of Pressure Pain Detection Thresholds in People With Chronic Low Back Pain and Volunteers Without Pain. *Phys. Ther., 85*(10), 1085-1092. doi:10.1093/ptj/85.10.1085

Giesecke, T., Gracely, R. H., Grant, M. A., Nachemson, A., Petzke, F., Williams, D. A., et al. (2004). Evidence of augmented central pain processing in idiopathic chronic low back pain. *Arthritis Rheum, 50*(2), 613-623. doi:10.1002/art.20063

Gisabella, B., Rowan, M. J., & Anwyl, R. (2003). Mechanisms underlying the inhibition of long-term potentiation by preconditioning stimulation in the hippocampus in vitro. *Neuroscience, 121*(2), 297-305. doi:10.1016/S0306-4522(03)00440-8

Glenton, C. (2003). Chronic back pain sufferers--striving for the sick role. *Soc Sci Med, 57*(11), 2243-2252. doi:10.1016/S0277-9536(03)00130-8

Glyn-Jones, S., Palmer, A. J. R., Agricola, R., Price, A. J., Vincent, T. L., Weinans, H., et al. (2015). Osteoarthritis. *The Lancet, 386*(9991), 376-387. doi:10.1016/S0140-6736(14)60802-3

Goel, A., & Lee, H. K. (2007). Persistence of experience-induced homeostatic synaptic plasticity through adulthood in superficial layers of mouse visual cortex. *J Neurosci, 27*(25), 6692-6700. doi:10.1523/jneurosci.5038-06.2007

Gogtay, N. J. (2010). Principles of sample size calculation. *Indian J. Ophthalmol., 58*(6), 517-518. doi:10.4103/0301-4738.71692

Goldsworthy, M. R., Muller-Dahlhaus, F., Ridding, M. C., & Ziemann, U. (2015). Resistant Against De-depression: LTD-Like Plasticity in the Human Motor Cortex Induced by Spaced cTBS. *Cereb Cortex, 25*(7), 1724-1734. doi:10.1093/cercor/bht353

Goldsworthy, M. R., Pitcher, J. B., & Ridding, M. C. (2012a). The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. *Eur. J. Neurosci., 35*(1), 125-134. doi:Doi 10.1111/J.1460-9568.2011.07924.X

Goldsworthy, M. R., Pitcher, J. B., & Ridding, M. C. (2012b). A comparison of two different continuous theta burst stimulation paradigms applied to the human primary motor cortex. *Clinical Neurophysiology, 123*(11), 2256-2263. doi:Doi 10.1016/J.Clinph.2012.05.001

Gomperts, S. N., Rao, A., Craig, A. M., Malenka, R. C., & Nicoll, R. A. (1998). Postsynaptically silent synapses in single neuron cultures. *Neuron, 21*(6), 1443-1451.

Gorczyca, R., Filip, R., & Walczak, E. (2013). Psychological aspects of pain. *Ann. Agric. Environ. Med., Spec no. 1*, 23-27.

Graven-Nielsen, T., Arendt-Nielsen, L., & Mense, S. (2002). Thermosensitivity of muscle: high-intensity thermal stimulation of muscle tissue induces muscle pain in humans. *J Physiol, 540*(Pt 2), 647-656.

Green, C. S., & Bavelier, D. (2008). Exercising your brain: A review of human brain plasticity and training-induced learning. *Psychol. Aging, 23*(4), 692-701. doi:10.1037/a0014345

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L. G., Mall, V., et al. (2012). A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol, 123*(5), 858-882. doi:10.1016/j.clinph.2012.01.010

Grutzendler, J., Kasthuri, N., & Gan, W. B. (2002). Long-term dendritic spine stability in the adult cortex. *Nature, 420*(6917), 812-816. doi:10.1038/nature01276

Gustin, S. M., Peck, C. C., Cheney, L. B., Macey, P. M., Murray, G. M., & Henderson, L. A. (2012). Pain and Plasticity: Is Chronic Pain Always Associated with Somatosensory Cortex Activity and Reorganization? *The Journal of Neuroscience, 32*(43), 14874-14884. doi:10.1523/jneurosci.1733-12.2012

Hackshaw, A. (2008). Small studies: strengths and limitations. *Eur Respir J, 32*(5), 1141-1143. doi:10.1183/09031936.00136408

Hallett, M. (2011). Neurophysiology of Dystonia: The Role of Inhibition. *Neurobiol. Dis., 42*(2), 177-184. doi:10.1016/j.nbd.2010.08.025

Hamada, M., Hanajima, R., Terao, Y., Okabe, S., Nakatani-Enomoto, S., Furubayashi, T., et al. (2009). Primary motor cortical metaplasticity induced by priming over the supplementary motor area. *J Physiol, 587*(Pt 20), 4845-4862. doi:10.1113/jphysiol.2009.179101

Hamada, M., Murase, N., Hasan, A., Balaratnam, M., & Rothwell, J. C. (2013). The role of interneuron networks in driving human motor cortical plasticity. *Cereb Cortex, 23*(7), 1593-1605. doi:10.1093/cercor/bhs147

Hamada, M., Terao, Y., Hanajima, R., Shirota, Y., Nakatani-Enomoto, S., Furubayashi, T., et al. (2008). Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. *J Physiol, 586*(16), 3927-3947. doi:10.1113/jphysiol.2008.152793

Hawker, G., A., Mian, S., Kendzerska, T., & French, M. (2011). Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and

Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). *Arthritis Care Res. (Hoboken), 63*(S11), S240-S252. doi:doi:10.1002/acr.20543

Hayashi, K., Shiozawa, S., Ozaki, N., Mizumura, K., & Graven-Nielsen, T. (2013). Repeated intramuscular injections of nerve growth factor induced progressive muscle hyperalgesia, facilitated temporal summation, and expanded pain areas. *Pain, 154*(11), 2344-2352. doi:10.1016/j.pain.2013.07.007

Hebb, D. O. (1949). *Organization and behavior* New York: Wiley.

Hell, J. W. (2014). CaMKII: claiming center stage in postsynaptic function and organization. *Neuron, 81*(2), 249-265. doi:10.1016/j.neuron.2013.12.024

Hinder, M. R., Goss, E. L., Fujiyama, H., Canty, A. J., Garry, M. I., Rodger, J., et al. (2014). Inter- and Intra-individual variability following intermittent theta burst stimulation: implications for rehabilitation and recovery. *Brain Stimul, 7*(3), 365-371. doi:10.1016/j.brs.2014.01.004

Hodges, P. W. (2011). Pain and motor control: From the laboratory to rehabilitation. *J. Electromyogr. Kinesiol., 21*(2), 220-228. doi:10.1016/j.jelekin.2011.01.002

Hodges, P. W., & Tucker, K. (2011). Moving differently in pain: a new theory to explain the adaptation to pain. *Pain, 152*(3 Suppl), S90-98. doi:10.1016/j.pain.2010.10.020

Hoheisel, U., Unger, T., & Mense, S. (2007). Sensitization of rat dorsal horn neurons by NGF-induced subthreshold potentials and low-frequency activation. A study employing

intracellular recordings in vivo. *Brain research, 1169*, 34-43. doi:10.1016/j.brainres.2007.06.054

Horvath, J. C., Forte, J. D., & Carter, O. (2015). Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: A systematic review. *Neuropsychologia, 66*, 213-236. doi:10.1016/j.neuropsychologia.2014.11.021

Hou, Q., Gilbert, J., & Man, H. Y. (2011). Homeostatic regulation of AMPA receptor trafficking and degradation by light-controlled single-synaptic activation. *Neuron, 72*(5), 806-818. doi:10.1016/j.neuron.2011.10.011

Huang, Y. Y., Colino, A., Selig, D. K., & Malenka, R. C. (1992). The influence of prior synaptic activity on the induction of long-term potentiation. *Science, 255*(5045), 730-733. doi:10.1126/science.1346729

Huang, Y. Z., Lu, M. K., Antal, A., Classen, J., Nitsche, M., Ziemann, U., et al. (2017). Plasticity induced by non-invasive transcranial brain stimulation: A position paper. *Clin Neurophysiol, 128*(11), 2318-2329. doi:10.1016/j.clinph.2017.09.007

Huang, Y. Z., Rothwell, J. C., Lu, C. S., Chuang, W. L., & Chen, R. S. (2011). Abnormal bidirectional plasticity-like effects in Parkinson's disease. *Brain, 134*(Pt 8), 2312-2320. doi:10.1093/brain/awr158

Hubel, D. H., & Wiesel, T. N. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *The Journal of Physiology, 160*(1), 106-154.102.

Hubel, D. H., & Wiesel, T. N. (1970). The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *The Journal of Physiology, 206*(2), 419-436.

Huber, R., Maatta, S., Esser, S. K., Sarasso, S., Ferrarelli, F., Watson, A., et al. (2008). Measures of cortical plasticity after transcranial paired associative stimulation predict changes in electroencephalogram slow-wave activity during subsequent sleep. *J Neurosci, 28*(31), 7911-7918. doi:10.1523/jneurosci.1636-08.2008

Hunter, D. J. (2011). Osteoarthritis. *Best Practice & Research Clinical Rheumatology, 25*(6), 801-814. doi:10.1016/j.berh.2011.11.008

Hunter, J. (2001). Medical history and chronic pain. *Clin J Pain, 17*(4 Suppl), S20-25.

Ialongo, C. (2016). Understanding the effect size and its measures. *Biochemia Medica, 26*(2), 150-163. doi:10.11613/BM.2016.015

Ibata, K., Sun, Q., & Turrigiano, G. G. (2008). Rapid synaptic scaling induced by changes in postsynaptic firing. *Neuron, 57*(6), 819-826. doi:10.1016/j.neuron.2008.02.031

Ilic, N. V., Sajic, J., Miskovic, M., Krstic, J., Milanovic, S., Vesovic-Potic, V., et al. (2009). The efficacy of two protocols for inducing motor cortex plasticity in healthy humans--TMS study. *General physiology and biophysics, 28 Spec No*, 228-234.

Imamura, M., Chen, J., Matsubayashi, S. R., Targino, R. A., Alfieri, F. M., Bueno, D. K., et al. (2013). Changes in pressure pain threshold in patients with chronic nonspecific low back pain. *Spine, 38*(24), 2098-2107. doi:10.1097/01.brs.0000435027.50317.d7

Innes, S. I. (2005). Psychosocial factors and their role in chronic pain: A brief review of development and current status. *Chiropractic & Osteopathy, 13*, 6-6. doi:10.1186/1746-1340-13-6

Isaac, J. T. R., Nicoll, R. A., & Malenka, R. C. (1995). Evidence for silent synapses: Implications for the expression of LTP. *Neuron, 15*(2), 427-434. doi:10.1016/0896-6273(95)90046-2

Iyer, M. B., Mattu, U., Grafman, J., Lomarev, M., Sato, S., & Wassermann, E. M. (2005). Safety and cognitive effect of frontal DC brain polarization in healthy individuals. *Neurology, 64*(5), 872-875. doi:10.1212/01.wnl.0000152986.07469.e9

Jackson, L. A., Jackson, M. L., Nelson, J. C., Neuzil, K. M., & Weiss, N. S. (2006). Evidence of bias in estimates of influenza vaccine effectiveness in seniors. *Int J Epidemiol, 35*(2), 337-344. doi:10.1093/ije/dyi274

Jacobs, K. M., & Donoghue, J. P. (1991). Reshaping the cortical motor map by unmasking latent intracortical connections. *Science, 251*(4996), 944-947. doi:10.1126/science.2000496

Jager, K. J., Zoccali, C., MacLeod, A., & Dekker, F. W. (2008). Confounding: What it is and how to deal with it. *Kidney Int., 73*(3), 256-260. doi:10.1038/sj.ki.5002650

Janetzki, L., Gussew, A., Malessa, R., Habenicht, U., Reichenbach, J. R., Strauss, B., et al. (2016). [Cerebral metabolic changes and chronic back pain: Study taking into

consideration clinical and psychological parameters]. *Schmerz, 30*(2), 134-140. doi:10.1007/s00482-015-0082-5

Jarvik, J. J., Hollingworth, W., Heagerty, P., Haynor, D. R., & Deyo, R. A. (2001). The Longitudinal Assessment of Imaging and Disability of the Back (LAIDBack) Study: baseline data. *Spine, 26*(10), 1158-1166.

Jeans, A. F., van Heusden, F. C., Al-Mubarak, B., Padamsey, Z., & Emptage, N. J. (2017). Homeostatic Presynaptic Plasticity Is Specifically Regulated by P/Q-type Ca(2+) Channels at Mammalian Hippocampal Synapses. *Cell Reports, 21*(2), 341-350. doi:10.1016/j.celrep.2017.09.061

Jensen, M. C., Brant-Zawadzki, M. N., Obuchowski, N., Modic, M. T., Malkasian, D., & Ross, J. S. (1994). Magnetic resonance imaging of the lumbar spine in people without back pain. *N Engl J Med, 331*(2), 69-73. doi:10.1056/nejm199407143310201

Jensen, M. P. (2010). A neuropsychological model of pain: research and clinical implications. *The journal of pain : official journal of the American Pain Society, 11*(1), 2-12. doi:10.1016/j.jpain.2009.05.001

Jensen, R., Kvale, A., & Baerheim, A. (2008). Is pain in patellofemoral pain syndrome neuropathic? *Clin J Pain, 24*(5), 384-394. doi:10.1097/AJP.0b013e3181658170

Jia, Z., & Yu, S. (2017). Grey matter alterations in migraine: A systematic review and meta-analysis. *NeuroImage : Clinical, 14*, 130-140. doi:10.1016/j.nicl.2017.01.019

Jones, C. B., Lulic, T., Bailey, A. Z., Mackenzie, T. N., Mi, Y. Q., Tommerdahl, M., et al. (2016). Metaplasticity in human primary somatosensory cortex: effects on physiology and tactile perception. *J Neurophysiol, 115*(5), 2681-2691. doi:10.1152/jn.00630.2015

Jones, S. R., Carley, S., & Harrison, M. (2003). An introduction to power and sample size estimation. *Emerg. Med. J., 20*(5), 453-458. doi:10.1136/emj.20.5.453

Joseph, C. (2013). Plasticity. *Handb Clin Neurol, 116*, 525-534. doi:10.1016/B978-0-444-53497-2.00041-3

Ju, W., Morishita, W., Tsui, J., Gaietta, G., Deerinck, T. J., Adams, S. R., et al. (2004). Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat Neurosci, 7*(3), 244-253. doi:10.1038/nn1189

Jung, P., & Ziemann, U. (2009). Homeostatic and nonhomeostatic modulation of learning in human motor cortex. *J Neurosci, 29*(17), 5597-5604. doi:10.1523/JNEUROSCI.0222-09.2009

Juottonen, K., Gockel, M., Silen, T., Hurri, H., Hari, R., & Forss, N. (2002). Altered central sensorimotor processing in patients with complex regional pain syndrome. *Pain, 98*(3), 315-323. doi:10.1016/S0304-3959(02)00119-7

Kaas, J. H. (2001). Neural Plasticity In P. B. Baltes (Ed.), *International Encyclopedia of the Social & Behavioral Sciences* (pp. 10542-10546). Oxford: Pergamon.

Kadam, P., & Bhalerao, S. (2010). Sample size calculation. *International Journal of Ayurveda Research, 1*(1), 55-57. doi:10.4103/0974-7788.59946

Kamen, G. (2004). Reliability of motor-evoked potentials during resting and active contraction conditions. *Med Sci Sports Exerc, 36*(9), 1574-1579. doi:10.1249/01.MSS.0000139804.02576.6A

Kang, J. S., Terranova, C., Hilker, R., Quartarone, A., & Ziemann, U. (2011). Deficient homeostatic regulation of practice-dependent plasticity in writer's cramp. *Cereb Cortex, 21*(5), 1203-1212. doi:10.1093/cercor/bhq204

Kapogiannis, D., & Wassermann, E. M. (2008). Transcranial magnetic stimulation in Clinical Pharmacology. *Cent. Nerv. Syst. Agents Med. Chem., 8*(4), 234-240.

Karabanov, A., & Siebner, H. R. (2012). Unravelling homeostatic interactions in inhibitory and excitatory networks in human motor cortex. *J Physiol, 590*(22), 5557-5558. doi:10.1113/jphysiol.2012.244749

Karabanov, A., Ziemann, U., Hamada, M., George, M. S., Quartarone, A., Classen, J., et al. (2015). Consensus Paper: Probing Homeostatic Plasticity of Human Cortex With Non-invasive Transcranial Brain Stimulation. *Brain Stimul, 8*(3), 442-454. doi:10.1016/j.brs.2015.01.404

Karlsson, J., Engebretsen, L., & Dainty, K. (2003). Considerations on sample size and power calculations in randomized clinical trials. *Arthroscopy, 19*(9), 997-999.

Keefe, F. J., Rumble, M. E., Scipio, C. D., Giordano, L. A., & Perri, L. M. (2004). Psychological aspects of persistent pain: current state of the science. *The Journal of Pain, 5*(4), 195-211. doi:10.1016/j.jpain.2004.02.576

Keel, J. C., Smith, M. J., & Wassermann, E. M. (2001). A safety screening questionnaire for transcranial magnetic stimulation. *Clin Neurophysiol, 112*(4), 720. doi:10.1016/S1388-2457(00)00518-6

Kerchner, G. A., & Nicoll, R. A. (2008). Silent synapses and the emergence of a postsynaptic mechanism for LTP. *Nature reviews. Neuroscience, 9*(11), 813. doi:10.1038/nrn2501

Kim, J., & Tsien, R. W. (2008). Synapse-specific adaptations to inactivity in hippocampal circuits achieve homeostatic gain control while dampening network reverberation. *Neuron, 58*(6), 925-937. doi:10.1016/j.neuron.2008.05.009

Kittelson, A. J., Thomas, A. C., Kluger, B. M., & Stevens-Lapsley, J. E. (2014). Corticospinal and Intracortical Excitability of the Quadriceps in Patients with Knee Osteoarthritis. *Experimental brain research, 232*(12), 3991-3999. doi:10.1007/s00221-014-4079-6

Klein, M. M., Treister, R., Raij, T., Pascual-Leone, A., Park, L., Nurmikko, T., et al. (2015). Transcranial Magnetic Stimulation (TMS) of the Brain: Guidelines for Pain Treatment Research. *Pain*. doi:10.1097/j.pain.0000000000000210

Klomjai, W., Katz, R., & Lackmy-Vallee, A. (2015). Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Ann Phys Rehabil Med, 58*(4), 208-213. doi:10.1016/j.rehab.2015.05.005

Knogler, L. D., Liao, M., & Drapeau, P. (2010). Synaptic scaling and the development of a motor network. *J Neurosci, 30*(26), 8871-8881. doi:10.1523/jneurosci.0880-10.2010

Kobayashi, Y., Kurata, J., Sekiguchi, M., Kokubun, M., Akaishizawa, T., Chiba, Y., et al. (2009). Augmented cerebral activation by lumbar mechanical stimulus in chronic low back pain patients: an FMRI study. *Spine, 34*(22), 2431-2436. doi:10.1097/BRS.0b013e3181b1fb76

Kolb, B., & Gibb, R. (2011). Brain Plasticity and Behaviour in the Developing Brain. *Journal of the Canadian Academy of Child and Adolescent Psychiatry, 20*(4), 265-276.

Kong, J., Spaeth, R. B., Wey, H. Y., Cheetham, A., Cook, A. H., Jensen, K., et al. (2013). S1 is associated with chronic low back pain: a functional and structural MRI study. *Mol Pain, 9*, 43. doi:10.1186/1744-8069-9-43

Kregel, J., Meeus, M., Malfliet, A., Dolphens, M., Danneels, L., Nijs, J., et al. (2015). Structural and functional brain abnormalities in chronic low back pain: A systematic review. *Semin Arthritis Rheum, 45*(2), 229-237. doi:10.1016/j.semarthrit.2015.05.002

Krivanekova, L., Lu, M. K., Bliem, B., & Ziemann, U. (2011). Modulation of excitability in human primary somatosensory and motor cortex by paired associative stimulation targeting the primary somatosensory cortex. *Eur J Neurosci, 34*(8), 1292-1300. doi:10.1111/j.1460-9568.2011.07849.x

Kuner, R., & Flor, H. (2017). Structural plasticity and reorganisation in chronic pain. *Nat Rev Neurosci, 18*(1), 20-30. doi:10.1038/nrn.2016.162

Lahr, J., Passmann, S., List, J., Vach, W., Floel, A., & Kloppel, S. (2016). Effects of Different Analysis Strategies on Paired Associative Stimulation. A Pooled Data Analysis from Three Research Labs. *PLoS One, 11*(5), e0154880. doi:10.1371/journal.pone.0154880

Lang, N., Siebner, H. R., Ernst, D., Nitsche, M. A., Paulus, W., Lemon, R. N., et al. (2004). Preconditioning with transcranial direct current stimulation sensitizes the motor cortex to rapid-rate transcranial magnetic stimulation and controls the direction of after-effects. *Biol Psychiatry, 56*(9), 634-639. doi:10.1016/j.biopsych.2004.07.017

Lang, N., Siebner, H. R., Ward, N. S., Lee, L., Nitsche, M. A., Paulus, W., et al. (2005). How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? *Eur J Neurosci, 22*(2), 495-504. doi:10.1111/j.1460-9568.2005.04233.x

Lazarevic, V., Pothula, S., Andres-Alonso, M., & Fejtova, A. (2013). Molecular mechanisms driving homeostatic plasticity of neurotransmitter release. *Frontiers in Cellular Neuroscience, 7*, 244. doi:10.3389/fncel.2013.00244

Lee, J. C., Croarkin, P. E., Ameis, S. H., Sun, Y., Blumberger, D. M., Rajji, T. K., et al. (2017). Paired-Associative Stimulation-Induced Long-term Potentiation-Like Motor Cortex Plasticity in Healthy Adolescents. *Frontiers in Psychiatry, 8*, 95. doi:10.3389/fpsyt.2017.00095

Lewin, G. R., Lechner, S. G., & Smith, E. S. (2014). Nerve growth factor and nociception: from experimental embryology to new analgesic therapy. *Handb Exp Pharmacol, 220*, 251-282. doi:10.1007/978-3-642-45106-5_10

Lewin, G. R., & Mendell, L. M. (1993). Nerve growth factor and nociception. *Trends Neurosci, 16*(9), 353-359.

Li, L. M., Uehara, K., & Hanakawa, T. (2015). The contribution of interindividual factors to variability of response in transcranial direct current stimulation studies. *Frontiers in Cellular Neuroscience, 9*, 181. doi:10.3389/fncel.2015.00181

Liao, D., Hessler, N. A., & Malinow, R. (1995). Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature, 375*(6530), 400-404. doi:10.1038/375400a0

Liao, D., Zhang, X., O'Brien, R., Ehlers, M. D., & Huganir, R. L. (1999). Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. *Nat Neurosci, 2*(1), 37-43. doi:10.1038/4540

Lieber, R. L. (1990). Statistical significance and statistical power in hypothesis testing. *J Orthop Res, 8*(2), 304-309. doi:10.1002/jor.1100080221

Liebetanz, D., Nitsche, M. A., Tergau, F., & Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain, 125*(Pt 10), 2238-2247.

Lisman, J., Yasuda, R., & Raghavachari, S. (2012). Mechanisms of CaMKII action in long-term potentiation. *Nature reviews. Neuroscience, 13*(3), 169-182. doi:10.1038/nrn3192

Lissin, D. V., Gomperts, S. N., Carroll, R. C., Christine, C. W., Kalman, D., Kitamura, M., et al. (1998). Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. *Proc Natl Acad Sci U S A, 95*(12), 7097-7102.

Liu, H., & Au-Yeung, S. S. Y. (2014). Reliability of transcranial magnetic stimulation induced corticomotor excitability measurements for a hand muscle in healthy and chronic stroke subjects. *Journal of the Neurological Sciences, 341*(1), 105-109. doi:10.1016/j.jns.2014.04.012

Lloyd, D., Findlay, G., Roberts, N., & Nurmikko, T. (2008). Differences in low back pain behavior are reflected in the cerebral response to tactile stimulation of the lower back. *Spine, 33*(12), 1372-1377. doi:10.1097/BRS.0b013e3181734a8a

Lluch, E., Torres, R., Nijs, J., & Van Oosterwijck, J. (2014). Evidence for central sensitization in patients with osteoarthritis pain: a systematic literature review. *Eur J Pain, 18*(10), 1367-1375. doi:10.1002/j.1532-2149.2014.499.x

Loeser, J. D., & Treede, R. D. (2008). The Kyoto protocol of IASP Basic Pain Terminology. *Pain, 137*(3), 473-477. doi:10.1016/j.pain.2008.04.025

Lomo, T. (1966). Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. *Acta Physiol. Scand, 68*(Suppl 277), 28. doi:citeulike-article-id:3133405

Lopez-Alonso, V., Cheeran, B., Rio-Rodriguez, D., & Fernandez-Del-Olmo, M. (2014). Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain Stimul, 7*(3), 372-380. doi:10.1016/j.brs.2014.02.004

Lopez-Alonso, V., Fernandez-Del-Olmo, M., Costantini, A., Gonzalez-Henriquez, J. J., & Cheeran, B. (2015). Intra-individual variability in the response to anodal transcranial direct current stimulation. *Clin Neurophysiol*. doi:10.1016/j.clinph.2015.03.022

Lu, W., Man, H., Ju, W., Trimble, W. S., MacDonald, J. F., & Wang, Y. T. (2001). Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron, 29*(1), 243-254.

Lüscher, C., & Frerking, M. (2001). Restless AMPA receptors: implications for synaptic transmission and plasticity. *Trends Neurosci., 24*(11), 665-670.

Lynch, G. S., Dunwiddie, T., & Gribkoff, V. (1977). Heterosynaptic depression: a postsynaptic correlate of long-term potentiation. *Nature, 266*(5604), 737-739.

Macdermid, J. (2005). Update: The Patient-rated Forearm Evaluation Questionnaire is now the Patient-rated Tennis Elbow Evaluation. *J Hand Ther, 18*(4), 407-410. doi:10.1197/j.jht.2005.07.002

Madison, D. V., Malenka, R. C., & Nicoll, R. A. (1991). Mechanisms Underlying Long-Term Potentiation of Synaptic Transmission. *Annu. Rev. Neurosci., 14*(1), 379-397. doi:10.1146/annurev.ne.14.030191.002115

Maeda, F., Keenan, J. P., Tormos, J. M., Topka, H., & Pascual-Leone, A. (2000a). Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. *Exp Brain Res, 133*(4), 425-430.

Maeda, F., Keenan, J. P., Tormos, J. M., Topka, H., & Pascual-Leone, A. (2000b). Modulation of corticospinal excitability by repetitive transcranial magnetic stimulation. *Clin Neurophysiol, 111*(5), 800-805. doi:10.1016/S1388-2457(99)00323-5

Maeda, Y., Kettner, N., Sheehan, J., Kim, J., Cina, S., Malatesta, C., et al. (2013). Altered brain morphometry in carpal tunnel syndrome is associated with median nerve pathology. *Neuroimage Clin, 2*, 313-319. doi:10.1016/j.nicl.2013.02.001

Maihofner, C., Handwerker, H. O., Neundorfer, B., & Birklein, F. (2004). Cortical reorganization during recovery from complex regional pain syndrome. *Neurology*, *63*(4), 693-701. doi:10.1212/01.WNL.0000134661.46658.B0

Maihöfner, C., Handwerker, H. O., Neundörfer, B., & Birklein, F. (2003). Patterns of cortical reorganization in complex regional pain syndrome. *Neurology*, *61*(12), 1707-1715. doi:10.1212/01.wnl.0000098939.02752.8e

Malcolm, M. P., Triggs, W. J., Light, K. E., Shechtman, O., Khandekar, G., & Gonzalez Rothi, L. J. (2006). Reliability of motor cortex transcranial magnetic stimulation in four muscle representations. *Clin Neurophysiol*, *117*(5), 1037-1046. doi:10.1016/j.clinph.2006.02.005

Malenka, R. C., & Bear, M. F. (2004). LTP and LTD: An Embarrassment of Riches. *Neuron, 44*(1), 5-21. doi:10.1016/j.neuron.2004.09.012

Maren, S., & Baudry, M. (1995). Properties and mechanisms of long-term synaptic plasticity in the mammalian brain: relationships to learning and memory. *Neurobiol Learn Mem, 63*(1), 1-18. doi:10.1006/nlme.1995.1001

Martin, S. J., Grimwood, P. D., & Morris, R. G. (2000). Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci, 23*, 649-711. doi:10.1146/annurev.neuro.23.1.649

Masse-Alarie, H., Beaulieu, L. D., Preuss, R., & Schneider, C. (2016). Corticomotor control of lumbar multifidus muscles is impaired in chronic low back pain: concurrent evidence from ultrasound imaging and double-pulse transcranial magnetic stimulation. *Exp Brain Res, 234*(4), 1033-1045. doi:10.1007/s00221-015-4528-x

Masse-Alarie, H., & Schneider, C. (2016). Revisiting the Corticomotor Plasticity in Low Back Pain: Challenges and Perspectives. *Healthcare (Basel), 4*(3). doi:10.3390/healthcare4030067

Massey, P. V., & Bashir, Z. I. (2007). Long-term depression: multiple forms and implications for brain function. *Trends Neurosci, 30*(4), 176-184. doi:10.1016/j.tins.2007.02.005

Mastroeni, C., Bergmann, T. O., Rizzo, V., Ritter, C., Klein, C., Pohlmann, I., et al. (2013). **Brain-derived neurotrophic factor--a major player in stimulation-induced**

homeostatic metaplasticity of human motor cortex? *PLoS One, 8*(2), e57957. doi:10.1371/journal.pone.0057957

Matamala, J. M., Howells, J., Dharmadasa, T., Trinh, T., Ma, Y., Lera, L., et al. (2018). Inter-session reliability of short-interval intracortical inhibition measured by threshold tracking TMS. *Neurosci Lett*. doi:10.1016/j.neulet.2018.02.065

Matheson, N. A., Shemmell, J. B. H., De Ridder, D., & Reynolds, J. N. J. (2016). Understanding the Effects of Repetitive Transcranial Magnetic Stimulation on Neuronal Circuits. *Frontiers in Neural Circuits, 10*, 67. doi:10.3389/fncir.2016.00067

May, A. (2008). Chronic pain may change the structure of the brain. *PAIN®, 137*(1), 7-15. doi:10.1016/j.pain.2008.02.034

Mayford, M., Siegelbaum, S. A., & Kandel, E. R. (2012). Synapses and Memory Storage. *Cold Spring Harb. Perspect. Biol., 4*(6), a005751. doi:10.1101/cshperspect.a005751

McCormick, D. A. (2014). Membrane Potential and Action Potential *From Molecules to Networks (Third Edition)* (pp. 351-376). Boston: Academic Press.

McGraw, K. O., & Wong, S. P. (1996). Forming inferences about some intraclass correlation coefficients. *Psychol. Methods, 1*(1), 30-46. doi:10.1037/1082-989X.1.1.30

Melzack, P. D. R. (2005). The McGill Pain QuestionnaireFrom Description to Measurement. *Anesthesiology, 103*(1), 199-202.

Melzack, R., & Wall, P. D. (1965). Pain mechanisms: a new theory. *Science, 150*(3699), 971-979. doi:10.1126/science.150.3699.971

Merrill, D. R., Bikson, M., & Jefferys, J. G. (2005). Electrical stimulation of excitable tissue: design of efficacious and safe protocols. *J Neurosci Methods, 141*(2), 171-198. doi:10.1016/j.jneumeth.2004.10.020

Merzenich, M. M., Nelson, R. J., Stryker, M. P., Cynader, M. S., Schoppmann, A., & Zook, J. M. (1984). Somatosensory cortical map changes following digit amputation in adult monkeys. *J Comp Neurol, 224*(4), 591-605. doi:10.1002/cne.902240408

Minhas, P., Datta, A., & Bikson, M. (2011). Cutaneous perception during tDCS: role of electrode shape and sponge salinity. *Clin Neurophysiol, 122*(4), 637-638. doi:10.1016/j.clinph.2010.09.023

Moliadze, V., Antal, A., & Paulus, W. (2010). Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. *Clin Neurophysiol, 121*(12), 2165-2171. doi:10.1016/j.clinph.2010.04.033

Morton, D. L., Sandhu, J. S., & Jones, A. K. P. (2016). Brain imaging of pain: state of the art. *J. Pain Res., 9,* 613-624. doi:10.2147/JPR.S60433

Moseley, G. L. (2003). A pain neuromatrix approach to patients with chronic pain. *Man Ther, 8*(3), 130-140. doi:10.1016/S1356-689X(03)00051-1

Moseley, G. L. (2007). Reconceptualising pain according to modern pain science. *Phys. Ther. Rev., 12*(3), 169-178. doi:10.1179/108331907X223010

Moseley, G. L., & Flor, H. (2012). Targeting cortical representations in the treatment of chronic pain: a review. *Neurorehabil Neural Repair, 26*(6), 646-652. doi:10.1177/1545968311433209

Moser, E. I., Krobert, K. A., Moser, M.-B., & Morris, R. G. M. (1998). Impaired Spatial Learning after Saturation of Long-Term Potentiation. *Science, 281*(5385), 2038-2042. doi:10.1126/science.281.5385.2038

Moser, M. B., Trommald, M., & Andersen, P. (1994). An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc. Natl. Acad. Sci. U. S. A., 91*(26), 12673-12675.

Mosier, D. R. (2010). CHAPTER 1 - Clinical Neuroscience A2 - Rolak, Loren A *Neurology Secrets (Fifth Edition)* (pp. 7-17). Philadelphia: Mosby.

Mulkey, R. M., & Malenka, R. C. (1992). Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron, 9*(5), 967-975.

Muller-Dahlhaus, F., & Ziemann, U. (2015). Metaplasticity in human cortex. *Neuroscientist, 21*(2), 185-202. doi:10.1177/1073858414526645

Müller-Dahlhaus, F., Ziemann, U., & Classen, J. (2010). Plasticity Resembling Spike-Timing Dependent Synaptic Plasticity: The Evidence in Human Cortex. *Front. Synaptic Neurosci., 2*, 34. doi:10.3389/fnsyn.2010.00034

Muller-Dahlhaus, J. F., Orekhov, Y., Liu, Y., & Ziemann, U. (2008). Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. *Exp Brain Res, 187*(3), 467-475. doi:10.1007/s00221-008-1319-7

Muller, J. F., Orekhov, Y., Liu, Y., & Ziemann, U. (2007). Homeostatic plasticity in human motor cortex demonstrated by two consecutive sessions of paired associative stimulation. *Eur J Neurosci, 25*(11), 3461-3468. doi:10.1111/j.1460-9568.2007.05603.x

Muller, M., & Davis, G. W. (2012). Transsynaptic control of presynaptic Ca(2)(+) influx achieves homeostatic potentiation of neurotransmitter release. *Curr Biol, 22*(12), 1102-1108. doi:10.1016/j.cub.2012.04.018

Murakami, T., Muller-Dahlhaus, F., Lu, M. K., & Ziemann, U. (2012). Homeostatic metaplasticity of corticospinal excitatory and intracortical inhibitory neural circuits in human motor cortex. *J Physiol, 590*(Pt 22), 5765-5781. doi:10.1113/jphysiol.2012.238519

Murase, N., Cengiz, B., & Rothwell, J. C. (2015). Inter-individual variation in the after-effect of paired associative stimulation can be predicted from short-interval intracortical inhibition with the threshold tracking method. *Brain Stimul, 8*(1), 105-113. doi:10.1016/j.brs.2014.09.010

Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol Rev Camb Philos Soc, 82*(4), 591-605. doi:10.1111/j.1469-185X.2007.00027.x

Napadow, V., Kettner, N., Ryan, A., Kwong, K. K., Audette, J., & Hui, K. K. (2006). Somatosensory cortical plasticity in carpal tunnel syndrome--a cross-sectional fMRI evaluation. *Neuroimage, 31*(2), 520-530. doi:10.1016/j.neuroimage.2005.12.017

Neogi, T., Frey-Law, L., Scholz, J., Niu, J., Arendt-Nielsen, L., Woolf, C., et al. (2015). Sensitivity and sensitisation in relation to pain severity in knee osteoarthritis: trait or state? *Ann Rheum Dis, 74*(4), 682-688. doi:10.1136/annrheumdis-2013-204191

Nettekoven, C., Volz, L. J., Leimbach, M., Pool, E.-M., Rehme, A. K., Eickhoff, S. B., et al. (2015). Inter-individual variability in cortical excitability and motor network connectivity following multiple blocks of rTMS. *NeuroImage, 118*, 209-218. doi:10.1016/j.neuroimage.2015.06.004

Newton, B. J., Southall, J. L., Raphael, J. H., Ashford, R. L., & LeMarchand, K. (2013). A Narrative Review of the Impact of Disbelief in Chronic Pain. *Pain Manag. Nurs., 14*(3), 161-171. doi:10.1016/j.pmn.2010.09.001

Neziri, A. Y., Manresa, J. B., Jüni, P., Arendt-Nielsen, L., Andersen, O. K., & Curatolo, M. (2011). F123 EXPANSION OF NOCICEPTIVE REFLEX RECEPTIVE FIELDS IN PATIENTS WITH LOW BACK AND NECK PAIN. *Eur. J. Pain Suppl., 5*(S1), 109-110. doi:10.1016/S1754-3207(11)70372-8

Ngodup, T., Goetz, J. A., McGuire, B. C., Sun, W., Lauer, A. M., & Xu-Friedman, M. A. (2015). Activity-dependent, homeostatic regulation of neurotransmitter release from

auditory nerve fibers. *Proc Natl Acad Sci U S A, 112*(20), 6479-6484. doi:10.1073/pnas.1420885112

Ni, Z., Gunraj, C., Kailey, P., Cash, R. F., & Chen, R. (2014). Heterosynaptic modulation of motor cortical plasticity in human. *J Neurosci, 34*(21), 7314-7321. doi:10.1523/JNEUROSCI.4714-13.2014

Nicoll, R. A. (2017). A Brief History of Long-Term Potentiation. *Neuron, 93*(2), 281-290. doi:10.1016/j.neuron.2016.12.015

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., et al. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimul, 1*(3), 206-223. doi:10.1016/j.brs.2008.06.004

Nitsche, M. A., Doemkes, S., Karakose, T., Antal, A., Liebetanz, D., Lang, N., et al. (2007a). Shaping the effects of transcranial direct current stimulation of the human motor cortex. *J Neurophysiol, 97*(4), 3109-3117. doi:10.1152/jn.01312.2006

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., et al. (2003a). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *J Physiol, 553*(Pt 1), 293-301. doi:10.1113/jphysiol.2003.049916

Nitsche, M. A., Jaussi, W., Liebetanz, D., Lang, N., Tergau, F., & Paulus, W. (2004). Consolidation of human motor cortical neuroplasticity by D-cycloserine. *Neuropsychopharmacology, 29*(8), 1573-1578. doi:10.1038/sj.npp.1300517

Nitsche, M. A., Liebetanz, D., Lang, N., Antal, A., Tergau, F., & Paulus, W. (2003b). Safety criteria for transcranial direct current stimulation (tDCS) in humans. *Clin Neurophysiol, 114*(11), 2220-2222; author reply 2222-2223. doi:10.1016/S1388-2457(03)00235-9

Nitsche, M. A., Muller-Dahlhaus, F., Paulus, W., & Ziemann, U. (2012). The pharmacology of neuroplasticity induced by non-invasive brain stimulation: building models for the clinical use of CNS active drugs. *J Physiol, 590*(19), 4641-4662. doi:10.1113/jphysiol.2012.232975

Nitsche, M. A., Nitsche, M. S., Klein, C. C., Tergau, F., Rothwell, J. C., & Paulus, W. (2003c). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clin Neurophysiol, 114*(4), 600-604. doi:10.1016/S1388-2457(02)00412-1

Nitsche, M. A., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol, 527 Pt 3*, 633-639. doi:10.1111/j.1469-7793.2000.t01-1-00633.x

Nitsche, M. A., & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology, 57*(10), 1899-1901. doi:10.1212/WNL.57.10.1899

Nitsche, M. A., & Paulus, W. (2011). Transcranial direct current stimulation--update 2011. *Restor Neurol Neurosci, 29*(6), 463-492. doi:10.3233/RNN-2011-0618

Nitsche, M. A., Roth, A., Kuo, M. F., Fischer, A. K., Liebetanz, D., Lang, N., et al. (2007b). Timing-dependent modulation of associative plasticity by general network excitability in the human motor cortex. *J Neurosci, 27*(14), 3807-3812. doi:10.1523/JNEUROSCI.5348-06.2007

Nitsche, M. A., Seeber, A., Frommann, K., Klein, C. C., Rochford, C., Nitsche, M. S., et al. (2005). Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *The Journal of Physiology, 568*(Pt 1), 291-303. doi:10.1113/jphysiol.2005.092429

Nudo, R. J. (2013). Recovery after brain injury: mechanisms and principles. *Frontiers in Human Neuroscience, 7*, 887. doi:10.3389/fnhum.2013.00887

O'Brien, R. J., & Fischbach, G. D. (1986). Modulation of embryonic chick motoneuron glutamate sensitivity by interneurons and agonists. *J Neurosci, 6*(11), 3290-3296.

O'Brien, R. J., Kamboj, S., Ehlers, M. D., Rosen, K. R., Fischbach, G. D., & Huganir, R. L. (1998). Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron, 21*(5), 1067-1078.

O'Connell, N. E., Wand, B. M., Marston, L., Spencer, S., & Desouza, L. H. (2010). Non-invasive brain stimulation techniques for chronic pain. *Cochrane Database Syst Rev*(9), Cd008208. doi:10.1002/14651858.CD008208.pub2

Okifuji, A., & Ackerlind, S. (2007). Behavioral medicine approaches to pain. *Med Clin North Am, 91*(1), 45-55. doi:10.1016/j.mcna.2006.10.008

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia, 9*(1), 97-113.

Olesen, A. E., Andresen, T., Staahl, C., & Drewes, A. M. (2012). Human experimental pain models for assessing the therapeutic efficacy of analgesic drugs. *Pharmacol Rev, 64*(3), 722-779. doi:10.1124/pr.111.005447

Opie, G. M., Post, A. K., Ridding, M. C., Ziemann, U., & Semmler, J. G. (2017a). Modulating motor cortical neuroplasticity with priming paired associative stimulation in young and old adults. *Clin Neurophysiol, 128*(5), 763-769. doi:10.1016/j.clinph.2017.02.011

Opie, G. M., Vosnakis, E., Ridding, M. C., Ziemann, U., & Semmler, J. G. (2017b). Priming theta burst stimulation enhances motor cortex plasticity in young but not old adults. *Brain Stimul, 10*(2), 298-304. doi:10.1016/j.brs.2017.01.003

Ottersen, O. P., & Helm, P. J. (2002). How hardwired is the brain? *Nature, 420*(6917), 751-752. doi:10.1038/420751a

Page, S. J., & Persch, A. C. (2013). Recruitment, Retention, and Blinding in Clinical Trials. *The American Journal of Occupational Therapy, 67*(2), 154-161. doi:10.5014/ajot.2013.006197

Pascual-Leone, A., Amedi, A., Fregni, F., & Merabet, L. B. (2005). The plastic human brain cortex. *Annu Rev Neurosci, 28*, 377-401. doi:10.1146/annurev.neuro.27.070203.144216

Pelletier, R., Higgins, J., & Bourbonnais, D. (2015). Is neuroplasticity in the central nervous system the missing link to our understanding of chronic musculoskeletal disorders? *BMC musculoskeletal disorders, 16*, 25. doi:10.1186/s12891-015-0480-y

Perl, E. R. (2007). Ideas about pain, a historical view. *Nat Rev Neurosci, 8*(1), 71-80. doi:10.1038/nrn2042

Petty, B. G., Cornblath, D. R., Adornato, B. T., Chaudhry, V., Flexner, C., Wachsman, M., et al. (1994). The effect of systemically administered recombinant human nerve growth factor in healthy human subjects. *Ann Neurol, 36*(2), 244-246. doi:10.1002/ana.410360221

Popa, T., Velayudhan, B., Hubsch, C., Pradeep, S., Roze, E., Vidailhet, M., et al. (2013). Cerebellar processing of sensory inputs primes motor cortex plasticity. *Cereb Cortex, 23*(2), 305-314. doi:10.1093/cercor/bhs016

Poreisz, C., Boros, K., Antal, A., & Paulus, W. (2007). Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain Res Bull, 72*(4-6), 208-214. doi:10.1016/j.brainresbull.2007.01.004

Potter-Nerger, M., Fischer, S., Mastroeni, C., Groppa, S., Deuschl, G., Volkmann, J., et al. (2009). Inducing homeostatic-like plasticity in human motor cortex through converging corticocortical inputs. *J Neurophysiol, 102*(6), 3180-3190. doi:10.1152/jn.91046.2008

Priori, A., Berardelli, A., Rona, S., Accornero, N., & Manfredi, M. (1998). Polarization of the human motor cortex through the scalp. *Neuroreport, 9*(10), 2257-2260. doi:10.1097/00001756-199807130-00020

Purpura, D. P., & McMurtry, J. G. (1965). INTRACELLULAR ACTIVITIES AND EVOKED POTENTIAL CHANGES DURING POLARIZATION OF MOTOR CORTEX. *J Neurophysiol, 28*, 166-185. doi:10.1152/jn.1965.28.1.166

Quartarone, A., Bagnato, S., Rizzo, V., Siebner, H. R., Dattola, V., Scalfari, A., et al. (2003). Abnormal associative plasticity of the human motor cortex in writer's cramp. *Brain, 126*(Pt 12), 2586-2596. doi:10.1093/brain/awg273

Quartarone, A., Morgante, F., Sant'angelo, A., Rizzo, V., Bagnato, S., Terranova, C., et al. (2008). Abnormal plasticity of sensorimotor circuits extends beyond the affected body part in focal dystonia. *J Neurol Neurosurg Psychiatry, 79*(9), 985-990. doi:10.1136/jnnp.2007.121632

Quartarone, A., & Pisani, A. (2011). Abnormal plasticity in dystonia: Disruption of synaptic homeostasis. *Neurobiol Dis, 42*(2), 162-170. doi:10.1016/j.nbd.2010.12.011

Quartarone, A., Rizzo, V., Bagnato, S., Morgante, F., Sant'Angelo, A., Romano, M., et al. (2005). Homeostatic-like plasticity of the primary motor hand area is impaired in focal hand dystonia. *Brain, 128*(Pt 8), 1943-1950. doi:10.1093/brain/awh527

Quartarone, A., Siebner, H. R., & Rothwell, J. C. (2006). Task-specific hand dystonia: can too much plasticity be bad for you? *Trends Neurosci, 29*(4), 192-199. doi:10.1016/j.tins.2006.02.007

Ragert, P., Camus, M., Vandermeeren, Y., Dimyan, M. A., & Cohen, L. G. (2009). Modulation of Effects of Intermittent Theta Burst Stimulation Applied Over Primary

Motor Cortex (M1) by Conditioning Stimulation of the Opposite M1. *Journal of Neurophysiology, 102*(2), 766-773. doi:10.1152/jn.00274.2009

Reddy, K. S. k., Naidu, M. U. R., Rani, P. U., & Rao, T. R. K. (2012). Human experimental pain models: A review of standardized methods in drug development. *Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences, 17*(6), 587-595.

Richardson, J. C. (2005). Establishing the (extra)ordinary in chronic widespread pain. *Health (London, England : 1997), 9*(1), 31-48. doi:10.1177/1363459305048096

Richardson, J. T. E. (2011). Eta squared and partial eta squared as measures of effect size in educational research. *Educational Research Review, 6*(2), 135-147. doi:10.1016/j.edurev.2010.12.001

Rioult-Pedotti, M. S., Friedman, D., & Donoghue, J. P. (2000). Learning-induced LTP in neocortex. *Science, 290*(5491), 533-536. doi:10.1126/science.290.5491.533

Rosenkranz, K., Kacar, A., & Rothwell, J. C. (2007). Differential Modulation of Motor Cortical Plasticity and Excitability in Early and Late Phases of Human Motor Learning. *The Journal of Neuroscience, 27*(44), 12058-12066. doi:10.1523/jneurosci.2663-07.2007

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., & Safety of, T. M. S. C. G. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol, 120*(12), 2008-2039. doi:10.1016/j.clinph.2009.08.016

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., et al. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol, 91*(2), 79-92. doi:10.1016/0013-4694(94)90029-9

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., et al. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin Neurophysiol, 126*(6), 1071-1107. doi:10.1016/j.clinph.2015.02.001

Rossini, P. M., Desiato, M. T., Lavaroni, F., & Caramia, M. D. (1991). Brain excitability and electroencephalographic activation: non-invasive evaluation in healthy humans via transcranial magnetic stimulation. *Brain Res, 567*(1), 111-119. doi:10.1016/0006-8993(91)91442-4

Rothwell, J. C., Hallett, M., Berardelli, A., Eisen, A., Rossini, P., & Paulus, W. (1999). Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl, 52*, 97-103.

Sacchetti, B., Lorenzini, C. A., Baldi, E., Bucherelli, C., Roberto, M., Tassoni, G., et al. (2001). Long-lasting hippocampal potentiation and contextual memory consolidation. *Eur J Neurosci, 13*(12), 2291-2298.

Sacktor, T. C. (2008). PKMzeta, LTP maintenance, and the dynamic molecular biology of memory storage. *Prog Brain Res, 169*, 27-40. doi:10.1016/s0079-6123(07)00002-7

Sauvé, W. M., & Crowther, L. J. (2014). The Science of Transcranial Magnetic Stimulation. *Psychiatric Annals, 44*(6), 279-283. doi:10.3928/00485713-20140609-05

Schabrun, S. M., Burns, E., & Hodges, P. W. (2015a). New Insight into the Time-Course of Motor and Sensory System Changes in Pain. *PLOS ONE, 10*(11), e0142857. doi:10.1371/journal.pone.0142857

Schabrun, S. M., Burns, E., Thapa, T., & Hodges, P. (2017a). The Response of the Primary Motor Cortex to Neuromodulation is Altered in Chronic Low Back Pain: A Preliminary Study. *Pain Med*. doi:10.1093/pm/pnx168

Schabrun, S. M., Christensen, S. W., Mrachacz-Kersting, N., & Graven-Nielsen, T. (2016). Motor Cortex Reorganization and Impaired Function in the Transition to Sustained Muscle Pain. *Cereb Cortex, 26*(5), 1878-1890. doi:10.1093/cercor/bhu319

Schabrun, S. M., Elgueta-Cancino, E. L., & Hodges, P. W. (2015b). Smudging of the Motor Cortex is related to the Severity of Low Back Pain. *Spine*. doi:10.1097/BRS.0000000000000938

Schabrun, S. M., Elgueta-Cancino, E. L., & Hodges, P. W. (2017b). Smudging of the Motor Cortex Is Related to the Severity of Low Back Pain. *Spine (Phila Pa 1976), 42*(15), 1172-1178. doi:10.1097/brs.0000000000000938

Schabrun, S. M., Hodges, P. W., Vicenzino, B., Jones, E., & Chipchase, L. S. (2015c). Novel adaptations in motor cortical maps: the relation to persistent elbow pain. *Med Sci Sports Exerc, 47*(4), 681-690. doi:10.1249/MSS.0000000000000469

Schabrun, S. M., Jones, E., Kloster, J., & Hodges, P. W. (2013). Temporal association between changes in primary sensory cortex and corticomotor output during muscle pain. *Neuroscience, 235*, 159-164. doi:10.1016/j.neuroscience.2012.12.072

Schabrun, S. M., Stinear, C. M., Byblow, W. D., & Ridding, M. C. (2009a). Normalizing motor cortex representations in focal hand dystonia. *Cereb Cortex, 19*(9), 1968-1977. doi:10.1093/cercor/bhn224

Schabrun, S. M., Stinear, C. M., Byblow, W. D., & Ridding, M. C. (2009b). Normalizing Motor Cortex Representations in Focal Hand Dystonia. *Cerebral cortex, 19*(9), 1968-1977. doi:10.1093/cercor/bhn224

Schambra, H. M., Ogden, R. T., Martinez-Hernandez, I. E., Lin, X., Chang, Y. B., Rahman, A., et al. (2015). The reliability of repeated TMS measures in older adults and in patients with subacute and chronic stroke. *Front Cell Neurosci, 9*, 335. doi:10.3389/fncel.2015.00335

Schliessbach, J., Vuilleumier, P. H., Siegenthaler, A., Bütikofer, L., Limacher, A., Juni, P., et al. (2017). Analgesic effect of clobazam in chronic low-back pain but not in experimentally induced pain. *European Journal of Pain, 21*(8), 1336-1345. doi:10.1002/ejp.1032

Schmidt-Wilcke, T., Leinisch, E., Ganssbauer, S., Draganski, B., Bogdahn, U., Altmeppen, J., et al. (2006). Affective components and intensity of pain correlate with structural differences in gray matter in chronic back pain patients. *Pain, 125*(1-2), 89-97. doi:10.1016/j.pain.2006.05.004

Schwenkreis, P., Janssen, F., Rommel, O., Pleger, B., Volker, B., Hosbach, I., et al. (2003). Bilateral motor cortex disinhibition in complex regional pain syndrome (CRPS) type I of the hand. *Neurology, 61*(4), 515-519. doi:10.1212/WNL.61.4.515

Schwenkreis, P., Witscher, K., Pleger, B., Malin, J.-P., & Tegenthoff, M. (2005). The NMDA antagonist memantine affects training induced motor cortex plasticity--a study using transcranial magnetic stimulation. *BMC neuroscience, 6*, 35-35. doi:10.1186/1471-2202-6-35

Sedgwick, P. (2014). Pitfalls of statistical hypothesis testing: type I and type II errors. *BMJ : British Medical Journal, 349*. doi:10.1136/bmj.g4287

Shadmehr, R., & Brashers-Krug, T. (1997). Functional stages in the formation of human long-term motor memory. *J Neurosci, 17*(1), 409-419.

Shanahan, C. J., Hodges, P. W., Wrigley, T. V., Bennell, K. L., & Farrell, M. J. (2015). Organisation of the motor cortex differs between people with and without knee osteoarthritis. *Arthritis Res Ther, 17*, 164. doi:10.1186/s13075-015-0676-4

Shors, T. J., Townsend, D. A., Zhao, M., Kozorovitskiy, Y., & Gould, E. (2002). Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus, 12*(5), 578-584. doi:10.1002/hipo.10103

Sidhu, S. K., Pourmajidian, M., Opie, G. M., & Semmler, J. G. (2017). Increasing motor cortex plasticity with spaced paired associative stimulation at different intervals in older adults. *Eur. J. Neurosci., 46*(11), 2674-2683. doi:10.1111/ejn.13729

Siebner, H. R., Auer, C., & Conrad, B. (1999). Abnormal increase in the corticomotor output to the affected hand during repetitive transcranial magnetic stimulation of the primary motor cortex in patients with writer's cramp. *Neurosci Lett, 262*(2), 133-136. doi:10.1016/S0304-3940(99)00056-7

Siebner, H. R., Lang, N., Rizzo, V., Nitsche, M. A., Paulus, W., Lemon, R. N., et al. (2004). Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *J Neurosci, 24*(13), 3379-3385. doi:10.1523/JNEUROSCI.5316-03.2004

Siebner, H. R., & Rothwell, J. (2003). Transcranial magnetic stimulation: new insights into representational cortical plasticity. *Exp Brain Res, 148*(1), 1-16. doi:10.1007/s00221-002-1234-2

Sorra, K. E., & Harris, K. M. (1998). Stability in synapse number and size at 2 hr after long-term potentiation in hippocampal area CA1. *J Neurosci, 18*(2), 658-671.

Soundara Rajan, T., Ghilardi, M. F. M., Wang, H.-Y., Mazzon, E., Bramanti, P., Restivo, D., et al. (2017). Mechanism of Action for rTMS: A Working Hypothesis Based on Animal Studies. *Front. Physiol., 8*, 457-457. doi:10.3389/fphys.2017.00457

Staahl, C., & Drewes, A. M. (2004). Experimental human pain models: a review of standardised methods for preclinical testing of analgesics. *Basic Clin Pharmacol Toxicol, 95*(3), 97-111. doi:10.1111/j.1742-7843.2004.950301.x

Stahnisch, F. W., & Nitsch, R. (2002). Santiago Ramon y Cajal's concept of neuronal plasticity: the ambiguity lives on. *Trends Neurosci, 25*(11), 589-591.

Stefan, K., Kunesch, E., Benecke, R., Cohen, L. G., & Classen, J. (2002). Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. *J Physiol, 543*(Pt 2), 699-708. doi:10.1113/jphysiol.2002.023317

Stefan, K., Kunesch, E., Cohen, L. G., Benecke, R., & Classen, J. (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain, 123 Pt 3*, 572-584. doi:10.1093/brain/123.3.572

Sterling, M., Jull, G., Vicenzino, B., & Kenardy, J. (2004). Characterization of acute whiplash-associated disorders. *Spine, 29*(2), 182-188. doi:10.1097/01.brs.0000105535.12598.ae

Sterling, M., Jull, G., & Wright, A. (2001). The effect of musculoskeletal pain on motor activity and control. *The Journal of Pain, 2*(3), 135-145. doi:10.1054/jpai.2001.19951

Strigaro, G., Hamada, M., Cantello, R., & Rothwell, J. C. (2016). Variability in response to 1Hz repetitive TMS. *Clinical Neurophysiology, 127*(3), e40. doi:10.1016/j.clinph.2015.11.126

Strutton, P. H., Catley, M., McGregor, A. H., & Davey, N. J. (2003). Corticospinal excitability in patients with unilateral sciatica. *Neurosci Lett, 353*(1), 33-36.

Strutton, P. H., Theodorou, S., Catley, M., McGregor, A. H., & Davey, N. J. (2005). Corticospinal excitability in patients with chronic low back pain. *J Spinal Disord Tech, 18*(5), 420-424.

Stuart, G., Spruston, N., Sakmann, B., & Häusser, M. (1997). Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends Neurosci., 20*(3), 125-131. doi:10.1016/S0166-2236(96)10075-8

Subramanian, J. (2011). Homeostatic plasticity of neurotransmitter release probability is determined by presynaptic calcium influx. *J Neurosci, 31*(31), 11097-11099. doi:10.1523/jneurosci.2734-11.2011

Südhof, T. C., & Malenka, R. C. (2008). Understanding Synapses: Past, Present, and Future. *Neuron, 60*(3), 469-476. doi:10.1016/j.neuron.2008.10.011

Suri, P., Morgenroth, D. C., & Hunter, D. J. (2012). Epidemiology of Osteoarthritis and Associated Comorbidities. *PM&R, 4*(5), S10-S19. doi:10.1016/j.pmrj.2012.01.007

Sutton, M. A., Ito, H. T., Cressy, P., Kempf, C., Woo, J. C., & Schuman, E. M. (2006). Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell, 125*(4), 785-799. doi:10.1016/j.cell.2006.03.040

Svensson, P., Beydoun, A., Morrow, T. J., & Casey, K. L. (1997). Non-painful and painful stimulation of human skin and muscle: analysis of cerebral evoked potentials. *Electroencephalogr Clin Neurophysiol, 104*(4), 343-350.

Sweatt, J. D. (2010). The NMDA Receptor *Mechanisms of Memory (Second Edition)* (pp. 190-207). London: Academic Press.

Te, M., Baptista, A. F., Chipchase, L. S., & Schabrun, S. M. (2017). Primary Motor Cortex Organization Is Altered in Persistent Patellofemoral Pain. *Pain Med, 18*(11), 2224-2234. doi:10.1093/pm/pnx036

Tecchio, F., Padua, L., Aprile, I., & Rossini, P. M. (2002). Carpal tunnel syndrome modifies sensory hand cortical somatotopy: a MEG study. *Human brain mapping, 17*(1), 28-36. doi:10.1002/hbm.10049

Thapa, T., Graven-Nielsen, T., Chipchase, L. S., & Schabrun, S. M. (2018a). Disruption of cortical synaptic homeostasis in individuals with chronic low back pain. *Clin Neurophysiol*. doi:10.1016/j.clinph.2018.01.060

Thapa, T., Graven-Nielsen, T., Chipchase, L. S., & Schabrun, S. M. (2018b). Disruption of cortical synaptic homeostasis in individuals with chronic low back pain. *Clinical Neurophysiology, 129*(5), 1090-1096. doi:10.1016/j.clinph.2018.01.060

Thapa, T., & Schabrun, S. M. (2018). Test-Retest Reliability of Homeostatic Plasticity in the Human Primary Motor Cortex. *Neural Plasticity, 2018*, 9. doi:10.1155/2018/6207508

Thiagarajan, T. C., Piedras-Renteria, E. S., & Tsien, R. W. (2002). alpha- and betaCaMKII. Inverse regulation by neuronal activity and opposing effects on synaptic strength. *Neuron, 36*(6), 1103-1114.

Thirugnanasambandam, N., Grundey, J., Paulus, W., & Nitsche, M. A. (2011). Dose-dependent nonlinear effect of L-DOPA on paired associative stimulation-induced neuroplasticity in humans. *J Neurosci, 31*(14), 5294-5299. doi:10.1523/jneurosci.6258-10.2011

Tinazzi, M., Zanette, G., Volpato, D., Testoni, R., Bonato, C., Manganotti, P., et al. (1998). Neurophysiological evidence of neuroplasticity at multiple levels of the somatosensory system in patients with carpal tunnel syndrome. *Brain, 121 (Pt 9)*, 1785-1794.

Todd, G., Flavel, S. C., & Ridding, M. C. (2009). Priming theta-burst repetitive transcranial magnetic stimulation with low- and high-frequency stimulation. *Exp Brain Res, 195*(2), 307-315. doi:10.1007/s00221-009-1791-8

Trachtenberg, J. T., Chen, B. E., Knott, G. W., Feng, G., Sanes, J. R., Welker, E., et al. (2002). Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature, 420*(6917), 788-794. doi:10.1038/nature01273

Treede, R.-D., Rief, W., Barke, A., Aziz, Q., Bennett, M. I., Benoliel, R., et al. (2015). A classification of chronic pain for ICD-11. *Pain, 156*(6), 1003-1007. doi:10.1097/j.pain.0000000000000160

Trommald, M., Hulleberg, G., & Andersen, P. (1996). Long-term potentiation is associated with new excitatory spine synapses on rat dentate granule cells. *Learn Mem, 3*(2-3), 218-228.

Tsao, H., Danneels, L., & Hodges, P. W. (2011a). Individual fascicles of the paraspinal muscles are activated by discrete cortical networks in humans. *Clin Neurophysiol, 122*(8), 1580-1587. doi:10.1016/j.clinph.2011.01.048

Tsao, H., Danneels, L. A., & Hodges, P. W. (2011b). ISSLS prize winner: Smudging the motor brain in young adults with recurrent low back pain. *Spine, 36*(21), 1721-1727. doi:10.1097/BRS.0b013e31821c4267

Tsao, H., Druitt, T. R., Schollum, T. M., & Hodges, P. W. (2010). Motor training of the lumbar paraspinal muscles induces immediate changes in motor coordination in patients with recurrent low back pain. *The journal of pain : official journal of the American Pain Society, 11*(11), 1120-1128. doi:10.1016/j.jpain.2010.02.004

Tsao, H., Galea, M. P., & Hodges, P. W. (2008). Reorganization of the motor cortex is associated with postural control deficits in recurrent low back pain. *Brain, 131*(Pt 8), 2161-2171. doi:10.1093/brain/awn154

Tsao, H., Tucker, K. J., & Hodges, P. W. (2011c). Changes in excitability of corticomotor inputs to the trunk muscles during experimentally-induced acute low back pain. *Neuroscience, 181*, 127-133. doi:10.1016/j.neuroscience.2011.02.033

Turrigiano, G. (2004). A competitive game of synaptic tag. *Neuron, 44*(6), 903-904. doi:10.1016/j.neuron.2004.12.011

Turrigiano, G. (2007). Homeostatic signaling: the positive side of negative feedback. *Curr Opin Neurobiol, 17*(3), 318-324. doi:10.1016/j.conb.2007.04.004

Turrigiano, G. (2011). Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement. *Annu Rev Neurosci, 34*, 89-103. doi:10.1146/annurev-neuro-060909-153238

Turrigiano, G. (2012). Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. *Cold Spring Harb Perspect Biol, 4*(1), a005736. doi:10.1101/cshperspect.a005736

Turrigiano, G., LeMasson, G., & Marder, E. (1995). Selective regulation of current densities underlies spontaneous changes in the activity of cultured neurons. *The Journal of Neuroscience, 15*(5), 3640-3652. doi:10.1523/jneurosci.15-05-03640.1995

Turrigiano, G. G. (1999). Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci, 22*(5), 221-227. doi:10.1016/S0166-2236(98)01341-1

Turrigiano, G. G. (2006). More than a sidekick: glia and homeostatic synaptic plasticity. *Trends Mol Med, 12*(10), 458-460. doi:10.1016/j.molmed.2006.08.002

Turrigiano, G. G. (2008). The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell, 135*(3), 422-435. doi:10.1016/j.cell.2008.10.008

Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C., & Nelson, S. B. (1998). Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature, 391*(6670), 892-896. doi:10.1038/36103

Turrigiano, G. G., & Nelson, S. B. (2000). Hebb and homeostasis in neuronal plasticity. *Curr Opin Neurobiol, 10*(3), 358-364. doi:10.1016/S0959-4388(00)00091-X

Turrigiano, G. G., & Nelson, S. B. (2004). Homeostatic plasticity in the developing nervous system. *Nat Rev Neurosci, 5*(2), 97-107. doi:10.1038/nrn1327

Uy, J., Ridding, M. C., & Miles, T. S. (2002). Stability of maps of human motor cortex made with transcranial magnetic stimulation. *Brain Topogr, 14*(4), 293-297. doi:10.1023/A:1015752711146

van Hecke, O., Torrance, N., & Smith, B. H. (2013). Chronic pain epidemiology – where do lifestyle factors fit in? *British Journal of Pain, 7*(4), 209-217. doi:10.1177/2049463713493264

van Hemmen, J. L. (2001). Chapter 18 Theory of synaptic plasticity. In F. Moss & S. Gielen (Eds.), *Handbook of Biological Physics* (Vol. 4, pp. 771-823): North-Holland.

Van Oosterwijck, J., Nijs, J., Meeus, M., & Paul, L. (2013). Evidence for central sensitization in chronic whiplash: A systematic literature review. *European Journal of Pain, 17*(3), 299-312. doi:10.1002/j.1532-2149.2012.00193.x

van Tulder, M. W., Assendelft, W. J., Koes, B. W., & Bouter, L. M. (1997). Spinal radiographic findings and nonspecific low back pain. A systematic review of observational studies. *Spine, 22*(4), 427-434.

Vidal-Dourado, M., Conforto, A. B., Caboclo, L. O., Scaff, M., Guilhoto, L. M., & Yacubian, E. M. (2014). Magnetic fields in noninvasive brain stimulation. *Neuroscientist, 20*(2), 112-121. doi:10.1177/1073858413491145

Vitureira, N., Letellier, M., & Goda, Y. (2012). Homeostatic synaptic plasticity: from single synapses to neural circuits. *Curr. Opin. Neurobiol., 22*(3), 516-521. doi:10.1016/j.conb.2011.09.006

Wagner, T., Valero-Cabre, A., & Pascual-Leone, A. (2007). Noninvasive human brain stimulation. *Annual review of biomedical engineering, 9*, 527-565. doi:10.1146/annurev.bioeng.9.061206.133100

Waldman, S. D. (2009). Pain Assessment Tools for Adults. In S. D. Waldman (Ed.), *Pain Review* (pp. 375-380). Philadelphia: W.B. Saunders.

Wand, B. M., Parkitny, L., O'Connell, N. E., Luomajoki, H., McAuley, J. H., Thacker, M., et al. (2011). Cortical changes in chronic low back pain: current state of the art and implications for clinical practice. *Man Ther, 16*(1), 15-20. doi:10.1016/j.math.2010.06.008

Wang, C. K., Myunghae Hah, J., & Carroll, I. (2009). Factors contributing to pain chronicity. *Curr Pain Headache Rep, 13*(1), 7-11.

Wang, G., Gilbert, J., & Man, H. Y. (2012). AMPA receptor trafficking in homeostatic synaptic plasticity: functional molecules and signaling cascades. *Neural Plast, 2012*, 825364. doi:10.1155/2012/825364

Wang, G., & Thompson, S. M. (2008). Maladaptive homeostatic plasticity in a rodent model of central pain syndrome: thalamic hyperexcitability after spinothalamic tract lesions. *J Neurosci, 28*(46), 11959-11969. doi:10.1523/jneurosci.3296-08.2008

Wankerl, K., Weise, D., Gentner, R., Rumpf, J. J., & Classen, J. (2010). L-type voltage-gated Ca2+ channels: a single molecular switch for long-term potentiation/long-term depression-like plasticity and activity-dependent metaplasticity in humans. *J Neurosci, 30*(18), 6197-6204. doi:10.1523/JNEUROSCI.4673-09.2010

Wassermann, E. M. (1998). Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. *Electroencephalogr Clin Neurophysiol, 108*(1), 1-16. doi:10.1016/S0168-5597(97)00096-8

Watson, D. J., Ostroff, L., Cao, G., Parker, P. H., Smith, H., & Harris, K. M. (2016). LTP enhances synaptogenesis in the developing hippocampus. *Hippocampus, 26*(5), 560-576. doi:10.1002/hipo.22536

Weise, D., Schramm, A., Beck, M., Reiners, K., & Classen, J. (2011). Loss of topographic specificity of LTD-like plasticity is a trait marker in focal dystonia. *Neurobiol Dis, 42*(2), 171-176. doi:10.1016/j.nbd.2010.11.009

Weise, D., Schramm, A., Stefan, K., Wolters, A., Reiners, K., Naumann, M., et al. (2006). The two sides of associative plasticity in writer's cramp. *Brain, 129*(Pt 10), 2709-2721. doi:10.1093/brain/awl221

Welch, K. M. (2003). Contemporary concepts of migraine pathogenesis. *Neurology, 61*(8 Suppl 4), S2-8. doi:10.1212/WNL.61.8_suppl_4.S2

Werner, A., & Malterud, K. (2003). It is hard work behaving as a credible patient: encounters between women with chronic pain and their doctors. *Soc Sci Med, 57*(8), 1409-1419. doi:10.1016/S0277-9536(02)00520-8

Weyhersmuller, A., Hallermann, S., Wagner, N., & Eilers, J. (2011). Rapid active zone remodeling during synaptic plasticity. *J Neurosci, 31*(16), 6041-6052. doi:10.1523/jneurosci.6698-10.2011

Wijma, A. J., van Wilgen, C. P., Meeus, M., & Nijs, J. (2016). Clinical biopsychosocial physiotherapy assessment of patients with chronic pain: The first step in pain neuroscience education. *Physiother Theory Pract, 32*(5), 368-384. doi:10.1080/09593985.2016.1194651

Wolters, A., Sandbrink, F., Schlottmann, A., Kunesch, E., Stefan, K., Cohen, L. G., et al. (2003). A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. *J Neurophysiol, 89*(5), 2339-2345. doi:10.1152/jn.00900.2002

Wolters, A., Schmidt, A., Schramm, A., Zeller, D., Naumann, M., Kunesch, E., et al. (2005). Timing-dependent plasticity in human primary somatosensory cortex. *J Physiol, 565*(Pt 3), 1039-1052. doi:10.1113/jphysiol.2005.084954

Wu, Z., & Yamaguchi, Y. (2006). Conserving total synaptic weight ensures one-trial sequence learning of place fields in the hippocampus. *Neural Netw, 19*(5), 547-563. doi:10.1016/j.neunet.2005.06.048

Xiong, W., Ping, X., Ripsch, M. S., Chavez, G. S. C., Elise Hannon, H., Jiang, K., et al. (2017). Enhancing excitatory activity of somatosensory cortex alleviates neuropathic pain through regulating homeostatic plasticity. *Scientific Reports, 7*(1), 12743. doi:10.1038/s41598-017-12972-6

Yau, S. Y., Li, A., & So, K. F. (2015). Involvement of Adult Hippocampal Neurogenesis in Learning and Forgetting. *Neural Plast, 2015*, 717958. doi:10.1155/2015/717958

Yee, A. X., Hsu, Y. T., & Chen, L. (2017). A metaplasticity view of the interaction between homeostatic and Hebbian plasticity. *Philos Trans R Soc Lond B Biol Sci, 372*(1715). doi:10.1098/rstb.2016.0155

Yger, P., & Gilson, M. (2015). Models of Metaplasticity: A Review of Concepts. *Front Comput Neurosci, 9*, 138. doi:10.3389/fncom.2015.00138

Yuste, R., & Bonhoeffer, T. (2001). Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annu Rev Neurosci, 24*, 1071-1089. doi:10.1146/annurev.neuro.24.1.1071

Zakzanis, K. K. (2001). Statistics to tell the truth, the whole truth, and nothing but the truth: formulae, illustrative numerical examples, and heuristic interpretation of effect size analyses for neuropsychological researchers. *Arch. Clin. Neuropsychol., 16*(7), 653-667.

Zenke, F., Hennequin, G., & Gerstner, W. (2013). Synaptic Plasticity in Neural Networks Needs Homeostasis with a Fast Rate Detector. *PLoS Comput. Biol., 9*(11), e1003330. doi:10.1371/journal.pcbi.1003330

Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell, 132*(4), 645-660. doi:10.1016/j.cell.2008.01.033

Zhao, C., Dreosti, E., & Lagnado, L. (2011). Homeostatic synaptic plasticity through changes in presynaptic calcium influx. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 31*(20), 7492-7496. doi:10.1523/JNEUROSCI.6636-10.2011

Zhao, C., Toni, N., & Gage, F. H. (2013). Synaptogenesis in the Adult CNS – Hippocampus In P. Rakic (Ed.), *Cellular Migration and Formation of Neuronal Connections* (pp. 723-738). Oxford: Academic Press.

Zhuo, M. (2008). Cortical excitation and chronic pain. *Trends Neurosci, 31*(4), 199-207. doi:10.1016/j.tins.2008.01.003

Ziemann, U., Iliać, T. V., Pauli, C., Meintzschel, F., & Ruge, D. (2004). Learning Modifies Subsequent Induction of Long-Term Potentiation-Like and Long-Term Depression-Like Plasticity in Human Motor Cortex. *The Journal of Neuroscience, 24*(7), 1666-1672. doi:10.1523/jneurosci.5016-03.2004

Ziemann, U., Muellbacher, W., Hallett, M., & Cohen, L. G. (2001). Modulation of practice-dependent plasticity in human motor cortex. *Brain, 124*(6), 1171-1181. doi:10.1093/brain/124.6.1171

Ziemann, U., Paulus, W., Nitsche, M. A., Pascual-Leone, A., Byblow, W. D., Berardelli, A., et al. (2008). Consensus: Motor cortex plasticity protocols. *Brain Stimul, 1*(3), 164-182. doi:10.1016/j.brs.2008.06.006

Ziemann, U., & Siebner, H. R. (2008). Modifying motor learning through gating and homeostatic metaplasticity. *Brain Stimul, 1*(1), 60-66. doi:10.1016/j.brs.2007.08.003

Zucker, R. S., & Regehr, W. G. (2002). Short-term synaptic plasticity. *Annu Rev Physiol, 64*, 355-405. doi:10.1146/annurev.physiol.64.092501.114547

Appendix A

Publication: Test-retest reliability of homeostatic plasticity induced and assessed using non-invasive brain stimulation in the human primary motor cortex

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Research Article

Test-Retest Reliability of Homeostatic Plasticity in the Human Primary Motor Cortex

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Homeostatic plasticity regulates synaptic activity by preventing uncontrolled increases (long-term potentiation) or decreases (long-term depression) in synaptic efficacy. Homeostatic plasticity can be induced and assessed in the human primary motor cortex (M1) using noninvasive brain stimulation. However, the reliability of this methodology has not been investigated. Here, we examined the test-retest reliability of homeostatic plasticity induced and assessed in M1 using noninvasive brain stimulation in ten, right-handed, healthy volunteers on days 0, 2, 7, and 14. Homeostatic plasticity was induced in the left M1 using two blocks of anodal transcranial direct current stimulation (tDCS) applied for 7 min and 5 min, separated by a 3 min interval. To assess homeostatic plasticity, 15 motor-evoked potentials to single-pulse transcranial magnetic stimulation were recorded at baseline, between the two blocks of anodal tDCS, and at 0 min, 10 min, and 20 min follow-up. Test-retest reliability was evaluated using intraclass correlation coefficients (ICCs). Moderate-to-good test-retest reliability was observed for the M1 homeostatic plasticity response at all follow-up time points (0 min, 10 min, and 20 min, ICC range: 0.43–0.67) at intervals up to 2 weeks. The greatest reliability was observed when the homeostatic response was assessed at 10 min follow-up (ICC > 0.61). These data suggest that M1 homeostatic plasticity can be reliably induced and assessed in healthy individuals using two blocks of anodal tDCS at intervals of 48 hours, 7 days, and 2 weeks.

1. Introduction

Synaptic plasticity is fundamental to learning and memory in the human brain. However, synaptic plasticity operates via a positive feedback loop and, as a result, has the potential to destabilise neural networks through excessive synaptic strengthening (long-term potentiation-like effects, LTP) or excessive synaptic weakening (long-term depression-like effects, LTD) [1]. To avoid destabilization, LTP-like and LTD-like changes are subject to homeostatic plasticity mechanisms that maintain the neural activity within an optimal physiological range.

Homeostatic plasticity is theorised to rely on the "sliding threshold" rule, such that the threshold for the induction of LTP or LTD is dependent on the activity in the postsynaptic neuron; high postsynaptic activity favors LTD, whereas low postsynaptic activity favors LTP [2–4]. Although early studies investigating homeostatic plasticity occurred in slice preparations in vitro, a growing body of research has used noninvasive brain stimulation to investigate this mechanism in the human cortex [2–8]. Typically, one noninvasive brain stimulation protocol is used to "prime" (or condition) the synaptic effects of a subsequent noninvasive brain stimulation protocol, and LTP-like and LTD-like effects are indexed using transcranial magnetic stimulation (TMS). For example, when a 5 min block of anodal transcranial direct current stimulation (tDCS) is preceded at a short interval (3 min) by an additional 7 min block of anodal tDCS, the LTP-like (facilitatory) effect of anodal tDCS on the primary motor cortex (M1) is reversed toward LTD (observed as a reduction in

corticomotor excitability to TMS) [9]. Similarly, the preconditioning of a 1 Hz repetitive transcranial magnetic stimulation (rTMS) paradigm (that has no overt effect on corticomotor excitability when applied alone) with anodal tDCS produces LTD-like (inhibitory) effects, whereas preconditioning with cathodal tDCS produces LTP-like (facilitatory) effects [10].

Noninvasive brain stimulation has been used to evaluate homeostatic plasticity in M1 in pathological conditions including focal hand dystonia, migraine, and chronic pain [11–14]. These studies demonstrate the impaired homeostatic control in these populations such that the threshold for synaptic plasticity fails to favor the induction of LTD when postsynaptic activity is high. For instance, in individuals with focal hand dystonia, a single block of anodal tDCS increases the corticomotor excitability consistent with the response observed in healthy controls. However, the application of a subsequent block of 1 Hz rTMS fails to reverse the corticomotor excitability toward LTD in this population [15]. Additional studies have provided evidence of paradoxical facilitation in both the visual cortex and M1 of individuals with migraine, observed as an increase in visual cortex and M1 excitability in response to 1 Hz rTMS (in contrast to a reduction in the excitability of both cortices in healthy controls) [16, 17].

Studies comparing M1 homeostatic plasticity between healthy individuals and those with pathology have been limited to cross-sectional designs, despite conditions such as migraine and low back pain being cyclical in nature [12, 14]. To allow the longitudinal evaluation of homeostatic plasticity, as well as the detailed evaluation of the relationship between impaired homeostatic plasticity and symptom status, it is necessary to determine whether homeostatic plasticity can be reliably induced and assessed over time. To our knowledge, no study has investigated the reliability of M1 homeostatic plasticity. Here we aimed to determine the test-retest reliability of M1 homeostatic plasticity, induced and assessed using noninvasive brain stimulation, at intervals of 48 hours, 7 days, and 2 weeks.

2. Methods and Materials

2.1. Subjects. As no previous multiday studies of homeostatic plasticity exist, a sample size calculation was performed using best available data of MEP amplitudes recorded from healthy individuals at 0, 10, and 20 minutes following an identical double tDCS protocol used to induce and assess homeostatic plasticity in M1 (effect size estimates of 0.4, alpha of 0.05, and power of 0.8) [14]. Using these parameters, ten participants were required to evaluate the test-retest reliability of noninvasive brain stimulation to induce and assess M1 homeostatic plasticity at intervals of 48 hours, 7 days, and 2 weeks. Accordingly, ten right-handed, healthy volunteers (mean ± standard deviation age: 23 ± 5 years, 5 males) were recruited. Handedness was assessed using the Edinburgh handedness questionnaire [18]. All participants were required to meet inclusion criteria as per transcranial magnetic stimulation (TMS) safety guidelines (i.e., no history of epilepsy, absence of metal implants in the skull) [19]. Individuals with a history of neurological, musculoskeletal, upper limb or psychiatric conditions were excluded. A verbal and written description of the experimental procedures was provided to all participants. Written, informed consent was obtained before testing. The study was approved by the institutional Human Research Ethics Committee (approval number: H10184) and performed in accordance with the Declaration of Helsinki.

2.2. Experimental Protocol. Based on intervals used in previous TMS reliability studies [20], corticomotor excitability was assessed, and plasticity was induced in M1, on day 0, 2, 7, and 14. Participants were seated comfortably with their right hand and arm at rest for each test session. To evaluate the change in corticomotor excitability across days, 15 motor-evoked potentials (MEPs) to single-pulse transcranial magnetic stimulation (TMS) were recorded at 120% of resting motor threshold (rMT) at the beginning of each test session. To account for any potential changes in the corticomotor excitability occurring across days that could influence the homeostatic response and to ensure a baseline level of corticomotor excitability that was consistent between individuals immediately prior to homeostatic plasticity induction, further 15 MEPs were recorded immediately prior to the induction of homeostatic plasticity (time point "baseline") at an intensity sufficient to evoke an average MEP of 1 mV peak-to-peak amplitude (S_{1mV}). This methodology is standard in studies of homeostatic plasticity [3, 4]. Homeostatic plasticity was induced in M1 using two blocks of anodal transcranial direct current stimulation (tDCS) applied for 7 min and 5 min, respectively and separated by a 3 min rest period ("double tDCS protocol"). This protocol has been used previously to induce homeostatic plasticity in human M1 [9, 14]. The corticomotor excitability in response to tDCS was monitored by recording 15 MEPs at S_{1mV} during the 3 min rest period between the two tDCS blocks (time point "between"), and at 0 min, 10 min, and 20 min follow-ups (see Figure 1). The number of MEPs was selected based on previous studies that have demonstrated good-to-excellent reliability when 15 MEPs are used to assess the corticomotor excitability within and between sessions [21–25].

2.3. Assessment of Corticomotor Excitability. Single-pulse transcranial magnetic stimulation (TMS) was delivered using a Magstim 200 stimulator (Magstim Co., Ltd., Dyfed, UK) and a standard 70 mm figure-of-eight coil. The coil was held over the left hemisphere, at a 45° angle to the sagittal plane to induce current in the posterior-anterior direction. The optimal coil position was determined by systematically moving the coil in 1 cm increments and locating the site that evoked the maximum response at the lowest stimulator intensity from the relaxed abductor pollicis brevis (APB) muscle (termed the "hotspot"). A soft-tip pen was used to mark the hotspot to allow accurate coil and tDCS electrode repositioning within and between testing sessions. Participants were requested to precisely remark their hotspot using a mirror and a soft-tipped pen or, if required, with assistance from a second person, on the days they did not attend the laboratory for testing. Surface electromyography was recorded

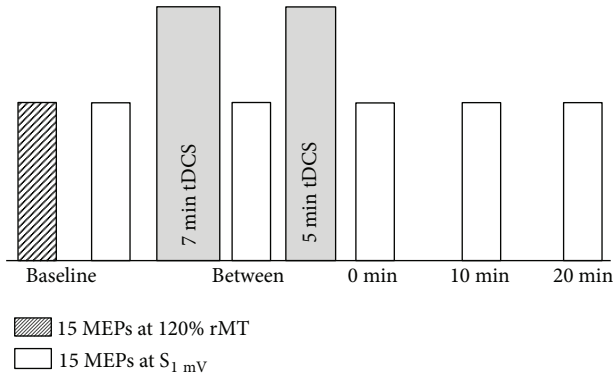

Figure 1: Experimental protocol for days 0, 2, 7, and 14. The corticomotor excitability was assessed at the beginning of each test session using 15 motor-evoked potentials (MEPs) recorded at 120% of resting motor threshold. To ensure a consistent level of baseline corticomotor excitability across subjects prior to the induction of plasticity, further 15 MEPs were recorded at an intensity sufficient to elicit an average MEP of 1 mV peak-to-peak amplitude (S_{1mV}) immediately before the first block of 7 min anodal transcranial direct current stimulation (tDCS). This intensity was kept consistent for the remainder of the test session. Plasticity was induced using a 7 min block of anodal tDCS, followed by a second 5 min block of anodal tDCS, separated by a 3 min rest period. Fifteen MEPs were recorded at S_{1mV} between the two blocks of anodal tDCS, and at 0 min, 10 min, and 20 min follow-ups.

using surface dual electrodes (Ag-AgCl, Noraxon dual electrodes, interelectrode distance: 2.0 cm) placed in a belly-tendon montage over the relaxed APB muscle [9, 11, 15]. The ground electrode was positioned over the ipsilateral olecranon. Raw EMG signals were amplified (1000 times), bandpass-filtered at 20–1000 Hz, and sampled at 2000 Hz (CED 1401 AD, Cambridge Electronic Design, Cambridge, United Kingdom) using Signal software (CED, version 5.08×86). To evaluate the change in the corticomotor excitability across days, 15 motor-evoked potentials (MEP) were recorded at 120% of resting motor threshold at the APB hotspot. The resting motor threshold (rMT) was defined as the minimum TMS intensity required to elicit at least five MEPs $\geq 50\,\mu$V in ten consecutive trials from the resting APB muscle [26].

2.4. Induction and Monitoring of M1 Synaptic and Homeostatic Plasticity. A battery-driven, ramp-controlled, constant current stimulator (DC-Stimulator Plus, NeuroConn, Ilmenau, Germany) delivered two blocks of excitatory, anodal transcranial direct current stimulation (tDCS) to the left primary motor cortex (M1). The left M1 was targeted to control for hand dominance, as only right-handed individuals were included in this study. The first anodal tDCS block lasted for 7 min, and the second, for 5 min. The two blocks were separated by a 3 min rest period. Rubber electrodes, placed in NaCl-soaked sponges (5×7 cm) were positioned over the hotspot corresponding to the right APB muscle (anode) as determined above and over the contralateral supraorbital region (cathode). Electrodes were fixed in position with two adjustable rubber straps. The current intensity was ramped up (0 mA–1 mA) and down (1 mA–0 mA) over ten seconds at the start and end of stimulation [27]. The single-pulse TMS was used to monitor the corticomotor excitability in response to the first and second blocks of anodal tDCS. This was achieved by setting the stimulator intensity to S_{1mV} at the previously determined optimal scalp site.

2.5. Data Analysis. Data are presented as means and standard deviations (SD) in text, tables, and figures. Statistical analyses were conducted using SPSS software for windows, version 22.

The data distribution was assessed using the Shapiro-Wilk test. A one-way repeated measure ANOVA with the factor "day" (0, 2, 7, 14) was performed to compare (i) resting motor threshold, (ii) TMS intensity used to elicit S_{1mV}, and (iii) corticomotor excitability (recorded at 120% rMT), between days. To examine the change in corticomotor response following the first block of anodal tDCS across days, the amplitude of the MEP at time point "between" was calculated as a proportion of the MEP amplitude at "baseline" and analysed using a one-way repeated measure ANOVA with the factor "day." To examine the change in the corticomotor response to the double tDCS protocol across days, the amplitude of the MEP at each of the follow-up time points (0 min, 10 min, and 20 min) was calculated as a proportion of the MEP amplitude at time points "baseline" and "between," and analysed using a one-way repeated measure ANOVA with the factor "day." This analysis was performed as the magnitude of the homeostatic response is likely to be dependent on the corticomotor excitability at "baseline," and the amount of facilitation achieved following the first block of anodal tDCS (i.e., time point "between"). Bonferroni post-hoc tests corrected for multiple comparisons were performed where appropriate. The Greenhouse-Geisser method was used to correct for nonsphericity. Effect sizes from the one-way repeated measure ANOVA are reported using partial eta squared. Cohen's benchmarks were used to define small (0.01), medium (0.06), and large effect sizes (0.14) [28, 29].

An intraclass correlation coefficient model (ICC 3,k) was used to evaluate the test-retest reliability of (i) the resting motor threshold, (ii) the TMS intensity used to elicit S_{1mV}, (iii) the corticomotor excitability (recorded at 120% rMT), (iv) the corticomotor response to the first block of anodal tDCS, and (v) the corticomotor (homeostatic) response recorded at 0 min, 10 min, and 20 min after the second block of anodal tDCS, across days 0, 2, 7, and 14. The ICC 3,k model was used to determine consistency between variables across days by accounting for fixed effects from the rater and random effects from study participants [30, 31]. ICC sc ores ≤ 0.20 were considered poor: 0.2–0.40, fair: 0.41–0.60, moderate; 0.61–0.80, good; and ≥ 0.81, excellent [32].

3. Results

3.1. Corticomotor Excitability and Homeostatic Plasticity in Healthy Individuals at Intervals of 48 Hours, 7 Days, and 2 Weeks. All data had normal distribution. There was no difference in the resting motor threshold ($F_{2,16} = 0.3$, $P = 0.7$, partial eta squared = 0.03), the TMS intensity used to elicit

S_{1mV} ($F_{3,27} = 0.4$, $P = 0.7$, partial eta squared = 0.04), or the corticomotor excitability (assessed at 120% rMT, $F_{2,16} = 0.4$, $P = 0.6$, partial eta squared = 0.05) between days (Table 1).

The magnitude of the increase in MEP amplitude following the first block of anodal tDCS was not different between days (corticomotor excitability at time point "between" calculated as a proportion of the MEP amplitude at "baseline"; $F_{3,27} = 0.4$, $P = 0.8$, partial eta squared = 0.04; Figure 2). Similarly, the magnitude of the decrease in MEP amplitude following the second block of anodal tDCS was not different between days at all follow-up time points (corticomotor excitability at time points 0, 10, and 20 min calculated as a proportion of the MEP amplitude at time point "baseline"; 0 min: $F_{2,16} = 0.5$, $P = 0.5$, partial eta squared = 0.06; 10-min: $F_{3,27} = 1.7$, $P = 0.2$, partial eta squared = 0.16; 20 min: $F_{3,27} = 0.8$, $P = 0.5$, partial eta squared = 0.08; and corticomotor excitability at time points 0, 10, and 20 min calculated as a proportion of the MEP amplitude at time point "between"; 0-min: $F_{3,27} = 1.2$, $P = 0.3$, partial eta squared = 0.12; 10 min: $F_{3,27} = 1.3$, $P = 0.3$, partial eta squared = 0.13; 20 min: $F_{3,27} = 1.2$, $P = 0.3$, partial eta squared = 0.12; Figure 2).

Small effect sizes were observed for rMT (partial eta squared = 0.03), the TMS intensity used to elicit S_{1mV} (partial eta squared = 0.04), the corticomotor excitability (assessed at 120% rMT, partial eta squared = 0.05), and the corticomotor response to the first block of anodal tDCS (partial eta squared = 0.04). Medium-to-large effect sizes were observed for homeostatic responses to the double tDCS protocol when normalised to "baseline" (0 min: partial eta squared = 0.06; 10 min: partial eta squared = 0.16; 20 min: partial eta squared = 0.08) and time point "between" (0 min: partial eta squared = 0.12; 10 min: partial eta squared = 0.13; 20 min: partial eta squared = 0.12).

3.2. Test-Retest Reliability. Excellent test-retest reliability was observed for rMT (ICC = 0.92, 95% CI 0.76–0.98; Table 1) and the TMS intensity used to elicit S_{1mV} (ICC = 0.95, 95% CI 0.87–0.99; Table 1) across days. Moderate-to-good reliability was observed for the corticomotor excitability assessed at 120% rMT across days (ICC = 0.80, 95% CI 0.47–0.94; Table 1).

The corticomotor response to the first block of anodal tDCS (ICC = 0.41, 95% CI −0.72–0.84; Table 1), and homeostatic responses to the double tDCS protocol at all follow-up time points across days, demonstrated moderate-to-good-reliability when data were normalised to time point "baseline" (0 min: ICC = 0.58, 95% CI −0.01–0.88; 10 min: ICC = 0.61, 95% CI −0.03–0.89; 20 min: ICC = 0.43, 95% CI −0.67–0.85; Table 1). Similarly, moderate-to-good-reliability was observed at all follow-up time points across days, when homeostatic responses were normalised to time point "between" (0 min: ICC = 0.61, 95% CI −0.03–0.89; 10 min: ICC = 0.67, 95% CI 0.12–0.91; 20 min: ICC = 0.60, 95% CI −0.06–0.89; Table 1). The highest ICCs were observed for the homeostatic plasticity response recorded at 10 min follow-up across days, (normalised to "baseline" ICC = 0.61, 95% CI −0.03–0.89; normalised to "between" ICC = 0.67, 95% CI 0.12–0.91; Table 1).

4. Discussion

This study is the first to examine the test-retest reliability of M1 homeostatic plasticity, induced and assessed using noninvasive brain stimulation, in the healthy human brain. The corticomotor response to single, and double, anodal tDCS demonstrated moderate-to-good test-retest reliability in healthy individuals over intervals up to 2 weeks. These data suggest that M1 homeostatic plasticity can be reliably induced and assessed over time using two blocks of anodal tDCS. This finding provides a foundation for the longitudinal evaluation of M1 homeostatic plasticity in humans using the double tDCS protocol.

Homeostatic plasticity regulates neuronal firing rates in the human brain and ensures that the neuronal activity is maintained within a stable physiological range [3, 4]. The Bienenstock-Cooper-Munro (BCM) theory of homeostatic plasticity proposes that neuronal firing rates are regulated based on the history of the postsynaptic activity, such that high levels of neuronal activity reduce the threshold for LTD induction and promote LTD-like plasticity (synaptic weakening, lower firing rates), while low levels of neuronal activity reduce the threshold for LTP induction and promote LTP-like plasticity (synaptic strengthening, higher firing rates) [2, 33].

Consistent with the BCM theory, studies exploring homeostatic plasticity using repetitive tetanic stimulation [5, 33–35] and noninvasive brain stimulation [3, 4, 36–38] have shown that neuronal activity is modified based on the level of postsynaptic activity [39–41]. For example, studies have shown that two blocks of anodal tDCS produce effects on M1 that follow a time-dependent rule consistent with homeostatic mechanisms [9]. Specifically, when 7 min of anodal tDCS is followed at 3 min interval by a second 5 min block of anodal tDCS, the increase in the corticomotor excitability observed with 7 min anodal tDCS applied alone is reversed toward inhibition [9]. The nature of this response mimics the homeostatic rule of a threshold that slides to favor the induction of LTD-like effects (the inhibitory response after the second block of anodal tDCS) when postsynaptic activity is high (following the first block of anodal tDCS) [2–4].

Our data confirm the direction and time course of these effects in the healthy brain (increased the corticomotor excitability in response to a single 7 min block of anodal tDCS; decreased the corticomotor excitability in response to double tDCS) and extend previous work by demonstrating moderate-to-good test-retest reliability with medium-to-large effect sizes when homeostatic plasticity is induced and assessed using noninvasive brain stimulation at intervals of 48 hours, 7 days, and 2 weeks. Specifically, moderate-to-good test-retest reliability with medium-to-large effect sizes was observed when the magnitude of the homeostatic response was considered relative to "baseline," (all ICC ≥ 0.43; all partial eta squared ≥ 0.06; Table 1) and when the magnitude of the response was considered relative to the level of facilitation produced following the first block of anodal tDCS (all ICC ≥ 0.60; all partial eta squared ≥ 0.12; Table 1). The greatest test-retest reliability (ICC ≥ 0.61) with

Table 1: Test-retest reliability (intraclass correlation coefficient [ICC]) estimates for cortical measures recorded across days 0, 2, 7, and 14.

Cortical measures	Cortical measures across days				ICC (95% CI)
	Day 0 (mean + SD)	Day 2 (mean + SD)	Day 7 (mean + SD)	Day 14 (mean + SD)	
rMT (% maximum stimulator output)	44 ± 7	45 ± 6	45 ± 7	44 ± 6	0.92 (0.76–0.98)
S_{1mV} (% maximum stimulator output)	54 ± 9	55 ± 11	56 ± 12	55 ± 12	0.95 (0.87–0.99)
Corticomotor excitability (mV)	1.0 ± 0.5	1.2 ± 0.9	1.0 ± 0.8	1.1 ± 0.9	0.80 (0.47–0.94)
Corticomotor $\text{response}_{\text{baseline}}$ (mV)	1.4 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.4 ± 0.5	0.41 (−0.72–0.84)
Homeostatic $\text{response}_{\text{baseline}}$ 0 min (mV)	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.4	0.8 ± 0.3	0.58 (−0.01–0.88)
Homeostatic $\text{response}_{\text{baseline}}$ 10 min (mV)	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.6 ± 0.1	0.61 (−0.03–0.89)
Homeostatic $\text{response}_{\text{baseline}}$ 20 min (mV)	0.8 ± 0.4	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.43 (−0.67–0.85)
Homeostatic $\text{response}_{\text{between}}$ 0 min (mV)	0.6 ± 0.1	0.6 ± 0.2	0.8 ± 0.4	0.7 ± 0.4	0.61 (−0.03–0.89)
Homeostatic $\text{response}_{\text{between}}$ 10 min (mV)	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.3	0.5 ± 0.2	0.67 (0.12–0.91)
Homeostatic $\text{response}_{\text{between}}$ 20 min (mV)	0.6 ± 0.3	0.5 ± 0.2	0.7 ± 0.3	0.6 ± 0.2	0.60 (−0.06–0.89)

Cortical measures: (i) resting motor threshold (rMT), (ii) transcranial magnetic stimulator (TMS) intensity needed to elicit an average peak-to-peak MEP amplitude of 1 mV(S_{1mV}), (iii) corticomotor excitability (motor-evoked potential (MEP) amplitude recorded at 120% of rMT), (iv) the corticomotor response to the first block of anodal tDCS normalised to baseline (corticomotor $\text{response}_{\text{baseline}}$), and (v) the corticomotor (homeostatic) response to the second block of anodal tDCS normalised to "baseline" (homeostatic $\text{response}_{\text{baseline}}$) and "between" (homeostatic $\text{response}_{\text{between}}$) at 0 min, 10 min, and 20 min follow-ups.

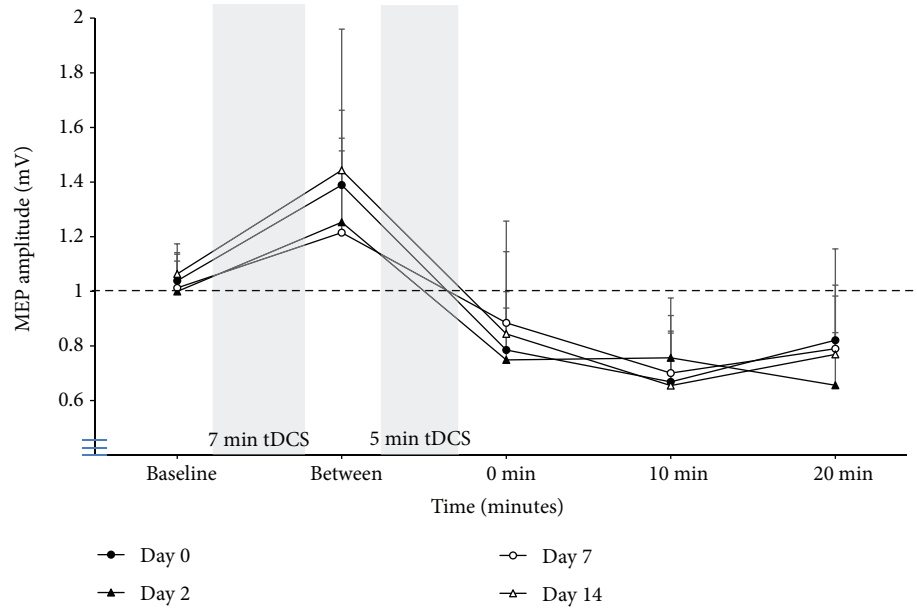

Figure 2: Group data (mean + SD) for motor-evoked potential (MEP) amplitude before the double tDCS protocol ("baseline"), after the first block of anodal tDCS ("between"), and at 0 min, 10 min, and 20 min follow-ups on days 0, 2, 7, and 14.

the largest effect size (partial eta squared ≥ 0.13) was observed when the homeostatic response was evaluated at the 10 min follow-up.

The current data also provide further evidence that the resting motor threshold (ICC = 0.92, 95% CI 0.76–0.98) and the corticomotor excitability (ICC = 0.80, 95% CI 0.47–0.94) are reliable at intervals of 48 hours, 7 days, and 2 weeks. This finding is in agreement with previous studies. For example, Malcolm et al. (2006) reported high reliability in motor thresholds (ICC = 0.90 – 0.97) in healthy volunteers over a period of 2 weeks [42]. Further, good reliability (ICC ≥ 0.75) for cortical excitability measures (resting motor threshold, TMS input-output curves, MEP amplitude, and cortical silent period) have been reported across two testing sessions, each 1 week apart, in healthy volunteers [43]. As changes in the resting motor threshold and/or baseline corticomotor excitability are likely to influence the homeostatic response, the reliability of these measures over time is an important consideration in the assessment of homeostatic plasticity in humans [3, 12, 44].

Previous studies have used a range of noninvasive brain stimulation protocols to probe the M1 homeostatic plasticity in both healthy and clinical populations [3, 4, 11, 13]. In people with nonspecific chronic low back pain (cLBP), homeostatic plasticity was assessed in M1 using a double tDCS protocol identical to that investigated here [14]. The authors demonstrated the impaired homeostatic plasticity in this population characterised by a failure to reverse high corticomotor excitability (induced by the first block of tDCS) toward inhibition (following the second block of tDCS). Using 5 Hz trains of repetitive TMS, the impaired homeostatic plasticity has been reported in individuals with episodic migraine during the preictal and postictal stages of the migraine cycle [12]. Although data were obtained from different individuals at different stages of the migraine cycle (i.e., the study did not utilise a repeated-measures design), impaired homeostatic plasticity was theorised to contribute to headache recurrence and migraine transformation from an episodic to a chronic condition [12]. Similar observations were reported in the M1 of individuals with focal hand dystonia where patients failed to reverse high corticomotor excitability toward inhibition when 1 Hz rTMS was primed by anodal tDCS [15]. Impaired M1 homeostatic plasticity in focal hand dystonia was later reported to correlate with the severity of symptoms and hypothesised to contribute to aberrant sensorimotor plasticity in this condition [13]. These data have been interpreted to suggest that impaired homeostatic plasticity may play a role in the pathogenesis of some clinical conditions. Further exploration of these findings using longitudinal and repeated measures study designs are needed to confirm these hypotheses.

It is noteworthy that some studies using repeated noninvasive brain stimulation techniques have demonstrated nonhomeostatic interactions in the human M1, where cumulative (rather than opposite) LTP- or LTD-like effects are induced [3, 45, 46]. For example, the application of two successive inhibitory continuous theta-burst stimulation protocols results in long-lasting MEP depression and not a reversal toward facilitation as would be hypothesised by the BCM theory [47, 48]. These data suggest that in addition to homeostatic mechanisms, nonhomeostatic interactions might also shape noninvasive brain stimulation-induced LTP-like and LTD-like effects. Future studies exploring the interplay between homeostatic and nonhomeostatic mechanisms over time are warranted in healthy and pathological populations.

This study has several limitations. First, the test-retest reliability in M1 homeostatic plasticity was assessed in one direction only, that is, with a facilitatory priming protocol (anodal tDCS). This approach was selected as previous studies in pathological conditions have shown failure to induce

LTD when postsynaptic activity is high [11, 13]. However, since the polarity and magnitude of synaptic plasticity varies as a function of activation history in the postsynaptic neuron, future studies should seek to determine whether inhibitory priming protocols (e.g., cathodal tDCS) are also reliable over time. Second, this study did not assess homeostatic plasticity in intracortical inhibitory or facilitatory networks. As tDCS is known to influence intracortical activity [27, 49–51], and homeostatic impairment has been demonstrated in intracortical inhibitory and facilitatory networks in individuals with migraine [16, 17, 44, 52], future studies should investigate homeostatic regulation in these networks over time. Third, although this study used noninvasive brain stimulation methods similar to previous studies in this field [3, 4], tDCS applied to M1 using electrodes of $5 \times 7\,cm^2$ may have resulted in the current spread to surrounding cortical regions [27, 53, 54]. Finally, our findings are limited to homeostatic plasticity in the healthy M1 using a double tDCS protocol. Further research is needed to determine the test-retest reliability of homeostatic plasticity induced using other noninvasive brain stimulation methodologies in M1, as well as homeostatic plasticity induced in other brain regions relevant to different pathologies [55–58].

5. Conclusion

These data demonstrate that M1 homeostatic plasticity, induced using two blocks of anodal tDCS and assessed using single-pulse TMS, has moderate-to-good reliability at intervals of 48 hours, 7 days, and 2 weeks, with the greatest reliability observed when the homeostatic response is assessed at the 10 min follow-up. These findings provide a foundation for the assessment of homeostatic plasticity in the primary motor cortex using repeated measures and longitudinal study designs in humans.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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References

[1] U. Ziemann and H. R. Siebner, "Modifying motor learning through gating and homeostatic metaplasticity," *Brain Stimulation*, vol. 1, no. 1, pp. 60–66, 2008.

[2] M. F. Bear, "Bidirectional synaptic plasticity: from theory to reality," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 358, no. 1432, pp. 649–655, 2003.

[3] A. Karabanov, U. Ziemann, M. Hamada et al., "Consensus paper: probing homeostatic plasticity of human cortex with non-invasive transcranial brain stimulation," *Brain Stimulation*, vol. 8, no. 3, pp. 442–454, 2015.

[4] F. Muller-Dahlhaus and U. Ziemann, "Metaplasticity in human cortex," *The Neuroscientist*, vol. 21, no. 2, pp. 185–202, 2015.

[5] G. Turrigiano, "Homeostatic signaling: the positive side of negative feedback," *Current Opinion in Neurobiology*, vol. 17, no. 3, pp. 318–324, 2007.

[6] G. G. Turrigiano, "Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same," *Trends in Neurosciences*, vol. 22, no. 5, pp. 221–227, 1999.

[7] B. Gisabella, M. J. Rowan, and R. Anwyl, "Mechanisms underlying the inhibition of long-term potentiation by preconditioning stimulation in the hippocampus *in vitro*," *Neuroscience*, vol. 121, no. 2, pp. 297–305, 2003.

[8] Y. Y. Huang, A. Colino, D. K. Selig, and R. C. Malenka, "The influence of prior synaptic activity on the induction of long-term potentiation," *Science*, vol. 255, no. 5045, pp. 730–733, 1992.

[9] K. Fricke, A. A. Seeber, N. Thirugnanasambandam, W. Paulus, M. A. Nitsche, and J. C. Rothwell, "Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex," *Journal of Neurophysiology*, vol. 105, no. 3, pp. 1141–1149, 2011.

[10] H. R. Siebner, N. Lang, V. Rizzo et al., "Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex," *The Journal of Neuroscience*, vol. 24, no. 13, pp. 3379–3385, 2004.

[11] A. Antal, N. Lang, K. Boros, M. Nitsche, H. R. Siebner, and W. Paulus, "Homeostatic metaplasticity of the motor cortex is altered during headache-free intervals in migraine with aura," *Cerebral Cortex*, vol. 18, no. 11, pp. 2701–2705, 2008.

[12] G. Cosentino, B. Fierro, S. Vigneri et al., "Cyclical changes of cortical excitability and metaplasticity in migraine: evidence from a repetitive transcranial magnetic stimulation study," *Pain*, vol. 155, no. 6, pp. 1070–1078, 2014.

[13] J. S. Kang, C. Terranova, R. Hilker, A. Quartarone, and U. Ziemann, "Deficient homeostatic regulation of practice-dependent plasticity in writer's cramp," *Cerebral Cortex*, vol. 21, no. 5, pp. 1203–1212, 2011.

[14] T. Thapa, T. Graven-Nielsen, L. S. Chipchase, and S. M. Schabrun, "Disruption of cortical synaptic homeostasis in individuals with chronic low back pain," *Clinical Neurophysiology*, vol. 129, no. 5, pp. 1090–1096, 2018.

[15] A. Quartarone, V. Rizzo, S. Bagnato et al., "Homeostatic-like plasticity of the primary motor hand area is impaired in focal hand dystonia," *Brain*, vol. 128, no. 8, pp. 1943–1950, 2005.

[16] F. Brighina, G. Giglia, S. Scalia, M. Francolini, A. Palermo, and B. Fierro, "Facilitatory effects of 1 Hz rTMS in motor cortex of patients affected by migraine with aura," *Experimental Brain Research*, vol. 161, no. 1, pp. 34–38, 2005.

[17] F. Brighina, A. Piazza, O. Daniele, and B. Fierro, "Modulation of visual cortical excitability in migraine with aura: effects of 1 Hz repetitive transcranial magnetic stimulation," *Experimental Brain Research*, vol. 145, no. 2, pp. 177–181, 2002.

[18] R. C. Oldfield, "The assessment and analysis of handedness: the Edinburgh inventory," *Neuropsychologia*, vol. 9, no. 1, pp. 97–113, 1971.

[19] J. C. Keel, M. J. Smith, and E. M. Wassermann, "A safety screening questionnaire for transcranial magnetic stimulation," *Clinical Neurophysiology*, vol. 112, no. 4, p. 720, 2001.

[20] J. Uy, M. C. Ridding, and T. S. Miles, "Stability of maps of human motor cortex made with transcranial magnetic stimulation," *Brain Topography*, vol. 14, no. 4, pp. 293–297, 2002.

[21] A. Bastani and S. Jaberzadeh, "A higher number of TMS-elicited MEP from a combined hotspot improves intra- and inter-session reliability of the upper limb muscles in healthy individuals," *PLoS One*, vol. 7, no. 10, article e47582, 2012.

[22] W. H. Chang, P. J. Fried, S. Saxena et al., "Optimal number of pulses as outcome measures of neuronavigated transcranial magnetic stimulation," *Clinical Neurophysiology*, vol. 127, no. 8, pp. 2892–2897, 2016.

[23] A. Christie, B. Fling, R. T. Crews, L. A. Mulwitz, and G. Kamen, "Reliability of motor-evoked potentials in the ADM muscle of older adults," *Journal of Neuroscience Methods*, vol. 164, no. 2, pp. 320–324, 2007.

[24] S. H. Doeltgen, M. C. Ridding, G. A. O'Beirne, J. Dalrymple-Alford, and M. L. Huckabee, "Test–retest reliability of motor evoked potentials (MEPs) at the submental muscle group during volitional swallowing," *Journal of Neuroscience Methods*, vol. 178, no. 1, pp. 134–137, 2009.

[25] S. Groppa, A. Oliviero, A. Eisen et al., "A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee," *Clinical Neurophysiology*, vol. 123, no. 5, pp. 858–882, 2012.

[26] P. M. Rossini, A. T. Barker, A. Berardelli et al., "Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee," *Electroencephalography and Clinical Neurophysiology*, vol. 91, no. 2, pp. 79–92, 1994.

[27] M. A. Nitsche, L. G. Cohen, E. M. Wassermann et al., "Transcranial direct current stimulation: state of the art 2008," *Brain Stimulation*, vol. 1, no. 3, pp. 206–223, 2008.

[28] J. T. E. Richardson, "Eta squared and partial eta squared as measures of effect size in educational research," *Educational Research Review*, vol. 6, no. 2, pp. 135–147, 2011.

[29] J. Cohen, *Statistical Power Analysis for the Behavioural Sciences*, Academic Press, New York, NY, USA, 1969.

[30] H. M. Schambra, R. T. Ogden, I. E. Martinez-Hernandez et al., "The reliability of repeated TMS measures in older adults and in patients with subacute and chronic stroke," *Frontiers in Cellular Neuroscience*, vol. 9, p. 335, 2015.

[31] K. O. McGraw and S. P. Wong, "Forming inferences about some intraclass correlation coefficients," *Psychological Methods*, vol. 1, no. 1, pp. 30–46, 1996.

[32] J. M. Matamala, J. Howells, T. Dharmadasa et al., "Inter-session reliability of short-interval intracortical inhibition measured by threshold tracking TMS," *Neuroscience Letters*, vol. 674, pp. 18–23, 2018.

[33] G. G. Turrigiano and S. B. Nelson, "Homeostatic plasticity in the developing nervous system," *Nature Reviews Neuroscience*, vol. 5, no. 2, pp. 97–107, 2004.

[34] W. C. Abraham, "Metaplasticity: tuning synapses and networks for plasticity," *Nature Reviews Neuroscience*, vol. 9, no. 5, p. 387, 2008.

[35] G. Turrigiano, "Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function," *Cold Spring Harbor Perspectives in Biology*, vol. 4, no. 1, article a005736, 2012.

[36] A. Karabanov and H. R. Siebner, "Unravelling homeostatic interactions in inhibitory and excitatory networks in human motor cortex," *The Journal of Physiology*, vol. 590, no. 22, pp. 5557-5558, 2012.

[37] N. Lang, H. R. Siebner, D. Ernst et al., "Preconditioning with transcranial direct current stimulation sensitizes the motor cortex to rapid-rate transcranial magnetic stimulation and controls the direction of after-effects," *Biological Psychiatry*, vol. 56, no. 9, pp. 634–639, 2004.

[38] S. K. Sidhu, M. Pourmajidian, G. M. Opie, and J. G. Semmler, "Increasing motor cortex plasticity with spaced paired associative stimulation at different intervals in older adults," *European Journal of Neuroscience*, vol. 46, no. 11, pp. 2674–2683, 2017.

[39] G. M. Opie, E. Vosnakis, M. C. Ridding, U. Ziemann, and J. G. Semmler, "Priming theta burst stimulation enhances motor cortex plasticity in young but not old adults," *Brain Stimulation*, vol. 10, no. 2, pp. 298–304, 2017.

[40] H. Fujiyama, M. R. Hinder, A. Barzideh et al., "Preconditioning tDCS facilitates subsequent tDCS effect on skill acquisition in older adults," *Neurobiology of Aging*, vol. 51, pp. 31–42, 2017.

[41] M. Christova, D. Rafolt, and E. Gallasch, "Cumulative effects of anodal and priming cathodal tDCS on pegboard test performance and motor cortical excitability," *Behavioural Brain Research*, vol. 287, pp. 27–33, 2015.

[42] M. P. Malcolm, W. J. Triggs, K. E. Light, O. Shechtman, G. Khandekar, and L. J. Gonzalez Rothi, "Reliability of motor cortex transcranial magnetic stimulation in four muscle representations," *Clinical Neurophysiology*, vol. 117, no. 5, pp. 1037–1046, 2006.

[43] H. Liu and S. S. Y. Au-Yeung, "Reliability of transcranial magnetic stimulation induced corticomotor excitability measurements for a hand muscle in healthy and chronic stroke subjects," *Journal of the Neurological Sciences*, vol. 341, no. 1-2, pp. 105–109, 2014.

[44] F. Brighina, G. Cosentino, S. Vigneri et al., "Abnormal facilitatory mechanisms in motor cortex of migraine with aura," *European Journal of Pain*, vol. 15, no. 9, pp. 928–935, 2011.

[45] P. Jung and U. Ziemann, "Homeostatic and nonhomeostatic modulation of learning in human motor cortex," *The Journal of Neuroscience*, vol. 29, no. 17, pp. 5597–5604, 2009.

[46] Y. Z. Huang, J. C. Rothwell, C. S. Lu, W. L. Chuang, and R. S. Chen, "Abnormal bidirectional plasticity-like effects in Parkinson's disease," *Brain*, vol. 134, no. 8, pp. 2312–2320, 2011.

[47] M. R. Goldsworthy, J. B. Pitcher, and M. C. Ridding, "The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex," *European Journal of Neuroscience*, vol. 35, no. 1, pp. 125–134, 2012.

[48] M. R. Goldsworthy, F. Muller-Dahlhaus, M. C. Ridding, and U. Ziemann, "Resistant against de-depression: LTD-like plasticity in the human motor cortex induced by spaced cTBS," *Cerebral Cortex*, vol. 25, no. 7, pp. 1724–1734, 2015.

[49] M. A. Nitsche, M. S. Nitsche, C. C. Klein, F. Tergau, J. C. Rothwell, and W. Paulus, "Level of action of cathodal DC polarisation induced inhibition of the human motor cortex," *Clinical Neurophysiology*, vol. 114, no. 4, pp. 600–604, 2003.

[50] M. A. Nitsche and W. Paulus, "Transcranial direct current stimulation—update 2011," *Restorative Neurology and Neuroscience*, vol. 29, no. 6, pp. 463–492, 2011.

[51] M. Biabani, M. Aminitehrani, M. Zoghi, M. Farrell, G. Egan, and S. Jaberzadeh, "The effects of transcranial direct current

stimulation on short-interval intracortical inhibition and intracortical facilitation: a systematic review and meta-analysis," *Reviews in the Neurosciences*, vol. 29, no. 1, pp. 99–114, 2017.

[52] F. Brighina, A. Palermo, O. Daniele, A. Aloisio, and B. Fierro, "High-frequency transcranial magnetic stimulation on motor cortex of patients affected by migraine with aura: a way to restore normal cortical excitability?," *Cephalalgia*, vol. 30, no. 1, pp. 46–52, 2010.

[53] A. Bastani and S. Jaberzadeh, "a-tDCS differential modulation of corticospinal excitability: the effects of electrode size," *Brain Stimulation*, vol. 6, no. 6, pp. 932–937, 2013.

[54] A. F. DaSilva, M. S. Volz, M. Bikson, and F. Fregni, "Electrode positioning and montage in transcranial direct current stimulation," *Journal of Visualized Experiments*, vol. 51, article e2744, 2011.

[55] T. Bocci, M. Caleo, S. Tognazzi et al., "Evidence for metaplasticity in the human visual cortex," *Journal of Neural Transmission*, vol. 121, no. 3, pp. 221–231, 2014.

[56] B. Bliem, J. F. Muller-Dahlhaus, H. R. Dinse, and U. Ziemann, "Homeostatic metaplasticity in the human somatosensory cortex," *Journal of Cognitive Neuroscience*, vol. 20, no. 8, pp. 1517–1528, 2008.

[57] A. Wolters, A. Schmidt, A. Schramm et al., "Timing-dependent plasticity in human primary somatosensory cortex," *The Journal of Physiology*, vol. 565, no. 3, pp. 1039–1052, 2005.

[58] C. B. Jones, T. Lulic, A. Z. Bailey et al., "Metaplasticity in human primary somatosensory cortex: effects on physiology and tactile perception," *Journal of Neurophysiology*, vol. 115, no. 5, pp. 2681–2691, 2016.

Appendix B

Publication: Disruption of cortical synaptic homeostasis in individuals with chronic low back pain

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Disruption of cortical synaptic homeostasis in individuals with chronic low back pain

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HIGHLIGHTS

- Primary motor cortex homeostatic plasticity is impaired in people with chronic low back pain.
- Homeostatic impairment manifests in the M1 representation of 'unaffected' hand muscles.
- Impaired homeostatic control could explain maladaptive synaptic plasticity, and symptom persistence.

ABSTRACT

Objective: Homeostatic plasticity mechanisms regulate synaptic plasticity in the human brain. Impaired homeostatic plasticity may contribute to maladaptive synaptic plasticity and symptom persistence in chronic musculoskeletal pain.
Methods: We examined homeostatic plasticity in fifty individuals with chronic low back pain (cLBP) and twenty-five pain-free controls. A single block (7-min) of anodal transcranial direct current stimulation ('single tDCS'), or two subsequent blocks (7-min and 5-min separated by 3-min rest; 'double tDCS'), were randomised across two experimental sessions to confirm an excitatory response to tDCS applied alone, and evaluate homeostatic plasticity, respectively. Corticomotor excitability was assessed in the corticomotor representation of the first dorsal interosseous muscle by transcranial magnetic stimulation-induced motor evoked potentials (MEPs) recorded before and 0, 10, 20, and 30-min following each tDCS protocol.
Results: Compared with baseline, MEP amplitudes increased at all time points in both groups following the single tDCS protocol ($P < 0.003$). Following the double tDCS protocol, MEP amplitudes decreased in pain-free controls at all time points compared with baseline ($P < 0.01$), and were unchanged in the cLBP group.
Conclusion: These data indicate impaired homeostatic plasticity in the primary motor cortex of individuals with cLBP.
Significance: Impaired homeostatic plasticity could explain maladaptive synaptic plasticity and symptom persistence in cLBP.

1. Introduction

Chronic low back pain (cLBP) is a prevalent and disabling musculoskeletal condition with few effective treatments (Balague et al., 2012). Although precise mechanisms remain unclear, structural and functional reorganisation of the sensorimotor cortex has been

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identified in cLBP, and is associated with pain severity, pain duration and movement dysfunction (Kregel et al., 2015; Masse-Alarie et al., 2016). Cortical reorganisation in cLBP is hypothesised to be a marker of maladaptive synaptic plasticity, and this concept provides the foundation for contemporary theories of pain persistence (Moseley and Flor, 2012). Importantly, synaptic plasticity is regulated by homeostatic mechanisms (termed *homeostatic plasticity*), that if impaired, could explain aberrant synaptic plasticity and potentially symptom persistence in cLBP. Despite this, a pathophysiological role for changes in homeostatic plasticity has been overlooked in musculoskeletal pain.

Hebbian or use-dependent synaptic plasticity involves the expression of lasting changes in synaptic efficacy underpinned by long-term potentiation (LTP; synaptic strengthening) and long-term depression (LTD; synaptic weakening) (Hebb, 1949). However, synaptic plasticity relies on a positive feedback loop that left unchecked would lead to either too much strengthening and excessive neuronal excitability (LTP), or too much weakening and neuronal silencing (LTD) (Karabanov et al., 2015). In the healthy brain, homeostatic plasticity mechanisms enforce stability and maintain brain excitability within a normal range by shifting the threshold for LTP and LTD based on the history of synaptic activity. For example, the threshold of a synapse with a history of high excitability will shift to favour induction of LTD (Bienenstock et al., 1982).

Homeostatic plasticity can be assessed in humans using non-invasive brain stimulation. For example, in healthy individuals a homeostatic response is elicited when two blocks of excitatory brain stimulation are applied at short intervals (Fricke et al., 2011). Homeostatic plasticity is observed as an increase in cortical excitability following the first block of excitatory stimuli (synaptic strengthening) that is reversed towards inhibition (synaptic weakening) when the second block of excitatory stimuli is applied after a few minutes. In this way, the brain corrects for exposure to excessive levels of excitation and prevents aberrant synaptic plasticity (Karabanov et al., 2015; Murakami et al., 2012).

Evidence from neurological conditions such as migraine and focal hand dystonia suggests a link between impaired homeostatic plasticity and symptoms. For example, these individuals exhibit reorganisation of the sensorimotor cortex (Jia and Yu, 2017; Schabrun et al., 2009) as well as excessive cortical excitability (Brighina et al., 2005; Quartarone et al., 2003; Welch, 2003). Impaired homeostatic plasticity is hypothesised to contribute to abnormal cortical reorganisation and sensorimotor symptoms in these conditions as a result of inappropriate and excessive LTP-like effects resulting from a failure to shift the threshold towards LTD when excitability is high (Brighina et al., 2005; Kang et al., 2011; Quartarone et al., 2008; Quartarone and Pisani, 2011). A comparable failure of homeostatic control in cLBP could explain similar observations of maladaptive cortical reorganisation and symptom persistence in this population yet, no study has investigated homeostatic plasticity in musculoskeletal disorders.

Importantly, impaired homeostatic plasticity has been shown to be generalised throughout the sensorimotor system and is not restricted to the cortical representations of affected muscles (Antal et al., 2008; Brighina et al., 2011; Quartarone et al., 2008, 2005). For instance, in migraine, impaired homeostatic plasticity is not restricted to the visual cortex and is also observed in M1 representations of 'unaffected' hand muscles (Antal et al., 2008; Brighina et al., 2011; Cosentino et al., 2014). Similarly, impaired homeostatic plasticity is present in the 'unaffected' median and ulnar innervated muscles in focal hand dystonia (Kang et al., 2011; Quartarone et al., 2008). Together, these findings indicate a global impairment in homeostatic plasticity that has been suggested to provide evidence for a primary role of impaired homeostatic plasticity in the pathophysiology of these conditions (Antal et al., 2008; Brighina et al., 2011; Kang et al., 2011; Quartarone and Pisani, 2011).

This study aimed to investigate homeostatic plasticity in the primary motor cortex representation of 'unaffected' hand muscles in individuals with cLBP and pain-free controls. Similar to findings in individuals with migraine and focal hand dystonia, it was hypothesised that individuals with cLBP would fail to display a reversal of excitation towards inhibition following a second block of excitatory non-invasive brain stimulation consistent with global impairment in homeostatic control. A secondary aim was to examine the relationship between the magnitude of impaired homeostatic plasticity (if present) and the intensity and duration of cLBP.

2. Materials and methods

2.1. Participants

To determine a sufficient sample size to detect a difference in homeostatic plasticity between those with and without cLBP should one exist (Aim 1), a power calculation was performed using a conservative effect size estimate of 0.2, an alpha of 0.05 and a power of 0.8. Using these parameters, 24 individuals were required in each group. However, to ensure there was also sufficient power to examine a relationship between impaired homeostatic plasticity and pain in the cLBP group, should one exist (Aim 2), a second power calculation using an r value of 0.4, an alpha of 0.05 and power of 0.8 was performed. Using these parameters, a sample size of 47 individuals with cLBP was required. Thus, 50 individuals with cLBP (mean ± standard deviation age: 45 ± 16 years, 26 men), and 25 healthy, pain-free controls (age: 43 ± 17 years; 13 men) were recruited. Chronic, non-specific low back pain was defined as the presence of continuous back pain lasting three months or more that was not due to a diagnosable pathology. A verbally administered 11-point numerical rating scale (NRS) anchored with 'no pain' at zero and 'worst pain possible' at 10, was used to determine pain intensity in the week prior to, and on the day of, testing. Participants were excluded if they presented with LBP due to lumbar surgery, fracture, lumbar puncture, malignancy, infection, facet denervation, neuropathic or mixed pain (where pain radiated below the gluteal fold), and pain-free controls were excluded if they had a history of cLBP. Any participant with a history of major circulatory, neurological, psychiatric, respiratory or cardiac diseases, who was taking central nervous system acting medication, or who presented with a cognitive deficit that impaired the ability to understand instructions or provide informed consent was excluded. All participants were required to meet inclusion criteria as per the transcranial magnetic stimulation (TMS) safety guidelines (i.e. no history of epilepsy, absence of metal implants in the skull) (Keel et al., 2001). Participant characteristics are summarised in Table 1.

A verbal and written description of the experimental procedures was provided to all participants. Written, informed consent was obtained before testing. The study was approved by the institutional

Table 1
Participant characteristics.

Demographics	Chronic low back pain	Pain-free controls
N	50	25
Age (years)	45 ± 16	43 ± 17
Male: Female	26:24	13:12
Side of worst pain (L:R)	22:28	-
Pain on day of testing (NRS)	4.9 ± 2.8	-
Pain in the week before (NRS)	4.1 ± 2.6	-
History of back pain (years)	12.7 ± 14.4	
Pain medication*	9	-

N: total number of participants; L: left; R: right; NRS: numerical rating scale.
* Taking paracetamol as required.

Human Research Ethics Committee (Approval number: H10184) and performed in accordance with the Declaration of Helsinki.

2.2. Experimental protocol

All participants attended two experimental sessions in random order, at least 7 days apart. Participants received a single 7-min block of anodal transcranial direct current stimulation (tDCS) applied to the primary motor cortex (M1) in one session to confirm the existence of an excitatory response ('single tDCS' protocol; Fig. 1A). In a separate session, participants were exposed to two blocks of anodal tDCS (7-min and 5-min separated by a 3-min rest period) to investigate homeostatic plasticity in M1 ('double tDCS'; Fig. 1B). This protocol has been shown to induce homeostatic plasticity (observed as a reduction in motor evoked potential (MEP) amplitude in response to TMS) in healthy individuals (Fricke et al., 2011). In both sessions, MEPs were elicited using single pulse TMS and recorded from the first dorsal interosseous (FDI) muscle ipsilateral to the side of worst pain in individuals with cLBP and the matched side for pain-free controls. The FDI muscle was chosen to allow investigation of global impairment in homeostatic plasticity consistent with previous studies in focal hand dystonia and migraine (Antal et al., 2008; Quartarone et al., 2005). MEPs were recorded at baseline, and immediately following each experimental protocol at 0, 10, 20 and 30-min follow-up (Cavaleri et al., 2017). Participants were comfortably seated with their hand and arm at rest throughout both sessions. No participant reported muscle spasm or discomfort during testing.

2.3. Assessment of corticomotor excitability

MEPs were recorded using surface dual electrodes (Ag-AgCl, Noraxon dual electrodes, product #272S, inter-electrode distance 2.0 cm) placed in a belly-tendon montage on the relaxed FDI muscle (Antal et al., 2008; Fricke et al., 2011; Quartarone et al., 2005). The ground electrode was positioned on the olecranon process. Raw EMG signals were amplified (1000 times), bandpass filtered within a range of 20 Hz (high-pass) to 1 kHz (low-pass) and sampled at 2 kHz (CED 1401 AD, Cambridge Electronic Design, Cambridge, United Kingdom) via acquisition software (CED, version 5.08 × 86). The MEP peak-to-peak amplitude was extracted and averaged for analysis.

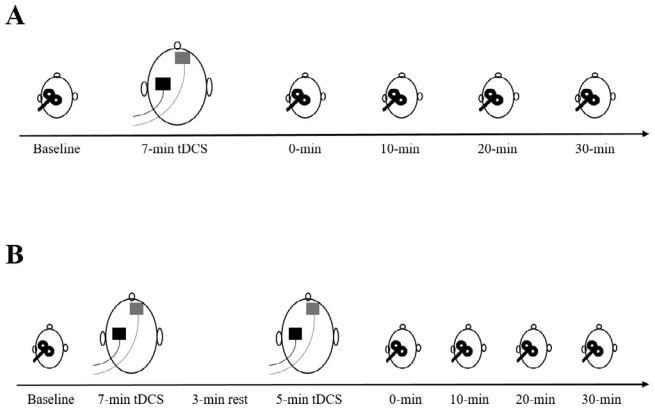

Fig. 1. (A) Single tDCS protocol: Anodal tDCS was applied to the primary motor cortex contralateral to the side of worst pain in individuals with cLBP and the matched side for pain-free controls in a single, 7-min block. (B) Double tDCS protocol: Anodal tDCS was applied to the primary motor cortex contralateral to the side of worst pain in individuals with cLBP and the matched side for pain-free controls for a 7-min block followed by a second 5-min block separated by a 3-min rest period. Motor evoked potentials (elicited using transcranial magnetic stimulation) were recorded at baseline and at 0, 10, 20 and 30-min follow-up in each experiment.

A standard 70 mm figure-of-eight coil connected to a magnetic stimulator (Magstim 200, Magstim Co. Ltd. Dyfed, UK) was used to provide single-pulse TMS. The coil was positioned tangentially to the scalp with the handle pointing posterolaterally at a 45° angle from the mid-sagittal plane. This orientation is optimal for the induction of posterior-to-anterior (PA) directed current for transsynaptic activation of horizontal cortical connections in M1 (Bashir et al., 2013; Brasil-Neto et al., 1992). The optimal site (hotspot) for eliciting MEPs from the relaxed FDI was determined before each experimental session by systematically moving the coil in 1 cm increments around the motor cortex. The hotspot was marked with a pen to allow accurate coil positioning. The stimulation intensity for TMS was adjusted to elicit an MEP amplitude of 1 mV peak-to-peak in the relaxed FDI at baseline, and this intensity was kept consistent throughout each test session. The average amplitude of 30 MEPs was used for analysis at each time-point.

2.4. Transcranial direct current stimulation

In both experiments, a battery driven direct current stimulator (DC-Stimulator Plus, NeuroConn, Ilmenau, Germany) was used to deliver a constant current of 1 mA through saline-soaked sponge electrodes (surface 7 × 5 cm). The active electrode (anode) was positioned over the motor cortical representation (hotspot) for FDI as determined by TMS in each participant. The reference electrode (cathode) was positioned over the contralateral supraorbital region. Electrodes were fixed with two adjustable rubber straps around the head. Stimulation in this montage has been reported to increase cortical excitability in the underlying M1 that outlasts the stimulation period by 20–60 min (Fricke et al., 2011). Current was ramped up and down over 10 s at the start and end of stimulation to avoid startling participants by alternating current transients that cause immediate neuronal firing during tDCS (Nitsche et al., 2008).

2.5. Statistical analysis

Data are presented as means and standard deviations (SD) throughout the text. For all analyses, SPSS software for windows, version 22 was used. A two-way analysis of variance (ANOVA) was performed to examine the TMS intensity used to elicit a 1 mV MEP at baseline between groups (cLBP and pain-free controls) and protocols (single vs. double tDCS). To examine the effect of each tDCS protocol (single vs. double) on raw (non-normalised) MEP amplitudes, separate two-way repeated measures ANOVAs were conducted for each protocol with factors 'Group' (cLBP vs. pain-free controls), and 'Time' (baseline, 0, 10, 20 and 30-min). Where appropriate, post-hoc testing was performed using t-tests with Bonferroni corrections for multiple comparisons. The Greenhouse-Geisser method was used to correct for non-sphericity as required. A Pearson product-moment correlation coefficient was used to assess the relationship between the magnitude of homeostatic plasticity (MEP amplitude) at each time-point after the double tDCS protocol and pain intensity, and pain duration, respectively. For all statistical tests a P-value of <0.05 was considered significant.

3. Results

3.1. TMS intensity at baseline

In the single tDCS protocol, the baseline TMS intensity (percent of maximum stimulator output) used to elicit MEPs of 1 mV peak-to-peak amplitude was 58 ± 15% and 58 ± 13% in the cLBP and pain-free control groups respectively. In the double tDCS protocol, the TMS intensity used to elicit MEPs of 1 mV peak-to-peak was 57 ± 14%, and 56 ± 15% in the cLBP and pain-free control groups

respectively. There was no difference in the baseline TMS intensity between groups ($F_{1,73} = 0.0$, $P = 0.8$) or protocols ($F_{1,73} = 3.8$, $P = 0.1$).

3.2. Single tDCS protocol

MEP amplitude increased in both groups following the single tDCS protocol (Fig. 2A; Table 2). There was a main effect of time ($F_{4,292} = 6.7$, $P < 0.001$), but no main effect of group ($F_{1,292} = 0.0$, $P = 0.9$) and no interaction effect ($F_{4,292} = 1.4$, $P = 0.2$). Compared with baseline, MEP amplitudes increased at all time points in both groups following 7-min of anodal tDCS (post-hoc all: $t > 3.6$, $P < 0.003$; Fig. 3A). Sixty-eight per cent of individuals with cLBP, and 76% of healthy, pain-free individuals exhibited an excitatory response (increased MEP amplitude relative to baseline) following the single tDCS protocol (Fig. 4A).

3.3. Double tDCS protocol

In the double tDCS protocol, the size of the MEP amplitude over time was dependent on the presence or absence of cLBP (Fig. 2B; Table 2). The ANOVA demonstrated no main effect of time ($F_{4,292} = 2.4$, $P = 0.1$) but a main effect of group ($F_{1,292} = 37.9$, $P < 0.001$) and an interaction effect between group and time ($F_{4,292} = 7.4$, $P < 0.001$). Compared with baseline, pain-free controls displayed a reduction in MEP amplitude at all time-points following the double tDCS protocol (post hoc vs. baseline; 0 min: $t = 3.4$, $P = 0.01$; 10 min: $t = 4.4$, $P < 0.001$; 20 min: $t = 4.3$, $P < 0.001$; 30 min: $t = 3.4$, $P = 0.01$; Fig. 3B). In contrast, individuals with cLBP demonstrated no change in MEP amplitude over time (post-hoc vs baseline; 0 min: $t = 1.1$, $P = 0.9$; 10 min: $t = 1.5$, $P = 0.7$; 20 min: $t = 1.8$, $P = 0.5$; 30 min: $t = 2.5$, $P = 0.1$; Fig. 3B). When individuals with and without cLBP were compared at each time-point, there was no difference in the MEP amplitude at baseline (post hoc: $t = 0.5$; $P = 0.7$). However, MEP amplitudes were higher in the cLBP group when compared to pain-free controls at all other time-points (0, 10, 20 and 30-min; post hoc all: $t > 4.3$, $P < 0.001$; Fig. 3B).

Thirty-two per cent of individuals with cLBP and 72% of healthy, pain-free individuals displayed a normal homeostatic plasticity response (decreased MEP amplitude relative to baseline) following the double tDCS protocol (Fig. 4B).

3.4. Relationship between the intensity and duration of cLBP and impairment in homeostatic plasticity

Neither NRS scores of pain intensity (all time-points: $r < 0.2$, $n = 50$, $P > 0.1$) nor pain duration (all time-points: $r < 0.1$, $n = 50$, $P > 0.5$) were correlated with the change in MEP amplitude in individuals with cLBP after the double tDCS protocol.

4. Discussion

This study is the first to investigate homeostatic plasticity in musculoskeletal pain. Although individuals with and without cLBP displayed typical increases in corticomotor excitability in response to the single tDCS protocol, only pain-free controls demonstrated a

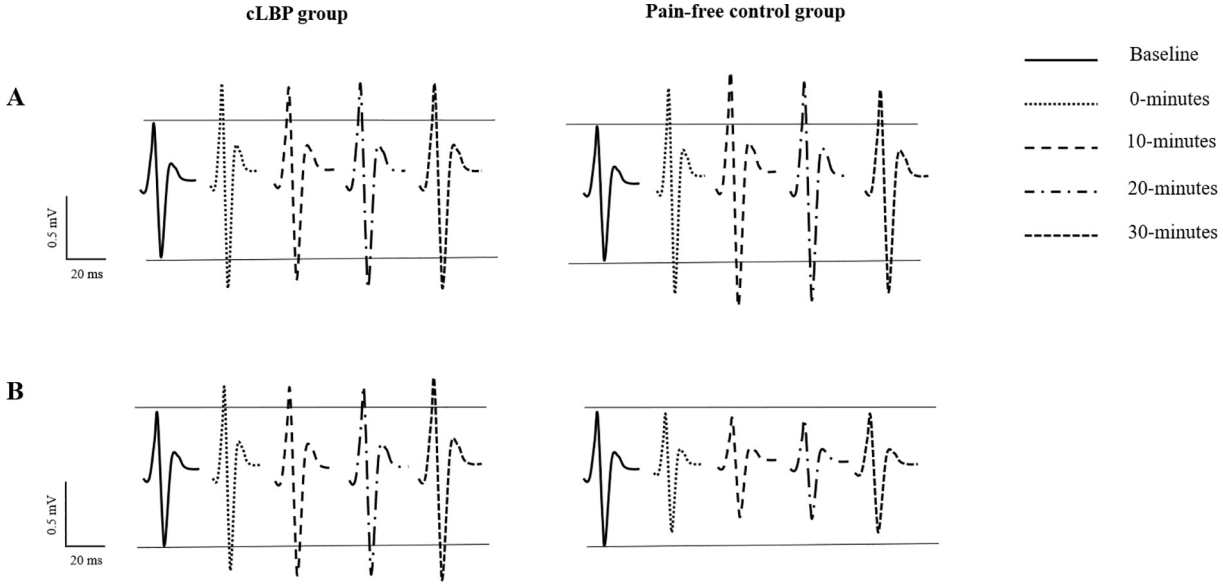

Fig. 2. Grand average raw MEP traces obtained at each time-point from participants in the cLBP and pain-free control group in response to the single (A), and double (B) tDCS protocols.

Table 2
Group data (mean ± standard deviation) for motor evoked potential amplitude (mV) recorded at each time-point (baseline, 0, 10, 20 and 30 min follow up) in the chronic low back pain and healthy, pain-free control groups in response to the single and double transcranial direct current stimulation (tDCS) protocols.

		Baseline	0 min	10 min	20 min	30 min
cLBP	Single tDCS	1.0 ± 0.1	1.4 ± 0.7*	1.3 ± 0.7*	1.4 ± 0.8*	1.4 ± 0.9*
	Double tDCS	1.0 ± 0.1	1.2 ± 0.6	1.2 ± 0.5	1.2 ± 0.5	1.3 ± 0.6
Controls	Single tDCS	1.0 ± 0.1	1.4 ± 0.9*	1.6 ± 0.9*	1.4 ± 0.6*	1.3 ± 0.5*
	Double tDCS	1.0 ± 0.1	0.7 ± 0.3*#	0.6 ± 0.3*#	0.6 ± 0.3*#	0.7 ± 0.4*#

cLBP: chronic low back pain. Significant difference relative to baseline (*$P < 0.01$) or the cLBP group at the same time-point (#$P < 0.001$).

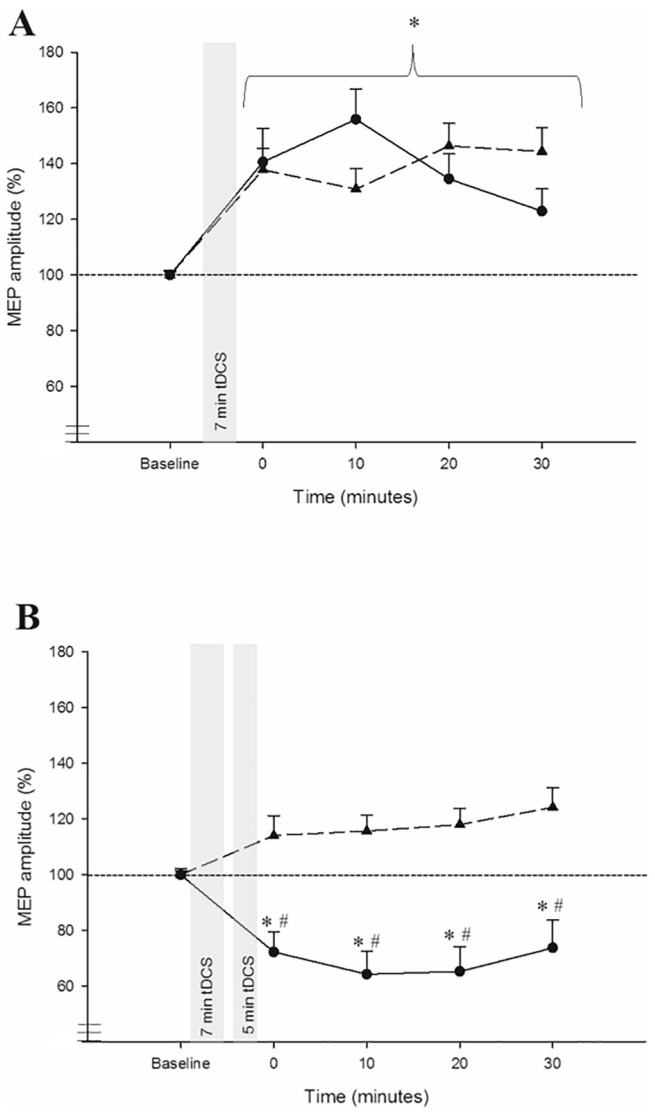

Fig. 3. Mean (+ SEM) motor evoked potential (MEP) amplitudes normalised to the baseline MEP amplitude (100%) in the cLBP (n = 50, closed triangles), and pain-free control (n = 25, closed circles) group in response to the single (A), and double (B) tDCS protocols. MEP amplitudes increased in both groups following the single tDCS protocol (main effect of time: $P < 0.003$). In the double tDCS protocol, the MEP amplitude was reduced at all time-points compared to baseline only in the pain-free control group ($^{*}P$ all < 0.01 relative to baseline). The cLBP group demonstrated no change in MEP amplitude compared to baseline ($^{\#}P$ all < 0.001 relative to cLBP group at the same time-point).

reversal of excitation towards inhibition following the double tDCS protocol consistent with normal homeostatic control. These novel data suggest a disruption of homeostatic plasticity in the primary motor cortex of individuals with cLBP that is present regardless of pain intensity or pain duration, and is not restricted to the representation of painful muscles. This mechanism could explain observations of maladaptive synaptic plasticity in cLBP, and could provide a pathophysiological mechanism to explain pain persistence in this condition.

Homeostatic plasticity is an essential form of plasticity in the human brain that ensures neuronal activity is maintained within a stable physiological range (Murakami et al., 2012; Ziemann and Siebner, 2008). Originally described by Bienenstock, Cooper and Munro, homeostatic plasticity prevents uncontrolled increases or decreases in synaptic efficacy by linking the effectiveness of LTP and LTD processes to the level of activity in the postsynaptic neuron (Bienenstock et al., 1982). When activity is high, LTP processes are less effective, favouring LTD and synaptic weakening. Similarly, when postsynaptic activity is low, LTD processes are less effective, favouring LTP and synaptic strengthening. It has previously been argued that changes in the amplitude of the MEP to transcranial magnetic stimulation after successive blocks of excitatory non-invasive brain stimulation reflect changes in the efficacy of synaptic relays within the corticomotor pathway (Siebner and Rothwell, 2003), and that reversal of the direction of these changes (towards inhibition) can be used to assess homeostatic plasticity (Fricke et al., 2011; Quartarone et al., 2005).

Using this model, the present data suggest individuals with cLBP have impaired homeostatic plasticity that manifests as a failure to regulate increases in corticomotor excitability since excitability is not reversed towards inhibition when postsynaptic activity is high. Failure to regulate synaptic plasticity in individuals with cLBP could lead to a disproportionately high rate of synaptic strengthening that in turn, produces abnormally high cortical excitability and maladaptive reorganisation of brain regions. Indeed, studies have shown increased cortical excitability (Kregel et al., 2015; Wand et al., 2011; Zhuo, 2008), reduced GABAergic inhibition (Janetzki et al., 2016; Schliessbach et al., 2017), and enlarged representations of the back muscles that are posteriorly shifted and show greater overlap in cLBP (Schabrun et al., 2015; Tsao et al., 2011, 2008) when compared with those of healthy controls. These cortical changes are associated with pain severity, impaired postural control and reduced coordination of trunk muscles (Janetzki et al., 2016; Tsao et al., 2011, 2008). In addition, the threshold where mechanical pressure turns to pain is lower in people with cLBP than controls (Giesbrecht and Battié, 2005; Giesecke et al., 2004; Imamura et al., 2013; Kobayashi et al., 2009), and people with cLBP exhibit reduced nociceptive withdrawal reflex thresholds (Biurrun Manresa et al., 2013), enlarged reflex receptive fields (Biurrun Manresa et al., 2013; Neziri et al., 2011), facilitated temporal summation (Biurrun Manresa et al., 2013), and increased S1 excitability (Flor et al., 1997; Kong et al., 2013). Together, these findings indicate an increase in spinal and cortical excitability in cLBP that could be explained by excessive synaptic strengthening as a result of impaired homeostatic control.

Although no studies have examined homeostatic plasticity in musculoskeletal pain, evidence from neurological populations supports this hypothesis. For example, focal hand dystonia is characterised by increased M1 excitability (Abbruzzese et al., 2001; Quartarone et al., 2003; Siebner et al., 1999), reduced GABAergic inhibition (Gallea et al., 2017; Hallett, 2011), and enlarged and overlapped M1 representations of the hand muscles (Schabrun et al., 2009) – cortical changes that are similar to those reported in cLBP. Several studies have shown impaired homeostatic plasticity in focal hand dystonia that is hypothesised to underpin the increased excitability and enlarged cortical representations observed in this condition (Kang et al., 2011; Quartarone et al., 2005, 2008; Quartarone and Pisani, 2011). Specifically, the failure of homeostatic plasticity to prevent the positive feedback cycle of synaptic plasticity is believed to produce unchecked increases in synaptic strength that consolidate maladaptive cortical reorganisation as well as the pathological sensorimotor interactions and movement patterns that manifest in focal hand dystonia (Quartarone and Pisani, 2011). Notably, excessive synaptic strengthening in focal hand dystonia is not restricted to the circuits clinically affected by dystonia, but is generalised throughout the sensorimotor system (Antal et al., 2008; Quartarone et al., 2008) – a finding consistent with the current observations in cLBP. This finding is also consistent with previous reports of a generalised alteration in cortical excitability that extends beyond the cortical representation of painful muscles in chronic pain conditions (termed 'pain-motor integration'), including cLBP (Flor et al., 1997; Juottonen et al., 2002; Schwenkreis et al., 2003; Tsao et al., 2011,

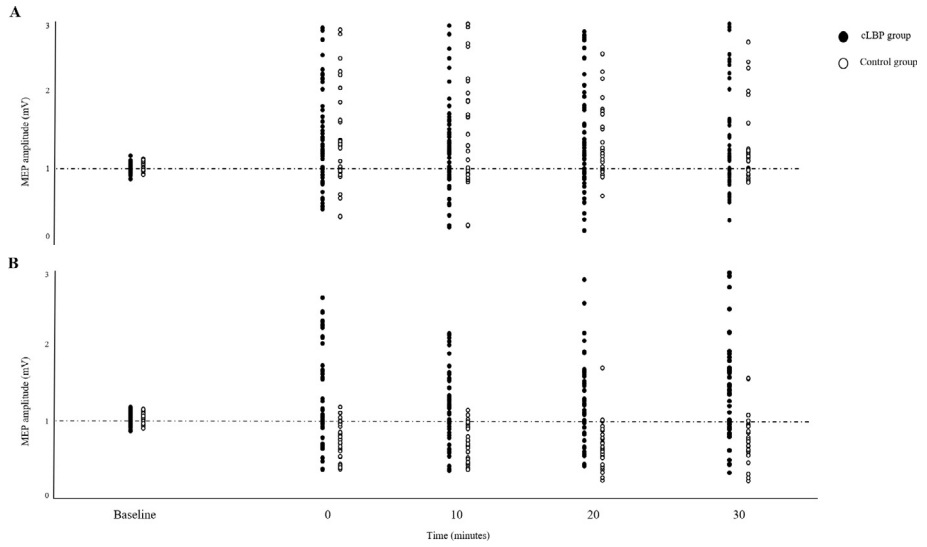

Fig. 4. Motor evoked potential (MEP) amplitude for each individual in the chronic low back pain (cLBP, closed circles) and healthy, pain-free control (open circles) group at each time-point in response to the single (A) and double (B) transcranial direct current stimulation (tDCS) protocols. Each circle represents the average MEP of 30 recordings at each time-point.

2008). As such, it is plausible that impaired homeostatic plasticity may also influence pain-motor integration in cLBP, driving increased cortical excitability, representational shifts, reinforcement of unwanted movement patterns (poor postural control, and coordination), and unpleasant sensory experiences.

Accordingly, should future studies confirm the relevance of impaired homeostatic plasticity to cLBP, therapies that seek to target neuroplasticity in persistent pain may need to target homeostatic, rather than synaptic, plasticity mechanisms. For example, previous studies have shown that patterned peripheral electrical stimulation applied to reduce cortical excitability (induce synaptic weakening; LTD) in focal hand dystonia where homeostatic plasticity is known to be impaired, improves cortical organisation and reduces symptoms (Schabrun et al., 2009). Similar treatments, designed specifically to counter excessive synaptic strengthening, could also be effective in cLBP. Further, an impaired ability to control increases in cortical excitability in cLBP may suggest that commonly used treatments known to promote synaptic strengthening (e.g. motor retraining, exercise) may require reconsideration in this population to avoid reinforcing aberrant synaptic plasticity, and inducing detrimental effects on symptoms. Further work is required to test these hypotheses.

Examination of individual level data revealed a portion of healthy individuals (38%) who displayed impaired homeostatic plasticity, and a portion of cLBP individuals (32%) whose homeostatic control was normal. Although the reasons for this are unclear it is possible that impaired homeostatic plasticity in healthy individuals could predispose to the development of chronic pain in future. However, longitudinal exploration of this mechanism within the same individual is needed to understand the relevance of individual variability in both the healthy and diseased brain. Notably, there was no association between the magnitude of impaired homeostatic plasticity and pain intensity or pain duration. However, all participants were experiencing LBP that had persisted for a substantial period of time (average duration of 12.7 ± 14.4 years). It is possible that homeostatic impairment develops in the sub-acute or early phases of cLBP and thus, was already present, regardless of small differences in symptom status in individuals tested in this study. Investigation of homeostatic plasticity in other brain areas, and during the acute, sub-acute and early phases of cLBP is warranted.

This study has several limitations. As the first exploration of this mechanism in cLBP we utilised a cross-sectional design. It is therefore not possible to determine whether homeostatic plasticity is impaired as a cause or a consequence of cLBP or to investigate the relationship between impaired homeostatic plasticity and fluctuations in pain over the clinical course of cLBP. Future studies should utilise longitudinal study designs with multiple measures of homeostatic plasticity and pain to provide comprehensive examination of this mechanism and the symptoms of cLBP. In the present study, the experimenter was not blinded to participant group and future studies should ensure blinding to reduce the risk of bias. In addition, our sample presented with a long history of cLBP. Different changes in homeostatic plasticity could be present in the acute or sub-acute phase of low back pain. Further studies are required to disentangle the relationship between different phases of cLBP and homeostatic plasticity mechanisms. Finally, this study did not include evaluation of spinal nociception (either through nociceptive withdrawal reflexes or laser evoked potentials). Previous studies have demonstrated spinal hyperexcitability in people with cLBP (Biurrun Manresa et al., 2013; Neziri et al., 2011). As transcranial magnetic stimulation provides an indication of excitability throughout the corticomotor pathway it is possible that changes in spinal excitability in people with cLBP may have contributed to the current findings. It is unclear whether changes in spinal excitability may drive altered homeostatic plasticity through the upregulation of afferent input to the cortex or whether impaired homeostatic plasticity drives an increase in spinal excitability. Future studies should seek to clarify the interaction between spinal mechanisms and homeostatic plasticity in people with and without cLBP.

5. Conclusion

This study is the first to explore homeostatic plasticity in musculoskeletal pain conditions. These unique data suggest a disruption of synaptic homeostasis in individuals with cLBP that manifests as an inability to counter excessive increases in corticomotor excitability. Further research is required to determine whether impaired homeostatic plasticity drives maladaptive synaptic plasticity and pain persistence in cLBP.

6. Disclosures

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References

Abbruzzese G, Marchese R, Buccolieri A, Gasparetto B, Trompetto C. Abnormalities of sensorimotor integration in focal dystonia: a transcranial magnetic stimulation study. Brain 2001;124:537–45. https://doi.org/10.1093/brain/124.3.537.

Antal A, Lang N, Boros K, Nitsche M, Siebner HR, Paulus W. Homeostatic metaplasticity of the motor cortex is altered during headache-free intervals in migraine with aura. Cereb Cortex 2008;18(11):2701–5. https://doi.org/10.1093/cercor/bhn032.

Balague F, Mannion AF, Pellise F, Cedraschi C. Non-specific low back pain. Lancet 2012;379(9814):482–91. https://doi.org/10.1016/S0140-6736(11)60610-7.

Bashir S, Perez JM, Horvath JC, Pascual-Leone A. Differentiation of motor cortical representation of hand muscles by navigated mapping of optimal TMS current directions in healthy subjects. J Clin Neurophysiol 2013;30(4):390–5. https://doi.org/10.1097/WNP.0b013e31829dda6b.

Bienenstock EL, Cooper LN, Munro PW. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci 1982;2(1):32–48.

Biurrun Manresa JA, Neziri AY, Curatolo M, Arendt-Nielsen L, Andersen OK. Reflex receptive fields are enlarged in patients with musculoskeletal low back and neck pain. Pain 2013;154(8):1318–24. https://doi.org/10.1016/j.pain.2013.04.013.

Brasil-Neto JP, Cohen LG, Panizza M, Nilsson J, Roth BJ, Hallett M. Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. J Clin Neurophysiol 1992;9(1):132–6.

Brighina F, Cosentino G, Vigneri S, Talamanca S, Palermo A, Giglia G, et al. Abnormal facilitatory mechanisms in motor cortex of migraine with aura. Eur J Pain 2011;15(9):928–35. https://doi.org/10.1016/j.ejpain.2011.03.012.

Brighina F, Giglia G, Scalia S, Francolini M, Palermo A, Fierro B. Facilitatory effects of 1 Hz rTMS in motor cortex of patients affected by migraine with aura. Exp Brain Res 2005;161(1):34–8. https://doi.org/10.1007/s00221-004-2042-7.

Cavaleri R, Schabrun SM, Chipchase LS. The number of stimuli required to reliably assess corticomotor excitability and primary motor cortical representations using transcranial magnetic stimulation (TMS): a systematic review and meta-analysis. Syst Rev 2017;6(1):48. https://doi.org/10.1186/s13643-017-0440-8.

Cosentino G, Fierro B, Vigneri S, Talamanca S, Paladino P, Baschi R, et al. Cyclical changes of cortical excitability and metaplasticity in migraine: evidence from a repetitive transcranial magnetic stimulation study. Pain 2014;155(6):1070–8. https://doi.org/10.1016/j.pain.2014.02.024.

Flor H, Braun C, Elbert T, Birbaumer N. Extensive reorganisation of primary somatosensory cortex in chronic back pain patients. Neurosci Lett 1997;224(1):5–8. https://doi.org/10.1016/S0304-3940(97)13441-3.

Fricke K, Seeber AA, Thirugnanasambandam N, Paulus W, Nitsche MA, Rothwell JC. Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. J Neurophysiol 2011;105(3):1141–9. https://doi.org/10.1152/jn.00608.2009.

Gallea C, Herath P, Voon V, Lerner A, Ostuni J, Saad Z, et al. Loss of inhibition in sensorimotor networks in focal hand dystonia. Neuroimage Clin 2017;17:90–7. https://doi.org/10.1016/j.nicl.2017.10.011.

Giesbrecht RJS, Battié MC. A comparison of pressure pain detection thresholds in people with chronic low back pain and volunteers without pain. Phys Ther 2005;85(10):1085–92. https://doi.org/10.1093/ptj/85.10.1085.

Giesecke T, Gracely RH, Grant MA, Nachemson A, Petzke F, Williams DA, et al. Evidence of augmented central pain processing in idiopathic chronic low back pain. Arthritis Rheum 2004;50(2):613–23. https://doi.org/10.1002/art.20063.

Hallett M. Neurophysiology of dystonia: the role of inhibition. Neurobiol Dis 2011;42(2):177–84. https://doi.org/10.1016/j.nbd.2010.08.025.

Hebb DO. The organization of behavior: a neuropsychological theory. New York: Wiley; 1949.

Imamura M, Chen J, Matsubayashi SR, Targino RA, Alfieri FM, Bueno DK, et al. Changes in pressure pain threshold in patients with chronic nonspecific low back pain. Spine 2013;38(24):2098–107. https://doi.org/10.1097/01.brs.0000435027.50317.d7.

Janetzki L, Gussew A, Malessa R, Habenicht U, Reichenbach JR, Strauss B, et al. Cerebral metabolic changes and chronic back pain: study taking into consideration clinical and psychological parameters. Schmerz 2016;30(2):134–40. https://doi.org/10.1007/s00482-015-0082-5.

Jia Z, Yu S. Grey matter alterations in migraine: a systematic review and meta-analysis. Neuroimage Clin 2017;14:130–40. https://doi.org/10.1016/j.nicl.2017.01.019.

Juottonen K, Gockel M, Silen T, Hurri H, Hari R, Forss N. Altered central sensorimotor processing in patients with complex regional pain syndrome. Pain 2002;98(3):315–23. https://doi.org/10.1016/S0304-3959(02)00119-7.

Kang JS, Terranova C, Hilker R, Quartarone A, Ziemann U. Deficient homeostatic regulation of practice-dependent plasticity in writer's cramp. Cereb Cortex 2011;21(5):1203–12. https://doi.org/10.1093/cercor/bhq204.

Karabanov A, Ziemann U, Hamada M, George MS, Quartarone A, Classen J, et al. Consensus paper: probing homeostatic plasticity of human cortex with non-invasive transcranial brain stimulation. Brain Stimul 2015;8(3):442–54. https://doi.org/10.1016/j.brs.2015.01.404.

Keel JC, Smith MJ, Wassermann EM. A safety screening questionnaire for transcranial magnetic stimulation. Clin Neurophysiol 2001;112(4):720. https://doi.org/10.1016/S1388-2457(00)00518-6.

Kobayashi Y, Kurata J, Sekiguchi M, Kokubun M, Akaishizawa T, Chiba Y, et al. Augmented cerebral activation by lumbar mechanical stimulus in chronic low back pain patients: an FMRI study. Spine 2009;34(22):2431–6. https://doi.org/10.1097/BRS.0b013e3181b1fb76.

Kong J, Spaeth RB, Wey HY, Cheetham A, Cook AH, Jensen K, et al. S1 is associated with chronic low back pain: a functional and structural MRI study. Mol Pain 2013;9:43. https://doi.org/10.1186/1744-8069-9-43.

Kregel J, Meeus M, Malfliet A, Dolphens M, Danneels L, Nijs J, et al. Structural and functional brain abnormalities in chronic low back pain: a systematic review. Semin Arthritis Rheum 2015;45(2):229–37. https://doi.org/10.1016/j.semarthrit.2015.05.002.

Masse-Alarie H, Beaulieu LD, Preuss R, Schneider C. Corticomotor control of lumbar multifidus muscles is impaired in chronic low back pain: concurrent evidence from ultrasound imaging and double-pulse transcranial magnetic stimulation. Exp Brain Res 2016;234(4):1033–45. https://doi.org/10.1007/s00221-015-4528-x.

Moseley GL, Flor H. Targeting cortical representations in the treatment of chronic pain: a review. Neurorehabil Neural Repair 2012;26(6):646–52. https://doi.org/10.1177/1545968311433209.

Murakami T, Muller-Dahlhaus F, Lu MK, Ziemann U. Homeostatic metaplasticity of corticospinal excitatory and intracortical inhibitory neural circuits in human motor cortex. J Physiol 2012;590:5765–81. https://doi.org/10.1113/jphysiol.2012.238519.

Neziri AY, Manresa JB, Jüni P, Arendt-Nielsen L, Andersen OK, Curatolo M. F123 expansion of nociceptive reflex receptive fields in patients with low back and neck pain. Eur J Pain Suppl 2011;5(S1):109–10. https://doi.org/10.1016/S1754-3207(11)70372-8.

Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: state of the art 2008. Brain Stimul 2008;1(3):206–23. https://doi.org/10.1016/j.brs.2008.06.004.

Quartarone A, Bagnato S, Rizzo V, Siebner HR, Dattola V, Scalfari A, et al. Abnormal associative plasticity of the human motor cortex in writer's cramp. Brain 2003;126:2586–96. https://doi.org/10.1093/brain/awg273.

Quartarone A, Morgante F, Sant'angelo A, Rizzo V, Bagnato S, Terranova C, et al. Abnormal plasticity of sensorimotor circuits extends beyond the affected body part in focal dystonia. J Neurol Neurosurg Psychiatry 2008;79(9):985–90. https://doi.org/10.1136/jnnp.2007.121632.

Quartarone A, Pisani A. Abnormal plasticity in dystonia: disruption of synaptic homeostasis. Neurobiol Dis 2011;42(2):162–70. https://doi.org/10.1016/j.nbd.2010.12.011.

Quartarone A, Rizzo V, Bagnato S, Morgante F, Sant'Angelo A, Romano M, et al. Homeostatic-like plasticity of the primary motor hand area is impaired in focal hand dystonia. Brain 2005;128:1943–50. https://doi.org/10.1093/brain/awh527.

Schabrun SM, Elgueta-Cancino EL, Hodges PW. Smudging of the motor cortex is related to the severity of low back pain. Spine 2015. https://doi.org/10.1097/BRS.0000000000000938.

Schabrun SM, Stinear CM, Byblow WD, Ridding MC. Normalizing motor cortex representations in focal hand dystonia. Cereb Cortex 2009;19(9):1968–77. https://doi.org/10.1093/cercor/bhn224.

Schliessbach J, Vuilleumier PH, Siegenthaler A, Bütikofer L, Limacher A, Juni P, et al. Analgesic effect of clobazam in chronic low-back pain but not in experimentally induced pain. Eur J Pain 2017;21(8):1336–45. https://doi.org/10.1002/ejp.1032.

Schwenkreis P, Janssen F, Rommel O, Pleger B, Volker B, Hosbach I, et al. Bilateral motor cortex disinhibition in complex regional pain syndrome (CRPS) type I of the hand. Neurology 2003;61(4):515–9. https://doi.org/10.1212/WNL.61.4.515.

Siebner HR, Auer C, Conrad B. Abnormal increase in the corticomotor output to the affected hand during repetitive transcranial magnetic stimulation of the primary motor cortex in patients with writer's cramp. Neurosci Lett 1999;262(2):133–6. https://doi.org/10.1016/S0304-3940(99)00056-7.

Siebner HR, Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res 2003;148(1):1–16. https://doi.org/10.1007/s00221-002-1234-2.

Tsao H, Danneels LA, Hodges PW. ISSLS prize winner: smudging the motor brain in young adults with recurrent low back pain. Spine 2011;36(21):1721–7. https://doi.org/10.1097/BRS.0b013e31821c4267.

Tsao H, Galea MP, Hodges PW. Reorganisation of the motor cortex is associated with postural control deficits in recurrent low back pain. Brain 2008;131:2161–71. https://doi.org/10.1093/brain/awn154.

Wand BM, Parkitny L, O'Connell NE, Luomajoki H, McAuley JH, Thacker M, et al. Cortical changes in chronic low back pain: current state of the art and implications for clinical practice. Man Ther 2011;16(1):15–20. https://doi.org/10.1016/j.math.2010.06.008.

Welch KM. Contemporary concepts of migraine pathogenesis. Neurology 2003;61(8 Suppl 4):S2–8. https://doi.org/10.1212/WNL.61.8_suppl_4.S2.

Zhuo M. Cortical excitation and chronic pain. Trends Neurosci 2008;31(4):199–207. https://doi.org/10.1016/j.tins.2008.01.003.

Ziemann U, Siebner HR. Modifying motor learning through gating and homeostatic metaplasticity. Brain Stimul 2008;1(1):60–6. https://doi.org/10.1016/j.brs.2007.08.003.

Appendix C

Editorial: Pain-motor integration and chronic pain: One step ahead

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Editorial

Pain-motor integration and chronic pain: One step ahead

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Pain-motor integration refers to physiological processes responsible for mutual interaction between nociceptive and motor information in the central nervous system. Two separate lines of evidence support the hypothesis that pain-motor integration mechanisms operate in the human cerebral cortex. First, epidural motor cortex stimulation (MCS), as well as non-invasive brain stimulation (NIBS) protocols delivered over the primary motor cortex (M1), can both improve pain in patients with drug-resistant chronic pain. MCS and NIBS are thought to modulate M1 corticofugal descending inhibitory inputs to structures involved in the central processing of pain such as the thalamus and the periaqueductal grey (PAG) (Cruccu et al., 2016; Lefaucheur, 2016). Second, experimental pain may affect the excitability as well as plasticity of specific circuits in the human motor system. Accordingly, over recent years, a growing number of authors have investigated experimentally the impact of chronic pain on long-term potentiation (LTP)- and depression (LTD)-like plasticity processes in M1, owing to pain-motor integration, in various neurological disorders (Suppa et al., 2013, 2017; Naro et al., 2015).

In this issue of *Clinical Neurophysiology*, Thapa et al. (2018) investigated possible changes in M1 LTP/LTD-like plasticity in individuals affected by chronic low back pain (cLBP). The study design consisted of two separate experimental sessions implying a single or double application of M1 anodal transcranial direct current stimulation (tDCS). As a measure of M1 plasticity, the authors measured and compared motor evoked potential (MEP) amplitudes, recorded at baseline and 0–30 min after tDCS ended. In healthy subjects, when delivering a single block of anodal tDCS (7 min of stimulation), as expected, MEPs increased in size at all the timepoints, suggesting M1 plasticity processes. By contrast, following the application of two blocks of anodal tDCS (first block of 7 min of tDCS followed by a second block of 5 min of tDCS, with an inter-block interval of 3 min), MEPs decreased in size in healthy controls, owing to homeostatic plasticity mechanisms, in line with a previous observation (Fricke et al., 2011). Conversely, in individuals with cLBP, although a single block of anodal tDCS led to normal MEP facilitation, following the two-block tDCS protocol, MEPs remained abnormally facilitated. By demonstrating normal responses to a single block of anodal tDCS but abnormal responses to the two-block tDCS protocol, the authors provided the first evidence of normal plasticity but abnormal homeostatic plasticity in M1, in individuals with cLBP. Finally, there was no correlation between the patient's clinical features (e.g. pain duration and intensity scored by means of the numerical rating scale – NRS) and the abnormal MEP changes observed after the two-block tDCS protocol.

The study of Thapa et al. (2018) is characterized by several strengths. First, the study included a relatively large number of individuals manifesting with cLBP, a common chronic musculoskeletal pain disorder with a rather unclear pathophysiology. Second, the study design was based on the experimental investigation of cortical pain-motor integration processes in cLBP, by means of an advanced NIBS protocol (Fricke et al., 2011). Third, by examining and comparing MEP changes induced by a single or double application of anodal tDCS, the authors compared mechanisms underlying plasticity and homeostatic plasticity in M1, both related to pain-motor integration, in individuals with cLBP.

Nonetheless, when interpreting the observations reported by Thapa et al. (2018), several methodological points should be taken into account. The experimental investigation of possible changes in M1 LTP/LTD-like plasticity driven by chronic pain would have benefitted from the evaluation of the integrity of the peripheral and central nociceptive pathway in individuals with cLBP. To this purpose, laser-evoked potentials (LEPs) are currently available to examine the integrity of structures involved in the transmission and central processing of pain (Cruccu et al., 2010). Peripheral or central nociceptive pathway alterations may induce M1 LTP/LTD-like plasticity changes, as demonstrated in different neurological conditions associated with pain of neuropathic as well as non-neuropathic origin (Chang et al., 2018). Another comment concerns the sub-optimal description of chronic drug treatments in the individuals with cLBP enrolled in the present study. Several pharmacological agents, commonly used for symptomatic improvement of chronic pain syndromes, potentially affect M1 excitability and LTP/LTD-like plasticity processes (Nitsche et al., 2012; Ziemann et al., 2015). For instance, gabapentinoids (gabapentin and pregabalin), antidepressant drugs (amitriptyline, duloxetine, etc.), opioid analgesics (oxycodone, tramadol, codeine, etc.) and finally, nonsteroidal anti-inflammatory drugs (NSAIDs) (e.g. acetaminophen/paracetamol), are all widely used in individuals with cLBP. Virtually all these pharmacological agents may affect measures of M1 excitability and LTP/LTD-like plasticity, as tested by NIBS protocols (Nitsche et al., 2012; Ziemann et al., 2015). A further comment concerns the physiological interpretation of the findings reported by Thapa et al. (2018). Homeostatic plasticity refers to high-order physiological processes able to prevent an

https://doi.org/10.1016/j.clinph.2018.02.005

uncontrolled strengthening or weakening of synaptic activity (LTP and LTD, respectively) by stabilizing it within physiological ranges (Karabanov et al., 2015; Müller-Dahlhaus and Ziemann, 2015). Differently from healthy controls, in individuals with cLBP, the two-block tDCS protocol failed to reverse MEPs from facilitation to inhibition, pointing to defective mechanisms of homeostatic plasticity in cLBP. However, the putative mechanisms through which cLBP affects homeostatic plasticity processes in M1 regions beyond those representing painful body representations, such as the intrinsic hand muscles examined here, remain still unclear. Finally, a subgroup of healthy subjects manifested homeostatic plasticity abnormalities similar to those seen in individuals with cLBP and, conversely, several individuals with cLBP were characterized by normal homeostatic plasticity processes. The most likely explanation for these findings is the well-known variability in responses to human NIBS protocols (Guerra et al., 2017a,b).

In conclusion, we believe that the study of Thapa et al. (2018) provides new important information on the effect of chronic pain on M1 LTP/LTD-like plasticity processes, through mechanisms of abnormal pain-motor integration, in patients with cLBP. Future studies will clarify the precise pathophysiological link between abnormal homeostatic plasticity processes in M1 and chronic pain generation or persistence, in individuals with cLBP.

Conflict of interest

Nothing to declare.

References

Chang WJ, O'Connell NE, Beckenkamp PR, Alhassani G, Liston MB, et al. Altered primary motor cortex structure, organization, and function in chronic pain: a systematic review and meta-analysis. J Pain 2018;12. 30769-1.

Cruccu G, Sommer C, Anand P, Attal N, Baron R, Garcia-Larrea L, et al. EFNS guidelines on neuropathic pain assessment: revised 2009. Eur J Neurol 2010;17:1010–8.

Cruccu G, Garcia-Larrea L, Hansson P, Keindl M, Lefaucheur JP, Paulus W, et al. EAN guidelines on central neurostimulation therapy in chronic pain conditions. Eur J Neurol 2016;23:1489–99.

Fricke K, Seeber AA, Thirugnanasambandam N, Paulus W, Nitsche MA, Rothwell JC. Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. J Neurophysiol 2011;105:1141–9.

Guerra A, López-Alonso V, Cheeran B, Suppa A. Variability in non-invasive brain stimulation studies: reasons and results. Neurosci Lett 2017. https://doi.org/10.1016/j.neulet.2017.12.058.

Guerra A, López-Alonso V, Cheeran B, Suppa A. Solutions for managing variability in non-invasive brain stimulation studies. Neurosci Lett 2017. https://doi.org/10.1016/j.neulet.2017.12.060.

Karabanov A, Ziemann U, Hamada M, George MS, Quartarone A, Classen J, et al. Consensus paper: probing homeostatic plasticity of human cortex with non-invasive transcranial brain stimulation. Brain 2015;8:993–1006.

Lefaucheur JP. Cortical neurostimulation for neuropathic pain: state of the art and perspectives. Pain 2016;157:81–9.

Müller-Dahlhaus F, Ziemann U. Metaplasticity in human cortex. Neuroscientist 2015;21:185–202.

Naro A, Leo A, Russo M, Quartarone A, Bramanti P, Calabrò RS. Shaping thalamo-cortical plasticity: a marker of cortical pain integration in patients with post-anoxic unresponsive wakefulness syndrome? Brain Stimul 2015;8:97–104.

Nitsche MA, Müller-Dahlhaus F, Paulus W, Ziemann U. The pharmacology of neuroplasticity induced by non-invasive brain stimulation: building models for the clinical use of CNS active drugs. J Physiol 2012;590:4641–62.

Suppa A, Biasiotta A, Belvisi D, Marsili L, La Cesa S, Truini A, et al. Heat-evoked experimental pain induces long-term potentiation-like plasticity in human primary motor cortex. Cereb Cortex 2013;23:1942–51.

Suppa A, Leone C, Di Stasio F, Marsili L, Di Santo A, Biasiotta A, et al. Pain-motor integration in the primary motor cortex in Parkinson's disease. Brain Stimul 2017;10:806–16.

Thapa T, Graven-Nielsen T, Chipchase L, Schabrun S. Disruption of cortical synaptic homeostasis in individuals with chronic low back pain. Clin Neurophysiol 2018;129:1090–6.

Ziemann U, Reis J, Schwenkreis P, Rosanova M, Strafella A, Badawy R, et al. TMS and drugs revisited 2014. Clin Neurophysiol 2015;126:1847–68.

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