THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

Is plasticity in the human motor cortices altered in healthy older adults?

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Bachelor of Psychological Science (Hons I)

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2015

The Queensland Brain Institute

Abstract

Neuroplasticity refers to the ability of the brain to alter its structure and function in response to the demands of its environment. Substantial evidence from studies in non-human animals suggests that neuroplasticity at the synaptic level is reduced in the aged brain. In humans, ageing is associated with cognitive and motor decline and some experimental evidence using non-invasive brain stimulation and motor training suggests that neuroplasticity is reduced in the motor cortex. It is also evident, however, that there is variability in age-related decline of motor performance and neuroplasticity across individuals. In the studies investigating neuroplasticity, little consideration has been given to changes occurring outside the targeted region, for example in homotopic regions of the contralateral hemisphere. This is an important limitation, as neuroimaging studies suggest that older adults exhibit more widespread activity both within and across the cerebral hemispheres, relative to young adults, when performing the same motor task. This finding suggests that additional neural populations may be recruited in older adults to assist in learning new motor skills. The aim of this thesis then, was to investigate whether the manifestation of neuroplasticity across the cerebral hemispheres is altered in older adults. To achieve this, three different protocols using skilled training or non-invasive brain stimulation were employed to experimentally induce neuroplasticity primarily in the primary motor cortex (M1). These protocols have been shown to induce changes with characteristics that resemble long-term potentiation (LTP) and long-term depression (LTD) in animal models, which is the candidate mechanism for synaptic plasticity. In each study neuroplasticity effects were probed by measuring changes in corticospinal excitability in bilateral coritcospinal pathways (corticospinal plasticity) using single-pulse transcranial magnetic stimulation (TMS) and motor-evoked potentials (MEPs).

In the first study young and older participants were trained on a simple and complex motor task. The degree to which performance improvement transferred from the trained to the untrained hand and the extent to which this reflected changes in the distribution of corticospinal plasticity across the hemispheres were assessed. Both groups demonstrated performance improvements in the trained and untrained hand, although performance improvement in the trained hand was smaller in older adults. The percentage transfer from the trained to the untrained hand was comparable between young and older adults. Importantly, after training on the simple task, corticospinal excitability increased bilaterally in both young and older adults, a similar trend was also observed with the complex task. This result indicates that corticospinal plasticity, its manifestation in bilateral corticospinal pathways, and its relation to behavioural performance was comparable between young and older adults.

In a second study, intermittent theta burst stimulation (iTBS) was employed. This protocol uses patterned TMS to induce rate-dependent LTP-like changes in excitability of the targeted M1. An attention task was implemented during iTBS to control for the putative effects of attention on corticospinal plasticity, which has been demonstrated previously in young adults. At the group level, MEP amplitude did not change significantly from baseline following iTBS. There was, however, large individual variability in responses, with half the participants demonstrating increases in MEP amplitude and the other half decreases. Participants were therefore classified into groups of LTP-like (MEP change >0%) and LTD-like (MEP change <0%) responders based on their average MEP change in the target muscle post iTBS. For each responder group it was found that MEP amplitude changed reliably in the targeted but not the non-targeted corticospinal pathway and this did not vary between young and older adults. The results of this analysis suggest that corticospinal plasticity induced by iTBS is similar in young and older adults in the targeted pathway and does not manifest over a more bilateral network in the aged brain. This study also highlighted the possibility that allocating spatial attention toward the limb undergoing the intervention might enhance LTP-like responses in the targeted pathway of older adults experiencing iTBS-induced corticospinal plasticity.

In the final study, TMS was administered in conjunction with stimulation to the median nerve in a protocol termed paired associative stimulation (PAS). An excitatory protocol known as PAS 25, which involved median nerve stimulation delivered 25 ms prior to TMS, was used. This protocol was used because the (associative) LTP-like neuroplasticity induced may be more comparable to that arising following motor training. The role of attention in facilitating neuroplasticity in older adults was also investigated by systematically varying the location of spatial attention during PAS to either the hand innervated by the targeted or the non targeted pathway across different sessions. Similar to the results of Experiment 2, post-PAS MEP amplitude did not change reliably from baseline at the group level due to large individual variability. However, after classifying participants into groups of LTP-like and LTD-like responders, MEP amplitude changed reliably in both groups in the targeted but not in the non-targeted pathway and this was comparable between the age groups. The bilateral manifestation of PAS-induced corticospinal plasticity was not reliably influenced by attention.

Together the results of these experiments suggest that corticospinal plasticity induced by various paradigms targeting slightly different neuroplasticity mechanisms is maintained in the aged brain. Importantly, the findings of all three experiments indicate that corticospinal plasticity does not manifest reliably over a more bilateral motor network in young or older adults. These findings

suggest that although synaptic plasticity may be altered at the single cell level by advancing age, neuroplasticity in more large-scale networks is not necessarily reduced in the aged brain.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Dickins D. S. E., Sale M. V., & Kamke M. R. (2015). Intermanual transfer and bilateral cortical plasticity is maintained in older adults after skilled motor training with simple and complex tasks. *Frontiers in Aging Neuroscience.* doi:10.3389/fnagi.2015.00073

Dickins D. S. E., Sale M. V., & Kamke M. R. (2015) Plasticity induced by intermittent theta burst stimulation (iTBS) in bilateral motor cortices is not altered in older adults. *Neural Plasticity.* doi:10.1155/2015/323409

Conference Abstracts:

Dickins, D. S. E., Sale, M. V., & Kamke, M. R. (2015). Inter-limb transfer of both simple and complex motor skills is maintained in elderly adults. *1st International Brain Stimulation Conference*, Singapore.

Dickins, D. S. E. (2014). Old dogs, new tricks, new tactics: Investigating age-related changes in the degree and distribution of neuroplasticity in the primary motor cortex. *Autumn School and Cognitive Neuroscience Workshop*, Brisbane & Heron Island.

Dickins, D. S. E., Sale, M. V., Mattingley, J. B., & Kamke, M. R. (2014). Neuroplasticity in the primary motor cortex is reduced in elderly adults. *Australasian Experimental Psychology Conference*, Brisbane.

Dickins, D. S. E., Sale, M. V., & Kamke, M. R. (2013). Bilateral plasticity in the human motor cortices of elderly and young adults and the effect of visual spatial attention. *Australasian Brain Stimulation Meeting*, Melbourne.

Dickins, D. S. E., Sale, M. V., Mattingley, J. B., & Kamke, M. R. (2012). The effect of visual spatial attention on bilateral plasticity in the human motor cortices. *Australasian Cognitive Neuroscience Conference*, Brisbane.

Publications included in this thesis

Dickins D. S. E., Sale M. V., & Kamke M. R. (2015). Intermanual transfer and bilateral cortical plasticity is maintained in older adults after skilled motor training with simple and complex tasks. *Frontiers in Aging Neuroscience.* doi: 10.3389/fnagi.2015.00073 Incorporated as Chapter 2

Contributor	**Statement of contribution**
Daina S. E. Dickins (Candidate)	Conceptualised & designed experiments (70%) Data collection, analysis & interpretation (80%) Wrote the paper (70%)
Martin V. Sale	Conceptualised & designed experiments (15%) Data collection, analysis & interpretation (10%) Wrote the paper (15%)
Marc R. Kamke	Conceptualised & designed experiments (15%) Data collection, analysis & interpretation (10%) Wrote the paper (15%)

Dickins D. S. E., Sale M. V., & Kamke M. R. (2015) Plasticity induced by intermittent theta burst stimulation (iTBS) in bilateral motor cortices is not altered in older adults. *Neural Plasticity.* doi: 10.1155/2015/323409 -Incorporated as Chapter 3

Contributor	**Statement of contribution**
Daina S. E. Dickins (Candidate)	Conceptualised & designed experiments (70%) Data collection, analysis & interpretation (80%) Wrote the paper (70%)
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Contributions by others to the thesis

Marc Kamke and Martin Sale contributed significantly to this thesis, in the conceptualisation, design and interpretation of experiments, and in proofreading individual manuscripts and the thesis in its entirety. David Lloyd also provided invaluable technical assistance.

Statement of parts of the thesis submitted to qualify for the award of another degree

None

Acknowledgements

First and foremost I would like to acknowledge and sincerely thank my primary advisor Dr Marc Kamke. Marc has devoted endless time and effort throughout my candidature to developing my skills as a researcher. Marc's standards of excellence, dedication, professionalism and modesty are inspirational. I will be forever grateful for the lessons Marc has taught me over the period of my candidature and will carry them with me in future endeavours.

I would like to acknowledge Dr Martin Sale, my secondary advisor who has provided substantial valuable feedback on numerous drafts and words of encouragement along the way. Thanks must also go to Professor Jason Mattingley for his efforts in bringing together and managing such a fabulous group of talented individuals. I would like to sincerely thank all members of the Mattingley and Cognitive Neuroscience labs, past and present, for their support, in the ring (lab meetings), at the watering hole, on the netball courts, and the soccer fields.

Thanks also to my family for instilling in me the determination and persistence required to complete this PhD, specifically my grandparents who continued to fuel my motivation to understand the ageing brain and to finish.

Last but by no means least I would like to thank my life partner Belinda Craig, for providing feedback on endless thesis drafts, putting up with my love of neuroscience, and for keeping me alive and sane over the past few years.

Keywords

neuroplasticity, ageing, transcranial magnetic stimulation, paired associative stimulation, intermittent theta burst stimulation, attention, motor cortices.

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 170101, Biological Psychology (Neuropsychology, Psychopharmacology, Physiological Psychology), 40%

ANZSRC code: 170102, Developmental Psychology and Ageing, 30%

ANZSRC code: 110903, Central Nervous System, 30%

Fields of Research (FoR) Classification

FoR code: 1109, Neurosciences, 50%

FoR code: 1701, Psychology, 50%

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List of Abbreviations

aMT	Active motor threshold
ANOVA	Analysis of variance
APB	Abductor pollicis brevis
cTBS	Continuous theta burst stimulation
DTI	Diffusion tensor imaging
EEG	Electroencephalography
EMG	Electromyography
GABA	Gamma-aminobutyric acid
GPAQ	General physical activity questionnaire
iTBS	Intermittent theta burst stimulation
LED	Light emitting diode
LTD	Long-term depression
LTP	Long-term potentiation
M1	Primary motor cortex
MEG	Magnetoencephalography
MEP	Motor-evoked potential
MRI	Magnetic resonance imaging
MSO	Maximum stimulator output
NMDA	N-methyl-D-aspartate
PAS	Paired associative stimulation
PET	Positron emission topography
rMT	Resting motor threshold
rTMS	Repetitive transcranial magnetic stimulation
TBS	Theta burst stimulation
TES	Transcranial electrical stimulation
TMS	Transcranial magnetic stimulation
VBM	Voxel based morphometry

Chapter 1 General Introduction

1.1 Introduction

Neuroplasticity refers to the ability of neural populations to change in structure and function (Kolb & Whishaw, 1998). Neuroplasticity has been demonstrated after cognitive and motor training, cortical lesions and peripheral nerve damage (Kleim et al., 1998, Kleim et al., 2002; Kleim et al., 2004; Draganski et al., 2004; Scholz et al., 2009; Rockstroch et al., 1998; Karni et al., 1995; Schmidt-Wilcke et al., 2010; Pons et al., 1991; Sanes et al., 1992; Nudo et al., 1996; Pascual-Leone et al., 1995), indicating the critical role of neuroplasticity in enabling animals to learn – to acquire knowledge and skills and to adapt to the environment, novel situations and experiences. Although neuroplasticity was, for a long time, thought to be restricted to infants and young adults, it is now considered to occur throughout the lifespan (Barnes & McNaughton, 1980; van Praag et al., 1999). However, relative to what is known about neuroplasticity in young and middle aged adults, little is understood about neuroplasticity in the aged brain (> 65 years), which is associated with substantial age-related change in structural and functional biology and declines in cognitive and motor learning (Finkbiner et al., 1991; Hashtroudi et al 1991; see Voelcker-Rehage et al., 2008 for review of changes in motor skill-learning).

Much of the research investigating neuroplasticity in the aged brain has focused on non-human animal models, using invasive methods wherein small electrodes are placed close to or within a single cell to record and stimulate neural activity (Barnes, 1979; Barnes & McNaughton, 1985). These studies contribute to the understanding of how aging influences plasticity at the sub-cellular level, or at the level of single or small groups of neurons. Neuroplasticity in the human brain is typically investigated indirectly using non-invasive recording and stimulation techniques targeting sensory and motor regions (Stefan et al., 2000; Wolters et al., 2003; Huang et al., 2005; Garry et al., 2004; McDonnell & Ridding, 2006. Carroll et al., 2008; Koeneke et al., 2006; Hinder et al., 2011, 2013; Rogasch et al., 2009; Cirillo et al., 2010; Perez et al., 2004). The sensorimotor cortex contains functional representations of different body parts that are relatively stable, but which change in response to learning or damage to the motor system. Studies using non-invasive brain stimulation investigate neuroplasticity within the neural pathway travelling from the motor cortex to the peripheral musculature (the corticospinal pathway). As will be discussed, studies using this technique suggest that plasticity in the motor system of older humans is reduced, but these studies have investigated corticospinal plasticity in only the unilateral or targeted corticospinal pathway. As will also be shown, older humans demonstrate age-related change in the lateralisation of neural activity during the performance of cognitive and motor tasks (Calautti et al., 2001; Carp et al., 2011; Heuninckx et al., 2005; Heuninckx et al., 2008; Hutchinson, 2002; Inuggi et al., 2011), but

consideration has not been given as to whether the distribution or manifestation of neuroplasticity is also altered. The aim of this thesis, then, was to investigate how the manifestation of neuroplasticity is altered in neurologically healthy older adults. The studies of this thesis approached this question using non-invasive transcranial magnetic stimulation (TMS). TMS uses a magnetic pulse to carry a small current into the brain to stimulate cells in the cortex. It was used within this thesis to both probe and induce neuroplasticity in networks of the primary motor cortices (M1s) in young and older adults.

This thesis has important implications for understanding how the brain works, and for facilitating neuroplasticity in both healthy older adults and those suffering motor learning impairments as a result of brain injury. Impaired motor functioning is a common side effect of brain injury and leaves individuals unable to complete the necessary activities of daily living. Understanding how neuroplasticity manifests in the motor system and the factors that can enhance or hinder neuroplasticity can aid the development of better rehabilitation strategies tailored to the needs of older adults. Moreover, the findings of this thesis may also reveal the potential for using non-invasive brain stimulation to facilitate plasticity in healthy and clinical populations of older adults. This chapter will begin with a brief review of what is known about neuroplasticity at the cellular level from studies using animal models. I then go on to discuss the focus of the thesis, which is TMS and neuroplasticity in the human brain, with specific emphasis on how the manifestation of neuroplasticity might be altered in older adults.

1.2 Underlying mechanisms of neuroplasticity

Psychologist William James (1890 in Pascual-Leone et al., 2005) was the first to use the term *plasticity* in reference to the modifiability of human behaviour. It was later accepted that changes in behaviour were likely underpinned by anatomical modifications and use of the term was extended to describe the modifiability of neural substrates (Cajal, 1904 in Pascual-Leone et al., 2005). As it was originally believed that the human brain was unable to generate new neurons, early theories of learning proposed that a change in the communication between existing neurons must underlie changes in behaviour (Hebb 1949). In his now famous postulate, Hebb proposed that neurons firing persistently at the same time with converging inputs at the same location would adapt to facilitate communication between one another, thus enhancing their synaptic efficacy (Hebb, 1949). The possibility that neighbouring neurons firing at temporally asynchronous intervals would reduce their synaptic strength was also recognised (Hebb 1932 in Brown & Milner 2002). Evidence for this theory of long term changes in synaptic efficacy, otherwise known as *synaptic plasticity*, first emerged in the late 1970s from investigations into the neural underpinnings of memory formation

and consolidation. Bliss and Lomo (1973) found that applying short trains of repetitive high frequency stimulation (100 Hz) to excitatory cells in the hippocampus triggered a lasting increase in the population excitatory post synaptic potential (EPSP) of neighbouring cells. The artificially induced increase in synaptic efficacy was termed *long-term potentiation* (LTP; Bliss & Lomo, 1973). It was not until some years later that Dudek and Bear (1992) demonstrated the bidirectional nature of neuroplasticity, wherein prolonged (3-15 mins) low frequency (1-5 Hz) stimulation induced a lasting decrease in the EPSP. This was termed *long-term depression* (LTD; Dudek & Bear, 1992). It became evident that the temporal correlation of pre- and post-synaptic spike activity was also important for LTP and LTD induction (Linden, 1999; Sjostrom et al., 2001; Sjostrom & Nelson, 2002). If the post-synaptic cell spiked 5-50 ms after the pre-synaptic cell, synaptic strength was increased; that is, LTP was induced (Markram et al., 1997; Bi & Poo, 1998; Debanne et al., 1998; Magee & Johnston 1997; Zhang et al., 1998). On the other hand, if the post-synaptic cell spiked prior to the pre-synaptic cell, synaptic strength was decreased and LTD was induced (Bi & Poo, 1998; Sjostrom & Nelson, 2002; Dan & Poo, 2004; Bi & Rubin, 2005). This type of synaptic plasticity was termed spike timing dependent plasticity (Abbott & Nelson, 2000).

In the case of rate-dependent and spike-timing dependent changes in synaptic efficacy, the expression of LTP and LTD depends on the level of intracellular calcium (Malenka & Bear 2004; Bi & Poo 1998, Debanneet al. 1998, Feldman 2000, Magee & Johnston 1997, Markram et al. 1997, Sjostromet al. 2001, Zhang et al. 1998). Lower intracellular calcium results in LTD (Cummings et al., 1996) whereas higher intracellular calcium results in LTP (Malenka, 1991). During both LTP and LTD the presynaptic cell releases glutamate, which binds with two types of receptors embedded in the membrane of the postsynaptic cell: - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid **(**AMPA) and N-methyl-D-aspartate (NMDA) receptors. Binding of glutamate with the AMPA receptor causes an influx of positively charged sodium ions into the postsynaptic cell. Once the change in membrane potential becomes sufficiently positive LTP is initiated. During LTP the magnesium ions that block access to the cell via NMDA channels at rest are released. This causes an influx of positive calcium ions into the postsynaptic neuron (Mayer et al., 1984, Nowak et al., 1984). The influx of calcium triggers the activation of calcium-dependent protein kinases. Protein kinases act both pre- and post- synaptically to modify synaptic efficacy by influencing gene expression and protein synthesis within the cell (Nguyen & Kandel, 1996; Pontzer et al., 1990) or by increasing the conductance of glutamate receptors, causing an increase in postsynaptic depolarisation (Derkach et al., 1999). When the post-synaptic cell is only weakly depolarised by a pre-synaptic cell, however, LTD is initiated. During LTD the magnesium ion is only partially displaced, which results in less calcium entering the post-synaptic neuron. The lower level of

intracellular calcium activates protein phosphatases, which dephosphorylate target proteins causing removal of AMPA receptors from the synaptic region. This reduces the sensitivity of the postsynaptic cell to glutamate and thereby results in LTD (for review Citri & Malenka, 2008).

It is important to note that a number of factors have been shown to modulate activity dependent synaptic plasticity such as LTP and LTD in animal models. These include homeostatic processes, which continuously monitor and modulate neuroplasticity in order to maintain a balance between excitation and inhibition (see Turrigiano & Nelson, 2004 for review). Other factors include physical exercise, neuromodulators, genetic polymorphisms in the brain-derived neurotrophic factor (BDNF) gene, oestrogen, and age (Korte et al., 1995; Vaynman et al., 2004). While age was the predominant focus of this thesis, some of these other factors will be discussed in the following sections in relation to neuroplasticity in the human brain.

1.3 Methods of quantifying synaptic plasticity in neurologically healthy humans

The methods used to quantify neuroplasticity in humans are relatively indirect compared with those used in animal models. In humans, neuroplasticity is most frequently quantified non-invasively by structural and functional changes within a single brain region or in large-scale systems involving multiple regions distributed across the entirety of the brain. Much of what is known about neuroplasticity in humans has come from investigations in the sensorimotor cortices. The motor cortex is advantageous for investigating neuroplasticity due to its strict topography and the fact that changes in cortical excitability can be measured by recording changes in voluntary and evoked muscle responses. Each cortical column within M1 represents functionally related muscles, with adjacent limbs neighbouring one another (Asanuma & Pavlides, 1997; Penfield & Welch, 1951; Sanes & Donoghue, 2000; Kakei et al., 1999). This arrangement allows one to stimulate a particular representation within the motor cortex, which activates descending corticospinal pathways, and record the resulting activity in peripheral muscles. The motor cortex has been mapped such that the location of the cortical representations of different body parts is now well known (Penfield and Rasmussen, 1950). This map is referred to as the *motor homunculus* and is depicted in Figure 1-1 .

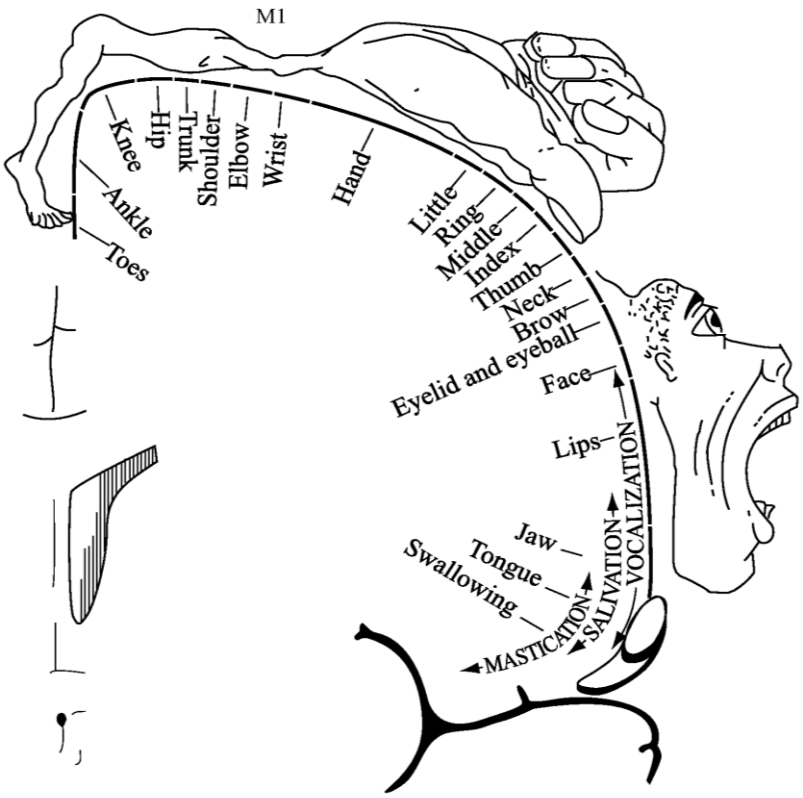

Figure 1-1 The motor homunculus. Representation of different body parts in the primary motor cortex (M1) based on direct cortical stimulation of awake humans during brain surgery.

The hand region is the most common target of neuroplasticity interventions due to its location, size and ease with which motor output can be quantified. Not only is the motor cortex a good model in which to study neuroplasticity, but understanding how neuroplasticity within the motor system is altered by advancing age is important for understanding motor function in health and disease. Hand function is critical for performing many activities of daily living and loss of function in the hand muscles may have significant implications for the well-being and independence of older adults. Loss of function in the hand muscles can arise due to peripheral or cortical damage, both of which older adults are at greater risk of experiencing. Neuroplasticity plays a key role in recovery of motor function following injury such as that induced by stroke (Rossini et al., 2003), but the majority of what is known about neuroplasticity in the motor system has been obtained through experiments involving young adult populations. Furthermore, studies that suggest the use of non-invasive brain stimulation techniques in rehabilitation to facilitate motor function also rely predominantly on young adult populations (Hendy & Kidgell, 2014; Hendy et al., 2015; Kidgell et al., 2013), with limited focus on older adults (Goodwill et al., 2015). In order to improve rehabilitative outcomes

for older adults with deficits in motor control, with specific focus on the hand region, it is important to understand how neuroplasticity in the motor cortices is affected by advancing age.

Before detailing the types of interventions used in humans it is important to understand how neuroplasticity is quantified in the human brain. The following section introduces some of the ways in which neuroplasticity in the motor cortex can be quantified in humans. Some of the methods used in humans are more direct than others, and the advantages and disadvantages of each technique will be discussed. A common strategy to combat the disadvantages of different techniques is to use a combination of methods, as employed in Chapter 2 and across the experiments of this thesis.

1.3.1 Behavioural performance

Learning can be quantified by changes in behavioural performance (Pereira et al., 2011; Andree & Maitra, 2002). A participant can demonstrate learning by showing a change in performance in line with a trained behaviour. For example, a participant might perform poorly on a novel juggling task before training, but becomes faster and more accurate after training due to motoric learning processes and a whole range of decisional processes related to the task. The problem with probing neuroplasticity with behaviour, however, is that it is difficult to identify the mechanism underpinning the change. Behavioural outcomes are not a direct reflection of neural processes occurring within the brain: contributions downstream of the brain, in the spinal cord and musculature, can also influence behavioural output. For example, while decisional processes may occur during training that might improve performance to some degree, performance improvement may be capped by limitations in the periphery, especially in older adults who demonstrate declines in peripheral musculature downstream of the cortex (Deschenes, 2011). In addition, behaviour is also influenced by motivation and effort, and/or characteristics of the task itself. If a participant is not motivated to learn the strategy required to perform the juggling task, they are unlikely to show large performance improvements. Therefore, although a change in behaviour may not be observed, it cannot be concluded that neuroplasticity did not occur or could occur with greater motivation or effort. It is also difficult to investigate the online effects (during training) of various factors that might influence neuroplasticity when probing it with behaviour. For example, neuroplasticity effects may be influenced by attention (Kamke et al., 2012; 2014). Investigating this possibility is problematic as it is impossible to disentangle attention from additional cognitive processes or remove attention from the task completely. To combat some of these limitations it is common to implement behavioural measures alongside techniques that can better measure training-induced neuroplasticity, such as neuroimaging or brain stimulation.

1.3.2 Neuroimaging- Magnetic Resonance Imaging (MRI) and Positron Emission Topography (PET)

Imaging methods such as MRI and PET are two of the most common methods used to measure changes in large-scale neural networks in humans. Structural MRI techniques such as voxel based morphometry (VBM) and diffusion tensor imaging (DTI) can be used to quantify changes in grey and white matter, respectively (Ashburner & Friston, 2000; Basser et al., 1994). MRI and PET can also be used to quantify changes in the functional activity of neural circuits by measuring the metabolic demands of neuronal populations (Buxton et al., 1998; Gevorski & Plotkin, 2006). These neuroimaging methods have revealed various plastic changes in the human brain. For example, enlarged grey matter volume after training on a juggling task (Draganski et al., 2004), increased white matter integrity after training on a visuomotor task (Scholz et al., 2009), enlarged neural representations of trained fingers following braille training (Rockstroch et al., 1998), and enlarged neural representation of a trained sequence after training on a sequential finger tapping task (Karni et al., 1995). Although these changes presumably reflect task-related change in brain structure and function, typically participants must perform a task in order to measure neural activity. One limitation with the approach then is that differences in motivation or strategy, which do not necessarily reflect neuroplasticity, may result in different patterns of activity. Some studies do quantify changes in functional activity and structure at rest, without participants having to perform a task. One limitation with this procedure, however, is that the degree to which those cells have become more excitable/closer to firing after a neuroplasticity intervention cannot be examined.

1.3.3 Electroencephalography (EEG) and Magnetoencephalography (MEG)

EEG (Luck, 2005) is a method of recording fluctuations in electrical potentials caused by ionic current flows through the neurons of the brain. The EEG signal reflects the summation of the synchronous activity of thousands or millions of neurons of similar spatial orientation. An advantage of EEG is its high temporal resolution, which enables one to measure changes in the latency and/or frequency of task-related increases and decreases in electrical activity after a neuroplasticity intervention (Chen et al., 2000; Shahin et al., 2008; Cooper et al., 2008). Due to its high temporal resolution EEG is more comparable to the recording methods used to quantify synaptic plasticity in animal models, in which changes in electrical potentials are recorded with implanted electrodes (Bliss & Lomo, 1973). However, the cortical sensorimotor regions are far more complex with many more different cell types than the hippocampus in animal models, where synaptic plasticity is typically quantified. Also, as the EEG signal reflects the activity of a complex network of a large number of neurons the signal-to-noise ratio is low. Therefore a disadvantage of

EEG is that small and focal changes in synaptic plasticity might not make a difference to overall cortical excitability and therefore may go undetected. Furthermore, unlike the specificity of implanted electrodes, the spatial resolution of EEG is low. Therefore, an additional disadvantage of EEG is that locating the source of the signal change is difficult. In addition, although it is possible to measure functional connectivity with EEG at rest, in order to measure changes in the active networks targeted by the neuroplasticity protocol, participants are usually required to perform a behavioural task.

MEG is similar to EEG but measures the magnetic, as opposed to the electrical, fields generated by neuronal activity in the brain (Cohen, 1968). Like EEG, MEG has higher temporal resolution compared with MRI, in the realm of milliseconds as opposed to seconds. An additional advantage of MEG compared with EEG is that the signal is far less affected by brain tissue, which results in higher spatial resolution and therefore better source localisation. Several studies demonstrate the value of MEG as a tool for investigating neuroplasticity non-invasively in the human brain by measuring changes in cortical excitability (Mary et al., 2015; Mogilner et al., 1993; Liu et al., 2004). However, as with MRI and PET, in order to measure neuroplasticity in the network targeted by the intervention participants are required to perform a task during EEG and MEG, introducing problems related to individual differences in motivation and strategy.

1.3.4 Transcranial Magnetic Stimulation (TMS)

A technique that can be used to quantify the magnitude of neuroplasticity non-invasively without requiring participants to perform a task is TMS. TMS is a method of stimulating neuronal cells via the principles of electromagnetic induction (Faraday, 1839). A coil of conductive wire is placed over the intact scalp directly above the cortex. A high voltage current is discharged through the coil, which yields a rapidly changing magnetic field perpendicular to the plane of the coil (Barker et al., 1985). This is depicted in Figure 1-2. Although stimulating at higher intensities can result in the direct stimulation of motor output cells, TMS most frequently activates motor output cells indirectly via intracortical interneurons (Hallet, 2000; Kobayashi & Pascual-Leone, 2003; Deng et al., 2012; Wagner et al., 2004; Roth et al., 2007). The rapidly changing magnetic field penetrates the surface of the skull and induces a flow of electrical current in the underlying tissue, which, in the case of the motor cortex, activates motor output cells trans-synaptically by stimulating the axons of interneurons in the superficial layers of the cortex (1.5-2 cm deep; Hallet, 2000; Kobayashi & Pascual-Leone, 2003; Deng et al., 2012; Wagner et al., 2004; Roth et al., 2007). This activation of the cortical interneurons triggers a descending propagation of action potentials along connecting

corticospinal neurons, motorneurons and the corresponding muscle fibres (Rothwell, 1991, Burke et al., 1993), resulting in muscle activity.

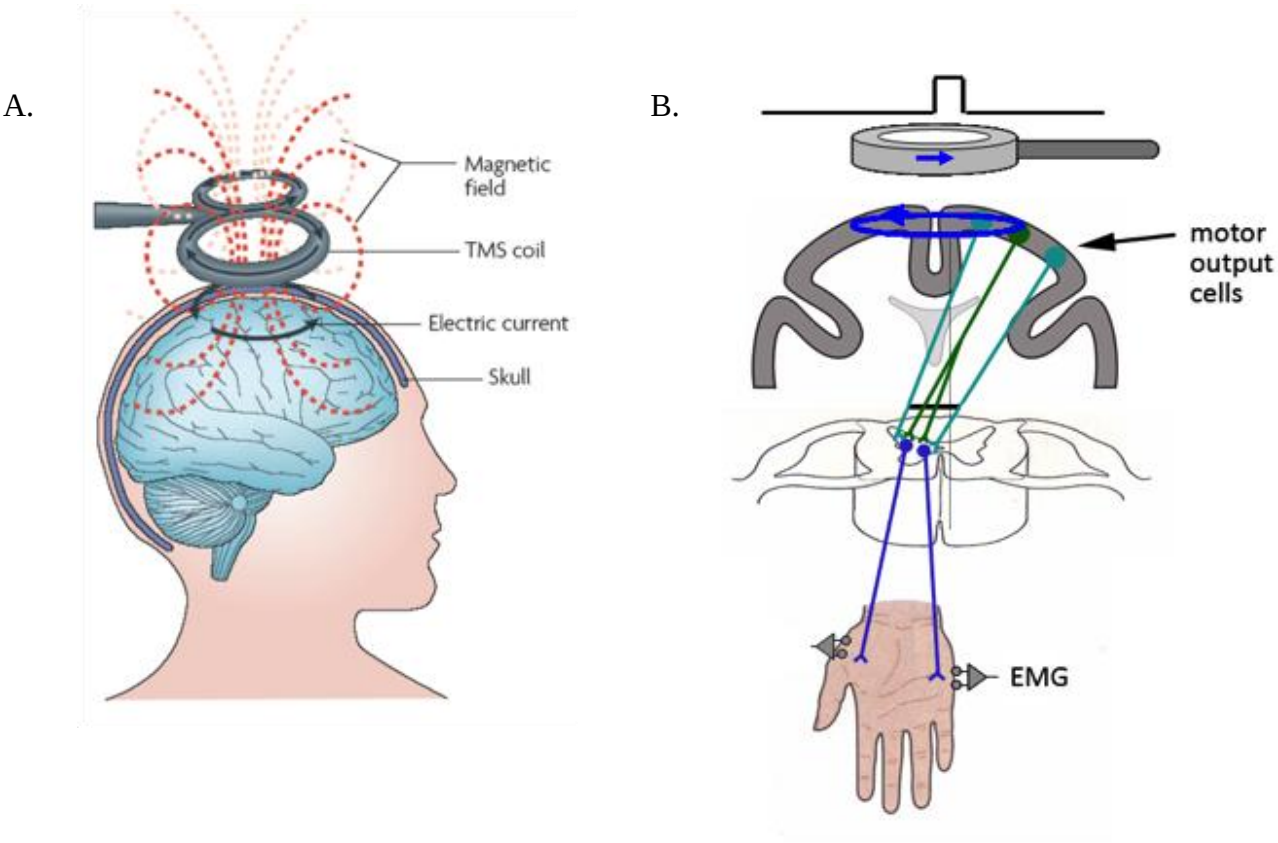

Figure 1-2 Illustration of TMS induced currents in the brain and the corticospinal pathway. A) When an electric current is passed through a figure-of-eight coil placed on the surface of the scalp, a magnetic field is generated that induces an electrical current in the underlying surface of the brain. B) The TMS pulse triggers muscle activity trans-synaptically. TMS excites cortical interneurons that synapse onto motor output cells. A cascade of neural activity is triggered by the TMS pulse, which travels from the cortex to the muscles corresponding to the representation targeted by the stimulation. TMS-evoked muscle activity can be quantified using EMG. Figure A was taken and adapted from Ridding and Rothwell (2007) with permission from Nature Publishing Group (license #3644550773144).

1.3.4.1 Motor-evoked potentials (MEPs)

Muscle activity triggered by a single TMS pulse can be quantified using electromyography (EMG). EMG is a technique that uses electrodes, typically placed on the surface of the skin to record changes in the electrical potential of a muscle. Immediately after the discharge of a TMS pulse, the electrical potential of the target muscle spikes; this is termed a *motor-evoked potential* (MEP) and is depicted in Figure 1-3. The amplitude of the TMS-induced MEP from the highest peak to the lowest

trough (peak-to-peak amplitude) is taken as an indirect measure of cortical excitability (Barker et al., 1985; Rothwell et al., 1987; Mills et al., 1987; Day et al., 1987). Studies using implanted electrodes in the epidural space of the spinal cord have revealed that MEPs are the product of a series of descending volleys, triggered by the direct and indirect stimulation of corticospinal output cells. The first volley is known as the direct wave (D-wave; Patton & Amassian 1954), and is triggered by direct axonal stimulation of pyramidal tract neurons. Subsequent volleys are termed indirect (I-) waves (Day et al., 1987; Patton & Amassian, 1954; Kaneko et al., 1996; Nakamura et al., 1997) and reflect trans-synaptic activation of pyramidal cells. Earlier I-waves are believed to reflect intra-cortical afferents onto pyramidal cells, while later I-waves are believed to reflect cortico-cortical afferents (Di Lazzaro et al., 2011).

We can infer the extent of neuroplasticity induced by a particular intervention in healthy humans from changes in MEP amplitude. If the same intensity stimulus is applied to the same location on the scalp before and after an intervention, a change in MEP amplitude suggests a change in cortical excitability. More specifically, because MEP amplitude depends on trans-synaptic activity, changes in amplitude are believed to reflect changes in synaptic efficacy. It must be acknowledged, however, that the MEP is a compound signal that consists of both cortical and spinal contributions (Groppa et al., 2012). Much of the evidence suggests that plasticity is underpinned by changes in the strength of synaptic connections of cortical interneurons (Stefan et al., 2002; Wolters et al., 2003; Muellbacher at el., 2001; Di Lazzaro et al., 2005), but spinal mechanisms may also play a role under some conditions (Meunier et al., 2007). As a consequence, neuroplasticity in M1 quantified by TMS (changes in MEP amplitude) is referred to as corticospinal plasticity to reflect the possible contribution of cortical and spinal adaptations to changes in MEP amplitude.

Probing neuroplasticity with TMS and MEPs provides several advantages over MRI, PET, MEG, and EEG. First, its high temporal and spatial resolution relative to MRI and EEG, respectively, enables one to probe neuroplasticity within specific pathways, such as that of the fingers after training on a finger-tapping sequence learning task. Second, with TMS, corticospinal plasticity can be quantified without participants having to perform a task. More recently TMS has been combined with EEG, to measure changes in excitability outside of the motor system (Kičić et al., 2008). Single pulse TMS is a well-established method of probing neuroplasticity in humans and is used to quantify the effects of a variety of neuroplasticity-inducing interventions. These are discussed in the following section.

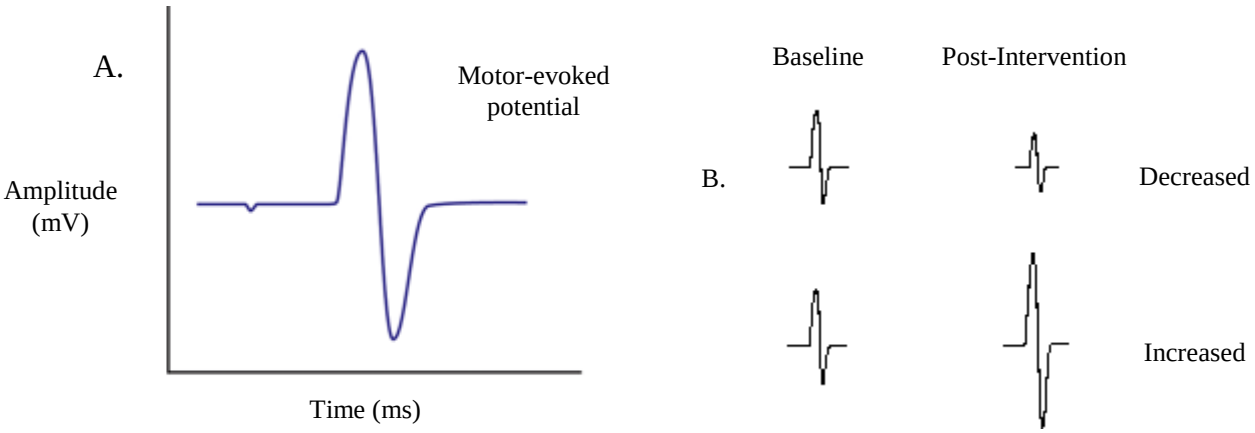

Figure 1-3 Motor-Evoked Potentials (MEPs). Surface EMG can be used to record TMS-evoked muscle activity. Changes in the peak-to-peak amplitude of the MEP reflect changes in cortical excitability. Permission not required.

1.4 Neuroplasticity-inducing interventions

1.4.1 Motor training

Training is the most obvious method of inducing behavioural change. Improvements in behavioural performance are used to quantify learning, while neuroimaging or brain stimulation is used to quantify neuroplasticity. Many different training tasks have been used to induce neuroplasticity. These vary from simple ballistic movements with single digits (Hlustik et al., 2004; Carroll et al., 2008; Koeneke et al., 2006; Classen et al., 1998; Sawaki et al., 2003; Hinder et al., 2011, 2013; Rogasch et al., 2009; Cirillo et al., 2010), to complex multi-digit sequential movements (Pascual-Leone et al., 1995; Karni et al., 1995; Nyberg et al., 2006; Ungerleider et al., 2002; Karni et al., 1998; Hlustik et al., 2004). Neuroplasticity has been demonstrated after training tasks involving juggling (Boyke et al., 2008; Draganski et al., 2004; Driemeyer et al., 2008), golf (Bezzola et al., 2011), whole body balancing (Taubert et al., 2010), and bimanual coordination (Heitger et al., 2012). Studies probing training-induced corticospinal plasticity using MEP amplitude often target a specific digit/group of muscles represented in a readily accessible region of M1, which can be targeted pre- and post-training with TMS. Some of the training tasks used in these studies include sensorimotor Purdue pegboard training (Garry et al., 2004; McDonnell & Ridding, 2006), goal-driven ankle flexion (Perez et al., 2004), ballistic pinch movements with the thumb and index finger (Muellbacher et al., 2001), and ballistic thumb movements (Carroll et al., 2008; Koeneke et al., 2006; Classen et al., 1998; Sawaki et al., 2003; Hinder et al., 2011, 2013; Rogasch et al., 2009; Cirillo et al., 2010). Evidence demonstrates that even short bursts of training within a single session

can induce behavioural performance improvements and changes in MEP amplitude (Carroll et al., 2008; Garry et al., 2004; McDonnell & Ridding, 2006; Muellbacher et al., 2001; Perez et al., 2004).

The studies mentioned above have been shown to induce changes in brain structure and/or function. Structural neuroimaging, and mapping of cortical representations with electrical stimulation or TMS, demonstrate changes in grey matter volume and in the size of cortical representations after motor training in humans (Boyke et al., 2008; Draganski et al., 2004; Driemeyer et al., 2008; Bezzola et al., 2011; Taubert et al., 2010) as well as in animal models (Black et al., 1990; Kleim et al., 2002; Nudo et al., 1996; Plautz et al., 2000). Functional neuroimaging has demonstrated changes in neural activation patterns and connectivity (Karni et al., 1995; Nyberg et al., 2006; Ungerleider et al., 2002; Karni et al., 1998; Hlustik et al., 2004; Heitger et al., 2012). Furthermore, single-pulse TMS has revealed functional changes in corticospinal excitability following motor training (Garry et al., 2004; McDonnell & Ridding, 2006. Carroll et al., 2008; Koeneke et al., 2006; Hinder et al., 2011, 2013; Rogasch et al., 2009; Cirillo et al., 2010; Perez et al., 2004). Interestingly, while this evidence demonstrates a link between motor-skill training and neuroplasticity, few studies have investigated this association at the level of an individual. The first experiment of this thesis, detailed in Chapter 2, combines single-pulse TMS and behavioural measures to quantify behavioural learning and corticospinal plasticity induced by motor training and attempts to identify the strength of their association at the level of the individual.

1.4.2 Repetitive TMS

Neuroplasticity can also be induced with non-invasive brain stimulation in the absence of a behavioural learning task (see Hoogendam et al., 2010 for review). Recall that in animal models LTP and LTD can be induced by repetitive electrical stimulation of neurons. High frequency stimulation results in LTP, whereas low frequency stimulation results in LTD. In humans, TMS is used to stimulate neural populations non-invasively. When administered repeatedly for an extended period it is known as repetitive TMS (rTMS), and can be used to mimic the synaptic plasticity-inducing protocols used in animal models. Similar to the bidirectional rate-dependent effects of electrical stimulation in animal models, in humans rTMS applied at higher frequencies (> 5 Hz) increases MEP amplitude (Pascual-Leone et al., 1994; Berardelli et al., 1998; Maeda et al., 2000; Peinemann et al., 2000; Jahanshahi et al., 1997), whereas lower frequencies (< 1Hz) decrease MEP amplitude (Chen et al., 1997; Maeda et al., 2000; Gerschlager et al., 2001; Fitzgerald et al., 2006; Siebner et al., 1999; Muellbacher, et al., 2001; Fitzgerald et al., 2002). These protocols are depicted in Figure 1-.

Evidence suggests the changes in MEP amplitude induced by rTMS are underpinned by LTP- and LTD-like mechanisms (see Hoogendam et al., 2010 for review). This evidence will be discussed below for each of the rTMS protocols used in this thesis. In addition to motor training, this thesis implements two of the best characterised forms of rTMS – theta burst stimulation (TBS) and paired associative stimulation (PAS) – to induce neuroplasticity in motor regions of the brain. As will be discussed, these protocols are believed to induce changes in MEP amplitude arising from similar, but slightly different mechanisms.

1.4.2.1 Theta Burst Stimulation

Theta burst stimulation (TBS) was developed some 30 years ago and was used exclusively in animal models to artificially induce the rhythmic oscillatory activity that had been associated with behavioural learning (Larson et al., 1986; see O'Keefe & Nadel, 1978 for review). Researchers demonstrated that learning to navigate a new environment was associated with increased hippocampal activity in the theta frequency band (4-7 Hz; see O'Keefe & Nadel, 1978 for review). Researchers attempted to induce this theta rhythm artificially in the hippocampus of animal models by applying repetitive short bursts of high frequency stimulation (50-200 Hz) at 200 ms intervals (5 Hz; the theta frequency). This stimulation resulted in the manifestation of LTP at synapses within hippocampal slices (Larson et al., 1986). TBS has since been used on many occasions to induce synaptic plasticity in animal models (e.g., Hess et al., 1996; Otani et al., 1998; Urban et al., 2002).

It was not until recently that an adapted form of TBS was developed that could be administered in humans (Huang & Rothwell, 2004), in accordance with safety guidelines (Wassermann, 1998). This protocol involved the presentation of short bursts (3-5 pulses) of low intensity (subthreshold) high frequency (50 Hz) stimulation, repeated five times a second (i.e., at the theta frequency; Huang et al., 2005). Two main types of TBS have been developed: continuous TBS (cTBS) and intermittent TBS (iTBS). During cTBS a short burst of 3 pulses (50 Hz) is applied every 200 ms for 40 seconds (Huang et al., 2005). iTBS involves a short bust of 3 pulses (50 Hz), which is applied every 200 ms for a total of 2 seconds. This occurs intermittently every 8 seconds, for a total of 600 pulses (Huang et al., 2005). These protocols are depicted in Figure 1-. Evidence demonstrates that cTBS decreases MEP amplitude, while iTBS increases MEP amplitude (Huang et al., 2005; Di Lazzaro et al., 2011; Kamke et al., 2012; Moliadze et al., 2014; Hinder et al., 2014; Nettekoven et al., 2014; Suppa et al., 2008).

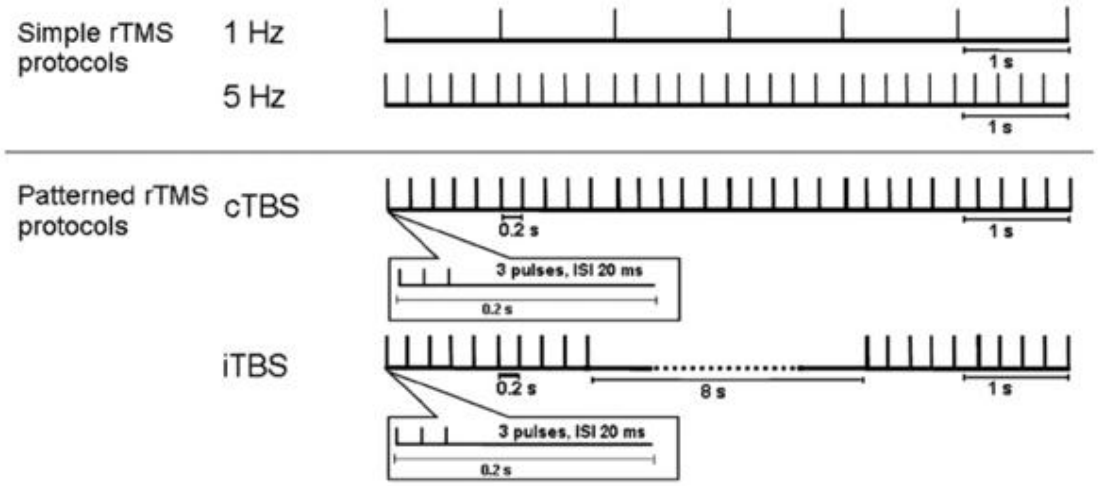

Figure 1-4 Schematic representation of rTMS protocols. During simple rTMS protocols stimuli are presented at the same interstimulus interval (ISI) for a prolonged period. Two examples are shown, a low frequency (1 Hz) and a high frequency (5 Hz) stimulation protocol. During patterned protocols stimuli are presented at different ISIs. During TBS 3 pulses are administered at 50 Hz, and repeated every 200 ms. Continuous theta burst stimulation (cTBS) involves a 40 second train of uninterrupted TBS given for a total of 600 pulses. Intermittent theta burst stimulation (iTBS) involves a 2 second train of TBS repeated every 10 seconds for a total of 190 seconds (600 pulses). This figure was reproduced and adapted from Hoogendam et al., (2010) with permission from Elsevier (license # 3644551372544).

In humans TBS is necessarily administered to a network of neurons encompassing many different types of cells, including inhibitory and excitatory interneurons. Evidence suggests that the high frequency component of TBS facilitates the activation of excitatory interneurons, while the theta component reduces the activity of GABA mediated inhibitory interneurons (Huang and Rothwell, 2004). Each subthreshold pulse of the high frequency burst triggers a gradual increase in excitation that leads to intracortical facilitation, which better entrains the theta oscillations associated with LTP induction (Huang & Rothwell, 2004). The theta component facilitates GABA mediated depression of inhibitory interneurons, which may further enhance intracortical facilitation (Mott & Lewis, 1991; Huang & Rothwell, 2004). Why iTBS and cTBS result in different effects remains largely unknown. Huang and colleagues (2005) suggest the difference reflects the activation of homeostatic processes, such that the prolonged excitation triggered by cTBS triggers subsequent inhibition (Huang et al., 2005). This idea is supported by the results of Gentner and colleagues (2008) who demonstrated facilitation after 300 pulses of cTBS but inhibition after 600 pulses. However, there is evidence to suggest that the two protocols induce different effects by modulating

different circuits (Di Lazzaro et al., 2008). Further research is needed to determine which of these possibilities is more likely.

At the synaptic level, several observations suggest the mechanisms underpinning TBS are similar to that of LTP and LTD in animal models. First, TBS induces changes in MEP amplitude (Huang & Rothwell, 2004), behavioural performance (Nyffeler et al., 2006) and brain activity (Hubl et al., 2008; Schindler et al., 2008) that outlast the stimulation period. This is a critical characteristic of synaptic plasticity (Abraham, 2003; Bliss and Lomo, 1973). Moreover, TBS effects also depend on the temporal pattern of the stimulation (Huang & Rothwell, 2004), which is consistent with the dependence of synaptic plasticity on the temporal order of activity that was discussed earlier in this chapter (Hernandez et al., 2005). Moreover, priming TBS with other forms of stimulation or physiologic activity alters subsequent effects (Gentner et al., 2008; Iezzi et al., 2008; Huang et al., 2008). This is consistent with the priming effects seen with synaptic plasticity in animal models (Abraham & Bear, 1996; Abraham, 2008). In addition, TBS effects appear to involve an NMDA-dependent mechanism, as the administration of an NMDA receptor antagonist abolishes TBS effects (Huang et al., 2007; Stagg et al., 2009; Tokay et al., 2009). Lastly, TBS effects, like synaptic plasticity, are influenced by genetic variations, for example the BDNF polymorphism (Cheeran et al., 2008). Although indirect, these observations together present a strong case for the involvement of LTP and LTD mechanisms in TBS-induced neuroplasticity. The second experiment of this thesis, detailed in Chapter 3, used iTBS to induce corticospinal plasticity in younger and older adults. Only one previous study has used iTBS to investigate how the manifestation of corticospinal plasticity might be altered in older adults (Di Lazzaro et al., 2008). This study is discussed in more detail later in this chapter.

1.4.2.2 Paired Associative Stimulation

As discussed previously synaptic plasticity can also be induced in animal models by spike-time dependent mechanisms (Bi & Poo, 1998) with paired-pulse protocols (Baranyi & Feher, 1981; Baranyi & Szente, 1987; Iriki et al., 1989; Baranyi et al., 1991; Iriki et al., 1991; Hess & Donoghue, 1994; Hess et al., 1996). In spike timing dependent synaptic plasticity the temporal order of the pre- and post-synaptic activity determines the direction of synaptic plasticity induced. When the presynaptic neuron fires just prior to a postsynaptic neuron LTP is induced (Bi & Poo, 1998). On the other hand, when the postsynaptic neuron fires before a presynaptic neuron, LTD is favoured (Bi & Poo, 1998). Stefan and colleagues (2000) developed a protocol, based on the paired stimulation protocols used in animal models, which could be used to induce changes in the excitability of the motor cortex non-invasively in humans. Low frequency TMS over primary motor

cortex was synchronously paired with peripheral stimulation of somatosensory afferents at the median nerve (Stefan et al., 2000). Figure 1-5 A depicts the typical PAS protocol. Somatosensory afferent signals require around 23 ms to travel from the median nerve to M1. Presentation of the TMS pulse around the time of the arrival of the somatosensory afferents in M1 results in coincidental inputs onto the postsynaptic pyramidal output cells (Rothwell, 1997; Porter, 1996; Kaneko et al., 1994). Figure 1-5 B depicts the change in MEP amplitude for different temporal pairings of the stimuli. The arrival of the afferent somatosensory input in M1 is indicated by the dotted line in Figure 1-5 B. When the TMS pulse is delivered after the arrival of the sensory signal in M1 (to the right of the dotted line), such that pre-synaptic activity occurs *before* post-synaptic activity, post-PAS MEP amplitude increases (Stefan et al., 2000). On the other hand, discharge of the TMS pulse prior to the arrival of the somatosensory afferents (left of the dotted line), triggers post-synaptic activity before pre-synaptic activity, which decreases post-PAS MEP amplitude (Wolters et al., 2003).

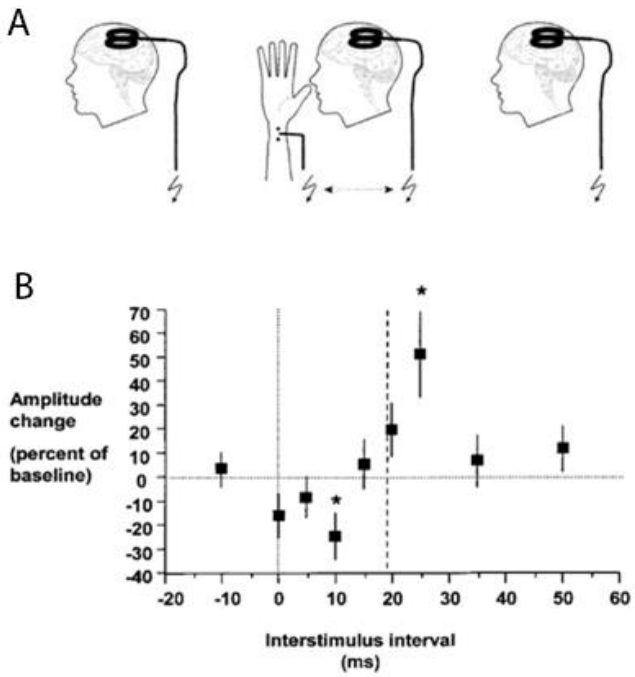

Figure 1-5 PAS protocol and effects on MEP amplitude. A) PAS protocol. Test MEP amplitudes were recorded at baseline and following the PAS protocol. PAS consisted of 90 pairs of stimuli, with TMS presented to left M1 and peripheral stimulation to the right median nerve. The interval at which these stimuli were presented varied from -10 to 50 ms across different sessions. B) The effect of PAS with ISIs from -10 to 50 on the amplitude of MEP in the right *abductor pollicis brevis* (APB) muscle. Asterisks indicate significant change in MEP amplitude ($p < .05$). Vertical broken line indicates approximate arrival of the afferent input from the nerve stimulation. This figure was reproduced and adapted from Wolters et al., (2003). Permission not required.

A number of observations suggest that the mechanisms underpinning the bidirectional change in MEP amplitude induced by PAS are similar to that of LTP/LTD synaptic plasticity in animal models. First, the increase in MEP amplitude is dependent on the inter-stimulus interval as well as the order of the stimulation (Wolters et al., 2003). Intervals where the TMS pulse is delivered at approximately the same time as the arrival of the afferent signals in motor cortex results in greater MEP amplitude facilitation (Stefan et al., 2000; Wolters et al., 2003). Conversely, intervals where the two signals are predicted to be asynchronous results in attenuated MEP amplitude (Wolters et al., 2003). Second, similar to spike-time dependent plasticity, PAS effects have been shown to arise rapidly and remain for prolonged durations after the stimulation period (~30 minutes; Stefan et al., 2000), an effect that is also evident with LTP and LTD in animal models (Bi & Poo, 1998). Moreover, the NMDA receptor antagonist dextromethorphan can attenuate PAS-induced corticospinal plasticity (Stefan et al., 2002; Wolters et al., 2003), which has also been shown with synaptic plasticity in animal models (Bi & Poo, 1998). Finally, evidence from animal models also indicates that LTP is limited to the afferent inputs that have been stimulated in the induction protocol (Bi & Poo, 1998). This topographic specificity is evident in humans also (Stefan et al., 2000; Quartarone et al., 2003; Weise et al., 2006), with PAS-induced corticospinal plasticity being most pronounced in the pathway targeted by the peripheral electrical stimulation (Stefan et al., 2000; Quartarone et al., 2003; Weise et al., 2006). Therefore, PAS-induced corticospinal plasticity is considered LTP/LTD-like in nature (Stefan et al., 2002; Ridding & Rothwell, 2007; Bi & Poo, 2001; Dan & Poo, 2004). Experiment 3 of this thesis implemented a PAS protocol.

1.5 Synaptic plasticity in the aged brain

Much of the work investigating neuroplasticity in the adult brain has been conducted in young animals. Consequently, relatively little is known about neuroplasticity in the aged brain. Advancing age is associated with significant structural and functional neurobiological change (see Greenwood, 2007 for review) and as yet, how this influences the capacity of the aged brain to undergo plastic change is not well understood.

1.5.1 Synaptic plasticity in aged animal models

Studies investigating synaptic plasticity in aged animals have demonstrated that LTP can be induced to levels comparable with those seen in young animals (Diana et al., 1994; Moore et al., 1993). However, the development of LTP is much slower, often requires stronger stimulation intensities, and decays much faster in older, relative to younger animals (Barnes, 1979; Barnes & McNaughton, 1985). Many factors have been shown to play a role in the alteration of LTP in the

aged brain of animal models. These include: fewer NMDA receptors (Kito et al., 1990; Liang & Lu, 1992; Magnusson, 1998; Magnusson & Cotman, 1993; Tamaru et al., 1991; Wenk et al., 1991), alterations in the activity-dependent redistribution of NMDA receptors (Clayton & Browning, 2001), greater involvement of non-NMDA dependent calcium channels (Shankar et al., 1998), impaired protein regulation (Davis et al., 2000;), impaired gene expression (Wagner et al., 2000; Ronn et al., 2000), reduced synaptic density (Markus & Petit, 1987), and reduced dendritic length (Vaughan, 1977; Wong et al., 2000; Markham & Juraska, 2002; Wong et al., 2006; Wang et al., 2009). Age-related changes in noradrenergic and cholinergic systems resulting in reduced levels of acetylcholine, serotonin, norepinephrine, and dopamine, due to reductions in neurotransmitter synthesis, release, and receptor availability also play a significant role in age-related alterations in LTP (Baxter et al., 1999: Chouinard et al., 1995; Friedman & Duckles, 1994; Isacson et al., 2002; Luine et al., 1990; Powers et al., 1988; Sherman & Friedman, 1990; Stemmelin et al., 2000).

In animal models, it is believed that the above mentioned age-related neurobiological and chemical changes not only reduce the ability of cells to sustain depolarisation for long enough to trigger LTP (Rosenzweig et al., 1997), but also prevent intracellular calcium levels from reaching the level required to activate calcium dependent kinases, critical for LTP induction (Foster, 1999; Foster & Norris, 1997). Instead, phosphatases are triggered which lead to an increased likelihood of LTD (Norris et al., 1998). Consistent with these effects, older animals require more time to acquire a motor skill and once acquired, these skills diminish faster in older relative to younger animals (Bickford, 1993; Churchill et al., 2003). Based on the evidence of age-related changes in synaptic plasticity in animal models, we might expect neuroplasticity and behavioural performance to be altered in older relative to younger adult humans.

1.5.2 Neuroplasticity in older human adults

In humans, behavioural learning is often used to infer neuroplasticity. Although some studies demonstrate that behavioural learning is maintained in old age (Cirillo et al., 2011; Parikh & Cole, 2013), most reveal impairments in the rate and magnitude of behavioural learning in older adults, comparable to those seen in the animal models (Parikh & Cole, 2013; Sawaki et al., 2003; Curran, 1997; Howard & Howard, 2001; Feeney et al., 2002; Howard et al., 2004, 2008; Bennett et al., 2007; McNay & Willingham, 1998; Fernandez-Ruiz et al., 2000; Buch et al., 2003; Bock, 2005; Bock & Girgenrath, 2006; Heuer & Hegele, 2008; Hegele & Heuer, 2010; Anguera et al., 2011). This evidence of impaired behavioural learning suggests that neuroplasticity might be altered in the aged brain.

Studies using neuroimaging have demonstrated experience-dependent changes in the structure of multiple regions of the older human brain. For example, in older adults aged between 50 - 67 years, grey matter volume increases in the visual cortex and hippocampus after training on a juggling task (Boyke et al., 2008) and white matter integrity increases in anterior regions of the corpus callosum after cognitive training (Lövdén et al. 2010). In older adults aged 60-75 years, studies using functional neuroimaging have also demonstrated training-induced changes in the activity of neural populations after working memory training (Brehmer et al., 2011; Heinzel et al., 2014), learning a motor sequence (Mary et al., 2015) and undergoing a combined cognitive, psychological and physical intervention (Zheng et al. 2015). These changes in brain function are associated with improvements in task performance (Brehmer et al., 2011; Heinzel et al., 2014; Mary et al., 2015; Zheng et al., 2015). These findings demonstrate that neuroplasticity does occur at the level of large-scale networks in the aged brain.

In comparison to young adults, however, structural and functional neuroplasticity is altered in the aged brain (Heinzel et al., 2014; Mary et al., 2015; Geerligs et al., 2014). For example, structural changes in cortical thickness are smaller in older than in younger adults after spatial navigation training (Wenger et al., 2012). Moreover, functional neuroplasticity, as measured by MEG and MRI, is reduced in older, relative to young adults after training on a motor sequence (Mary et al., 2015) and a working memory task (Heinzel et al., 2014). More specifically, Mary and colleagues (2015) demonstrated that beta desynchronisation in the left somatosensory cortex was attenuated after learning a sequential motor task. Beta desynchronisation is suggested to reflect inhibition within the somatosensory cortex (Klimesch et al., 2007; Salmelin et al., 1995). Thus, these authors suggest the change in functional activation in older, relative to younger, adults may reflect reduced inhibition and may account for reductions in motor learning in older adults (Mary et al., 2015). Together, this evidence demonstrates large-scale alterations in structural and functional neuroplasticity in older human adults. As mentioned previously, however, it is difficult to ascertain the degree to which changes in synaptic plasticity contribute to these large-scale changes due to the influence of motivational and task-related factors.

To overcome limitations associated with the task-dependency of neuroimaging, many studies have implemented TMS alongside neuroplasticity interventions such as motor training and non-invasive brain stimulation methods to probe neuroplasticity more directly. After motor training, corticospinal plasticity quantified by single-pulse TMS is shown to be reduced in older relative to younger adults, and such reductions are associated with reduced performance gains (Rogasch et al., 2009; Tecchio et al., 2008). For example, Rogasch and colleagues (2009) trained young and older adults on a

ballistic thumb abduction task. Although corticospinal plasticity, as measured by an increase in MEP amplitude, was evident in the pathway innervating the trained limb in young adults, it was not evident in older adults. Moreover, performance gains in peak acceleration in the trained limb following training were significantly smaller in older adults, suggesting a relationship between reduced corticospinal plasticity and performance. Corticospinal plasticity is not always reduced in older adults, however. For example, Cirillo and colleagues (2010) demonstrated that after a similar ballistic finger abduction task, changes in MEP amplitudes recorded from the trained limb were comparable between young and older adults, even though performance improvements in the trained hand were smaller in older adults. These results demonstrate the variability in observations pertaining to the relationship between corticospinal plasticity and behaviour in young and older adults, even when considering similar tasks employed within the same laboratory. Consequently, it can be concluded that the evidence pertaining to whether corticospinal plasticity is reduced in the aged brain is inconclusive.

Because the performance of a motor task is heavily dependent on factors related to motivation and the ability of participants to perform the task, studies using TMS to induce neuroplasticity may provide a more objective means by which to assess changes in neuroplasticity with advancing age and the causal effects of TMS-induced neuroplasticity on behaviour. Few studies have investigated neuroplasticity in the aged brain using such TMS-based protocols, which include rTMS, PAS and TBS. Inhibitory rTMS was found to not suppress MEP amplitude in the target muscle of adults over 60 years of age (Todd et al, 2010; Bashir et al., 2014). Moreover, excitatory PAS did not increase MEP amplitude in the target muscle of older adults (50-79 years old), despite significantly enhancing MEPs in the young (Fathi et al, 2010; Müller-Dahlhaus et al., 2008; Tecchio et al., 2008). In addition, the magnitude and duration of MEP change induced by cTBS was reduced in older relative to younger adults (Freitas et al., 2011). The evidence from these TMS experiments suggests that corticospinal plasticity is reduced in the aged brain, which is consistent with the findings from animal models. However, these TMS studies have focused solely on investigating changes in the balance of excitation and inhibition within the hemisphere targeted by the stimulation and have predominantly quantified corticospinal plasticity in the pathway innervating the targeted limb. Few studies have investigated TMS-induced changes in the balance between excitation and inhibition in the opposite non targeted hemisphere or quantified corticospinal plasticity in the non targeted corticospinal pathway. This limitation is important, as evidence suggests that ageing may alter the manifestation of neuroplasticity, such that it occurs across a more diffuse network in the aged brain relative to that of young adults (Calautti et al., 2001; Carp et al., 2011; Heuninckx et al., 2005; Heuninckx et al., 2008; Hutchinson, 2002; Inuggi et al., 2011). The

section to follow will develop the idea that the manifestation of neuroplasticity may be altered in the aged brain.

1.6 Age-related changes in functional neural activity and implications for neuroplasticity

There is an abundance of evidence that demonstrates age-related alterations in neural activity during the performance of motor tasks. Studies using PET, fMRI and EEG have demonstrated that cortical activity occurs over a larger region with advancing age, including bilateral prefrontal, premotor and sensorimotor regions during the performance of motor tasks (Calautti et al., 2001; Carp et al., 2011; Heuninckx et al., 2005; Heuninckx et al., 2008; Hutchinson, 2002; Inuggi et al., 2011). For example, Naccarato and colleagues (2006) measured the brain's hemodynamic response using fMRI while participants performed a right index finger-thumb tapping task paced to an auditory tone. Task-related activity occurred more bilaterally such that there was greater task-related activity in the M1 ipsilateral to the trained hand in older, relative to younger participants.

These findings suggest that older individuals may recruit additional brain regions to assist in performing a motor task, perhaps due to the age-related structural and biochemical changes mentioned earlier. This idea, however, is a topic of debate. In some cases age-related reductions in lateralisation have been demonstrated when the performance of older adults was comparable with that of young adults (Hutchinson, 2002; Naccarato et al., 2006). These observations suggest that functional changes in the lateralisation of activity may be compensatory, responsible for maintaining motor performance in the aged brain. However, reduced lateralisation has also been demonstrated when behavioural performance is impaired (Heuninckx, et al., 2005; Inuggi et al., 2011). This observation suggests that functional changes in the lateralisation of activity reflects a reduction in the selectivity of neural activity, otherwise known as *dedifferentiation* (Park et al., 2001), which could be hindering behaviour. This thesis does not address the question of whether a reduction in the lateralisation of neural activity in the aged brain is compensatory or maladaptive, but instead considers the implications of such changes for neuroplasticity.

This thesis proposes that because task-related activity is less lateralised with age (Calautti et al., 2001; Carp et al., 2011; Heuninckx et al., 2005; Heuninckx et al., 2008; Hutchinson, 2002; Inuggi et al., 2011), neuroplasticity too may manifest over a more bilateral network in older relative to young adults. Neuroplasticity is activity dependent; therefore, if activity occurs bilaterally it might also be expected that neuroplasticity would manifest over a more bilateral network in older relative to younger adults. The studies discussed previously, reporting reduced corticospinal plasticity in older adults after motor training (Rogasch et al., 2009; Tecchio et al., 2008; Cirillo et al., 2010) and TMS

interventions (Fathi et al, 2010; Müller-Dahlhaus et al., 2008; Tecchio et al., 2008; Todd et al., 2010; Freitas et al., 2011), are limited in that they measure changes in behavioural performance and MEP amplitude only in the trained hand and corresponding corticospinal pathway. Therefore, those studies cannot address the question of whether neuroplasticity manifests over a more distributed (bilateral) network.

1.6.1 Redistribution of neuroplasticity in the aged brain after TMS-based neuroplasticity-inducing interventions

As argued above, to assess whether the distribution of neuroplasticity is altered in the aged brain, studies need to probe neuroplasticity bilaterally. Only a few studies have measured MEPs in both the stimulated and non-stimulated hemispheres, focusing predominately on young adults (Shin & Sohn, 2011; Suppa et al., 2008; Ishikawa et al 2007, Stefan et al., 2008; Bashir et al., 2014). These studies have demonstrated changes in corticospinal excitability in both the stimulated and unstimulated corticospinal pathways after unilateral TMS-based neuroplasticity interventions (Shin & Sohn, 2011; Suppa et al., 2008; Ishikawa et al 2007, Stefan et al., 2008). For example, Suppa and colleagues (2008) demonstrated that iTBS enhanced excitability in the stimulated corticospinal pathway, yet suppressed excitability in the unstimulated pathway. This pattern has been replicated by subsequent research (Di Lazzaro et al., 2008; Di Lazzaro et al., 2011). In addition, Shin and Sohn (2011) demonstrated that PAS 25 induced an increase in cortical excitability in both the stimulated and unstimulated corticospinal pathways. Although the direction of change in the unstimulated pathway varies across these few studies, seemingly depending on the neuroplasticity intervention used, the evidence suggests that TMS-based interventions influence the excitability of the opposite, unstimulated corticospinal pathway. Only one study to date has examined TMS-induced corticospinal plasticity in bilateral pathways in older adults (Di Lazzaro et al., 2008). These authors demonstrated bilateral corticospinal plasticity in young and older adults, such that iTBS enhanced excitability in the stimulated pathway but reduced excitability in the opposite unstimulated pathway. Importantly, only six older participants were tested, suggesting that this study was underpowered to detect differences between the different age groups. As such, it remains unclear how advancing age, which has been associated with a reduction in lateralisation of neural activity, might alter the manifestation of neuroplasticity across the hemispheres. Chapters 3 and 4 of this thesis addressed this question by assessing bilateral corticospinal plasticity following iTBS and PAS, respectively, in young and older adults. In order to increase statistical power in the studies of this thesis relative to that of Di Lazzaro and colleagues (2008), larger participants pools of 20 individuals were tested.

1.7 Task-dependent modulation of neuroplasticity in young and older adults

Given the possibility that neuroplasticity manifests more diffusely in older adults, studies investigating training induced adaptations within the bilateral motor system, as opposed to the unilateral system, may be more informative in revealing the extent of age-related change in neuroplasticity. It is well established that performance can be improved bilaterally in both the trained and untrained limbs after unilateral strength and skill training (Caroll et al., 2008; Rogasch et al., 2009; Kidgell et al., 2015: Hinder et al., 2011; Cirillo et al., 2010; Parikh & Cole, 2013). This phenomenon is referred to as cross-limb transfer (Scripture et al., 1894). While many studies have quantified performance improvements bilaterally following strength and skill training, in young and older adults, the mechanism driving cross-limb transfer is not well understood. Studies have begun to investigate the candidate mechanisms of cross-limb transfer by quantifying training-induced adaptations within the central and peripheral nervous system. So far, evidence indicates there is no change in the peripheral musculature of the untrained limb or in the activity of spinal motor neurons innervating the untrained limb following training (Hortobágyi et al., 1996; Houston et al., 1983; Dragert & Zehr, 2011; Lagerquist et al., 2006; Firmland et al., 2009). This suggests that the adaptations driving cross-limb transfer manifest in the brain.

Two models have been developed, which speculate as to the precise location of these training-induced adaptations within the brain (see Ruddy & Carson, 2013 and Hendy et al., 2012 for review). The *cross-activation* or *spill-over hypothesis* suggests that adaptations developed during unilateral training occur bilaterally, in the representation of the target muscle in the contralateral motor cortex and in the homologous region of the ipsilateral M1. The adaptations within the ipsilateral M1 are proposed to increase neural drive to the representative untrained limb, which increases strength and skill performance in that limb. Evidence in support of the cross-activation hypothesis demonstrates significant changes in the balance between excitation and inhibition in the cortical representations and corresponding corticospinal tracts innervating the trained and untrained limb. For example, corticospinal excitability, as indexed by MEP amplitude, is increased bilaterally after unilateral training (Carroll et al., 2008; Lee et al., 2010; Hinder et al., 2011; Poh et al., 2013; Koeneke et al., 2006; Latella et al., 2012; Hendy et al., 2015; Leung et al., 2015). Furthermore, cortical inhibition, as indexed by cortical silent period duration and the degree of short interval intracortical inhibition induced by paired-pulse stimulation, is decreased in both representations of the trained and untrained limb following unilateral training (Leung et al., 2015; Kidgell et al., 2015; Goodwill et al., 2015; Hendy et al., 2015). In addition, application of anodal tDCS to the hemisphere ipsilateral to the trained limb during training can enhance cross-limb transfer (Hendy &

Kidgell, 2014) and enhance corticospinal excitability in the pathway innervating the untrained limb in both young (Hendy & Kidgell, 2014; Hendy et al., 2015) and older adults (Goodwill et al 2015). These findings suggest that neural adaptations induced by unilateral training occur bilaterally in the corticospinal pathway innervating the trained limb as well as that of the untrained limb. Based on these observations of spill-over or cross-activation, it could be hypothesised that any additional activity occurring within the hemisphere ipsilateral to the trained limb as a result of age-related change may disrupt or interfere with the bilateral adaptations supporting cross-limb transfer.

Alternatively, the *bilateral access model* (see Ruddy & Carson, 2013 and Hendy et al., 2012 for review) proposes that the motor engram developed during unilateral motor training occurs in a location that is accessible to both primary motor cortices. There are several lines of evidence supporting the bilateral access model. First, behavioural changes in motor performance are often not correlated with changes in excitability (Carroll et al., 2008; Hinder et al., 2011; Hortobagyi et al., 2011). This suggests that the changes that occur in the untrained M1 may occur as a secondary effect of adaptations outside of the motor cortex and may not reflect increased neural drive to the untrained limb. Second, undertaking a voluntary grip force contraction with one hand while simultaneously training on a five-finger sequential tapping task with the other hand, does not affect cross-limb transfer of the sequential task (Parlow & Dewey, 1991). If both primary motor cortices were encoding the motor engram for the sequence task, as the cross hemisphere model suggests, one might expect interference from engaging the ipsilateral cortex in a voluntary contraction during training. This was not the case. It has been suggested and indeed demonstrated, however, that strength and skill training may rely on different brain networks, which may explain the lack of interference between the tasks in the study by Parlow and Dewey (1991; see Ruddy and Carson, 2013 for review). In line with the proposal that cross-limb transfer of simple and complex tasks is underpinned by different mechanisms, it is possible that age-related change modulates cross-limb transfer of simple and complex tasks differently. Few studies have used TMS to directly compare the effect of simple and complex motor training on bilateral cortical excitability, and none have investigated this effect in older adults.

Hinder and colleagues (2011) trained young and older adults on a simple ballistic finger abduction task similar to that of studies previously discussed (Rogasch et al., 2009; Cirillo et al., 2010). Following training, both performance improvements in the trained hand and MEP changes in the trained hemisphere were comparable between young and older adults. Performance improvements in the untrained hand were also investigated. Hinder and colleagues (2011) found that cross-limb transfer occurred in young but not older adults with this simple task. However, MEP amplitudes

were increased in both hemispheres in both age groups. In line with the cross-activation hypothesis the increase in cortical excitability in the ipsilateral hemisphere might suggest that the motor engram for the simple task was encoded bilaterally in both the contralateral and ipsilateral motor cortices. Alternatively, increased excitability in the ipsilateral cortex may have occurred as a consequence of adaption in non-motor regions or changes in the balance of excitation and inhibition within the contralateral motor cortex. Interestingly, contrary to what might be expected with the cross activation model, the increase in excitability did not manifest as an increase in performance in the untrained limb in older adults. One possible explanation for this effect is that adaptation did occur in the ipsilateral motor cortex but that capacity limits in the peripheral musculature prevented the increase in neural drive from increasing performance. Alternatively, increased excitability in the ipsilateral motor cortex may not be functionally related to cross-hemisphere transfer. In light of the evidence of age-related reductions in the lateralisation of neural activity there is an additional explanation that may account for these findings. It could be speculated that age related change resulted in a greater spread of neural activity across bilateral motor cortices during unilateral training. The increase in bilateral neural activity may have led to corticospinal plasticity in the ipsilateral hemisphere. In this instance the change in excitability might reflect the compensatory involvement of the ipsilateral motor cortex in assisting learning in the trained hand instead of the untrained hand.

The experiment detailed in Chapter 2 of this thesis was conducted to try and determine the degree to which changes in the excitability of the ipsilateral hemisphere supported learning in the trained hand in young and older adults. In difference to the study by Hinder and colleagues (2011), wherein cross-limb transfer and cortical excitability were compared between young and older adults after training on a simple task, the study detailed in Chapter 2 of this thesis also trained participants on a complex task. Interestingly, age-related effects on cross-limb transfer are present only under particular task conditions. In particular, some evidence shows that transfer is attenuated in older adults with simple tasks, but not complex tasks (Parikh & Cole, 2013). This result suggests that task complexity is important in modulating the effect of advancing age on cross-limb transfer and bilateral corticospinal plasticity.

1.7.1 Task complexity

Increasing the complexity of a task increases the demand on cognitive processes, encompassing a larger network of regions including the frontal cortices (Davare et al., 2010; Ehrsson et al., 2000, 2001; Holmstrom et al., 2011; Mima et al., 1999; Parikh & Cole, 2013; Sadato et al., 1996; Solodkin et al., 2001; Verstynen et al., 2005; Halsband & Lange, 2006). Activity in frontal regions

may be important in regulating lower-level sensory processing in various posterior brain regions in accordance with ones goals (Cohen et al., 1988; Pardo et al., 1991; Kastner, 2004; Shulman et al., 2003; Simpson et al., 2011; Hopfinger et al., 2000). Importantly, frontal regions are the most susceptible to age-related declines in grey and white matter, with these changes occurring more rapidly than in any other region (Haug, 1983; Haug & Eggers, 1991; Head et al., 2004; Pfefferbaum & Sullivan, 2005; Raz et al., 1997). Frontal regions also show changes in functional activity with advancing age. Similar to age-related change in activity during the performance of motor tasks, activity in frontal regions is less lateralised during cognitive tasks (Cabeza, 2002; Reuter-Lorenz et al., 2000; Grady et al., 1999). The extent to which this change in functional activity reflects compensation versus dedifferentiation is also a topic of much debate in the cognitive domain (Cabeza, 2002; Grady, 2002; Grady et al., 1999; Madden et al., 1999; Cabeza et al., 2004; Li & Lindenberger, 1999; Li & Lindenberger, 2001). It is possible, however, that increased reliance on frontal regions may assist learning under conditions of increased task complexity. In the simple task condition, frontal regions may play less of a role and age-related changes in the lateralisation of neural activity within M1 may hinder learning and neuroplasticity. Evidence of changes in interhemispheric inhibition after unilateral motor training with and without concurrent non-invasive brain stimulation during training suggests that the corpus callosum may play a key role in cross-limb transfer (Avanzino et al., 2014; Williams et al., 2010). This might be especially the case during training with simpler tasks, which rely primarily on neural activity within, and communication between, the motor cortices (Mima et al., 1999; Sadato et al., 1996). The corpus callosum, in particular the anterior portion, is found to deteriorate with advancing age (see Fling et al., 2011 for review; Hou et al., 2012). As discussed previously, in comparison to simple tasks, complex tasks rely on a larger network of regions spanning frontal-parietal networks (Davare et al., 2010; Ehrsson et al., 2000, 2001; Holmstrom et al., 2011; Mima et al., 1999; Sadato et al., 1996; Verstynen et al., 2005). The larger more diffuse network involved in the performance of a complex task may potentially enable signals to bypass age-affected regions such as the anterior corpus callosum (Parikh & Cole, 2013). Therefore, age-related degeneration may have less impact on cross-limb transfer of complex than of simple motor tasks.

No studies to date have compared simple and complex tasks directly whilst incorporating measures of both corticospinal plasticity and behaviour bilaterally in both young and older adults. Without measuring bilateral corticospinal plasticity and behaviour in young and older adults before and after training, the role of the ipsi- and contra-lateral motor cortices in transfer of simple and complex motor skills cannot be determined. Therefore, the extent to which the manifestation of neuroplasticity across the hemispheres is altered in the aged brain remains unclear. Chapter 2 of this

thesis replicates and builds upon the study by Hinder and colleagues (2011) by assessing the extent to which age-related change interferes with cross-limb transfer and the distribution of neuroplasticity under conditions of increasing task complexity.

1.7.2 Attention

One major difference between studies using motor training versus TMS to induce neuroplasticity is that the former engages cognitive processes, as noted in the previous section. In this context attention is a cognitive process that varies with task complexity (Schwartz et al., 2005). Interestingly, studies have demonstrated that under conditions of increased task demands corticospinal plasticity induced by PAS (Stefan et al., 2004) and transcranial direct current stimulation (TDCS; Antal et al., 2007) is reduced. Kamke and colleagues (2012) demonstrated that variations in the demand on attentional resources (attentional load) could account for this effect. These authors instructed participants to perform a well-established attentional load task, while undergoing PAS and iTBS (in separate sessions). Reliable LTP-like corticospinal plasticity effects were evident when attentional resources were not occupied with the task (low load condition) but were abolished in the high-load task. This finding was present for both PAS and iTBS neuroplasticity inducing paradigms, suggesting that attentional load modulates corticospinal plasticity within the motor system.

It has also been established that the spatial location of attention can influence corticospinal plasticity (Stefan et al., 2004; Kamke et al., 2014). Stefan and colleagues (2004) demonstrated that when attention is directed to the limb targeted by the intervention, corticospinal plasticity effects induced by PAS are enhanced. In that study participants were tasked with detecting a number of weak electrical stimuli randomly applied to either the limb targeted by PAS or the opposite limb, with both hands occluded. MEP amplitudes were enhanced with attention directed to the target hand. However, it is possible that the afferent input to the sensorimotor cortex arising from the tactile stimulation enhanced corticospinal plasticity. Kamke and colleagues (2014) eliminated this possibility by manipulating the location of spatial attention with a visual task. Participants were instructed to covertly attend to a stream of LEDs on the side of the target or non-target hand. Participants silently counted the number of targets (twin-flashes) that appeared in the attended location. Participants reported the number of targets detected at the end of the trial by fixating with their eyes on one of three response panels. Consistent with previous findings, PAS-induced LTP-like effects (MEP amplitudes) were enhanced with attention allocated to the side of the target limb relative to when attention was allocated to the side of the non-target limb.

Evidence demonstrates that attention to the target limb might facilitate corticospinal plasticity by enhancing the strengthening of neural connections within the representation of the target limb location (Kamke et al., 2014). The mechanism by which attention might alter corticospinal plasticity remains unclear, but may involve the up-regulation of neuromodulators such as dopamine (Kamke et al., 2014). Dopamine plays an important role in the modulation of neural responses by attention (Moore, 2006) and also influences LTP/LTD-like effects induced by non-invasive brain stimulation (Kuo et al., 2008; Monte-Silva et al., 2010; Thirugnanasambandam et al., 2011). The dopaminergic system is often altered in the aged brain (Reeves et al., 2002; Stark & Pakkenberg, 2004; Suhara et al., 1991; van Dyck et al., 2002), yet no studies to date have investigated whether the attentional modulation of corticospinal plasticity is altered in older adults. Moreover, the majority of studies investigating age-related changes in corticospinal plasticity in older adults do not report even controlling for the effects of attention. As a result, the degree to which attention functions to protect or hinder corticospinal plasticity in the aged brain is unknown. Importantly, older adults have been shown to experience declines in attention (see Drag & Bieliauskas, 2010 for review). If age-related change disrupts attention and results in deficits in the processing of relevant and irrelevant information, the influence of attention may be altered in the aged brain. Some attentional abilities, however, remain intact in older adults (Tales et al., 2002; Nebes & Brady, 1993; Folk & Hoyer, 1992; Hartley et al., 1990; Lincourt et al. 1997; Danckert et al., 1998), suggesting that the modulation of corticospinal plasticity by attention may be maintained. The second empirical study of this thesis, detailed in Chapter 3, controlled for the effect of attention on neuroplasticity induction in bilateral corticospinal pathways. The final empirical study detailed in Chapter 4 manipulated the spatial location of attention to determine the role of attention in modulating corticospinal plasticity in young and older adults.

1.8 Aims and Hypotheses

In summary, neuroplasticity is reported to be reduced in non-human animals and evidence suggests similar effects in humans. Advancing age has been associated with great neurobiological change, including a reduction in the lateralisation of neural activity. Few studies have investigated the implications of such reductions in the lateralisation of activity for neuroplasticity and behavioural learning. While young adults demonstrate bilateral performance improvements after unilateral training (cross-limb transfer) these effects are significantly reduced in older adults, especially with simple training tasks. The implication of this is that neuroplasticity is not simply reduced in older adults but instead is redistributed. This thesis investigated the possibility that age-related changes in cross-hemisphere activity might alter the manifestation of neuroplasticity and behaviour in older

adults. Specifically, it is hypothesised that neuroplasticity may occur across a more diffuse network of motor and non-motor regions within the targeted- and non-targeted hemisphere. This thesis focused on investigating neuroplasticity in the targeted and non-targeted M1, as neuroplasticity induced by neurobiological change or brain injury typically manifests in regions that perform the same or similar functions as that experiencing decline/damage (Rossini et al., 2003). In addition, corticospinal plasticity was measured in bilateral motor cortices as the methods required to probe intrahemispheric neuroplasticity (twin coil TMS) are much more complex, time consuming, and would not be able to be administered alongside measures of interhemispheric effects in the same experiment.

Understanding age differences in the manifestation of neuroplasticity across bilateral motor pathways is informative because if neuroplasticity supporting one limb manifests more bilaterally in older adults this has implications for learning bilateral motor tasks, or for transfer of learning from one limb to the other. Moreover, the rehabilitative potential of pairing unilateral motor training with non-invasive brain stimulation for facilitating strength and skill gain in individuals with peripheral and central nervous system damage is gaining increasing focus within the literature (Hendy et al., 2015; Goodwill et al., 2015; Hendy & Kidgell, 2014). Non-invasive brain stimulation presents as a potentially effective and efficient technique to modulate excitability in many cortical regions. When applied to the motor cortex specifically, non-invasive brain stimulation has been shown to induce corticospinal plasticity (Huang et al., 2005; Stefan et al., 2000; Nitsche et al., 2008). Therefore, non-invasive brain stimulation applied in isolation or alongside motor training may potentially improve clinical outcomes in health and disease (Tanaka et al. 2011; Fregni et al. 2006). For example, after stroke, an inhibitory non-invasive brain stimulation protocol might be applied to the overactive, intact hemisphere to try and reduce interhemispheric inhibitory effects on the lesioned hemisphere before motor training is undertaken (Takeuchi et al., 2008; Khedr et al., 2009; Emara et al., 2010; Nair et al., 2011). Despite the elderly being the most prominent target for such interventions, much of the knowledge used for applying non-invasive brain stimulation in clinical settings has been gained from younger adults; it is not clear if the effects readily translate to use in older adults who demonstrate great age-related neurobiological structural and functional change within the motor system. Put another way, it is not yet well understood how plasticity in the motor system of neurologically healthy adults is altered by advancing age. Using changes in corticospinal excitability probed by TMS as a model of neuroplasticity, this thesis provides a comprehensive investigation into how the manifestation of corticospinal plasticity across the bilateral motor system is altered in the aged brain. In each of the three experiments of this thesis, single-pulse TMS was applied to the left and right motor cortices, prior to and following a

neuroplasticity intervention. Changes in bilateral corticospinal excitability, as indexed by MEP amplitude, were used as an indirect measure of neuroplasticity.

The first empirical study detailed in Chapter 2 assessesed how well older, compared with younger, adults were able to learn a motor skill with one hand and transfer performance gains from the trained hand to the opposite, untrained hand. Transfer of performance gains is a well-established phenomenon in young adults but has not been extensively investigated in older adults or in conjunction with measures of corticospinal plasticity. In particular, no studies have compared age differences in transfer and corticospinal plasticity after training on a simple and a complex task. With simple tasks it is possible that age-related reductions in the lateralisation of neural activity lead to neuroplasticity in bilateral motor cortices. This could interfere with learning in the untrained hand and associated neuroplasticity in M1, which might reduce cross-limb transfer in older adults. With complex tasks, however, the greater involvement of premotor and higher-order cognitive regions may result in neuroplasticity over a larger network, potentially in brain regions outside M1, and thereby age-related change in M1 activity may cause little interference to cross-limb transfer in older adults. These ideas are developed further in Chapter 2.

The second experiment of this thesis examined how neuroplasticity and its distribution across bilateral motor pathways might be altered in the aged brain using external non-invasive brain stimulation to induce neuroplasticity. In Chapter 3, iTBS was applied unilaterally to the left M1 of young and older adults. During stimulation attention was controlled with a visuo-spatial attention task located on the target hand. It was hypothesised that if functional networks are less lateralised in the aged brain then corticospinal plasticity might be induced bilaterally by iTBS in older but not young adults. Moreover, it was expected that corticospinal plasticity in the stimulated pathway would be reduced in older adults.

Experiment 3 employed a different form of TMS-based intervention - PAS. The mechanisms underpinning PAS are proposed to be slightly different to the mechanisms underpinning iTBS and motor training. Therefore, Experiment 3 provided additional information regarding how neuroplasticity may be altered in the aged brain. The role of attention in modulating neuroplasticity was also investigated in Experiment 3. PAS was applied unilaterally to the left M1, and in separate sessions attention was allocated to the target and non-target hands during PAS. Attention to the target-hand has been shown to facilitate corticospinal plasticity in the target pathway on a number of occasions in young adults, but this effect has not been investigated in older adults. Once again, based on the hypothesis that functional networks are less lateralised in the aged brain and span across bilateral motor cortices, it was predicted that older, relative to younger, adults would

demonstrate greater bilateral corticospinal plasticity following PAS. Furthermore, if attentional modulation of neuroplasticity is reduced in the aged brain, older adults would be expected to show similar corticospinal plasticity across the two attention conditions. Young adults, however, were predicted to demonstrate attentional modulation of corticospinal plasticity such that PAS-induced effects would be enhanced when attention was allocated to the target hand.

To summarise, advancing age is associated with a reduction in the lateralisation of neural activity. The experiments of this thesis investigated whether neuroplasticity, as indexed by changes in corticospinal excitability (corticospinal plasticity) within the motor system, also becomes less lateralised with advancing age. As three different methods were used to induce neuroplasticity, which have similar but slightly different underlying mechanisms, together the experiments of this thesis provide a comprehensive account of changes in neuroplasticity in the aged motor cortex. In addition, the experiments of this thesis begin to highlight the extent to which the involvement of cognition and attention influences neuroplasticity in the aged brain.

Chapter 2 Cross-limb transfer and bilateral corticospinal plasticity is maintained in older adults after skilled motor training with simple and complex tasks

This chapter is based on a paper published during candidature, which is cited below. Deviations from the published paper include replacing the term “Intermanual transfer” with with “Cross-limb transfer”. Effect sizes have been included in the analyses and the results interpreted in light of these. Section and Figure numbers have been modified for consistency with the rest of the thesis. Consistent with the published paper, the chapter is written in American English.

Dickins, D. S. E., Sale, M. V., & Kamke, M. R. (2015). Intermanual transfer and bilateral cortical plasticity is maintained in older adults after skilled motor training with simple and complex tasks. *Front. Aging Neurosci.* 2015. doi: 10.3389/fnagi.2015.00073.

Prior to Experiment 1 a pilot study was conducted to ensure that the chosen training paradigms and feedback schedules were sufficient to induce performance improvements in the trained hand in young and older adults. The details and results of this pilot are provided in Appendix A.

2.1 Abstract

Cross-limb transfer refers to the phenomenon whereby unilateral motor training induces performance gains in both the trained limb and in the opposite, untrained limb. Evidence indicates that cross-limb transfer is attenuated in older adults following training on a simple ballistic movement task, but not after training on a complex task. This study investigated whether differences in plasticity in bilateral motor cortices underlie these differential cross-limb transfer effects in older adults. Twenty young (<35 years old) and older adults (>65 years) trained on a simple (repeated ballistic thumb abduction) and complex (sequential finger-thumb opposition) task in separate sessions. Behavioral performance was used to quantify cross-limb transfer between the dominant (trained) and non-dominant (untrained) hands. The amplitude of motor-evoked potentials (MEPs) induced by single pulse transcranial magnetic stimulation (TMS) was used to investigate excitability changes in bilateral motor cortices. Contrary to predictions, both age groups exhibited performance improvements in both hands after unilateral skilled motor training with simple and complex tasks. These performance gains were accompanied by significant bilateral increases in corticospinal excitability in both groups for the simple task, and a similar trend in the complex task. The findings suggest that advancing age does not necessarily influence the capacity for cross-limb transfer after training with the dominant hand.

2.2 Introduction

Neuroplasticity refers to the ability of the brain to alter its structure and function, allowing for adaptation to changing demands of the environment. Such neural flexibility plays a fundamental role in the formation and storage of memories, in learning new skills and behaviors, and in recovery of function following brain injury. Evidence suggests that, compared with younger adults, neuroplasticity is reduced in older individuals. For example, studies inducing neuroplasticity experimentally by motor training or using non-invasive brain stimulation have shown that corticospinal plasticity is reduced in the motor cortex of older adults (Rogasch et al., 2009; Sawaki et al., 2003; Todd et al., 2010; Fathi et al., 2010; Müller-Dahlhaus et al., 2008). It has also been shown, however, that older adults frequently exhibit more diffuse neural activity, both within and between hemispheres, than do younger adults when performing the same task (Heuninckx et al., 2005). Thus, neuroplasticity supporting a given function may manifest differently across neural networks in young and older individuals. The aim of the current study was to investigate whether the distribution of neuroplasticity is altered in older compared with younger adults and to determine if any change in neuroplasticity impacts the transfer of the learned motor skill to the untrained hand.

Advancing age is associated with great functional change in multiple neural systems (see Seidler et al., 2010 for review). In the motor system, aging is associated with a reduction in the specificity of neural activity during the performance of motor tasks. Specifically, when performing the same task older adults demonstrate greater activity across the hemispheres than do young adults (Calcautti et al., 2001; Carp et al., 2011; Heuninckx et al., 2005; Hutchinson, 2002; Inuggi et al., 2011; Naccarato et al., 2006; Mattay et al., 2002; Ward & Frackowiak, 2003). It has been proposed that such bilateral over-activation may reflect a reduction in the ability of the aged brain to regulate activity in specific motor networks, which can disrupt normal brain function and lead to a decline in motor performance (Bernard & Seidler, 2011; Inuggi et al., 2011; Langan et al., 2010; Li & Lindenberger, 1999; Riecker et al., 2006). Alternatively, over-activation may reflect a compensatory mechanism, whereby additional brain regions are recruited to compensate for the effects of age-related neurobiological change, thereby assisting in maintaining motor function (Goble et al., 2010; Heuninckx et al., 2008; Kim et al., 2010; Naccarato et al., 2006). In either case, evidence of over-activation suggests that neuroplasticity induced by skilled motor training may manifest more diffusely across bilateral motor pathways in older relative to younger adults. Moreover, activity (and hence neuroplasticity) in both hemispheres may be necessary to support learning of a unilateral motor skill in older adults.

Few studies have compared bilateral corticospinal plasticity in young and older adults following training on a unilateral motor task. Evidence from studies using transcranial magnetic stimulation (TMS) to probe corticospinal plasticity, however, suggests that a bilateral change in corticospinal excitability is not a characteristic unique to the older brain. For example, there is a wealth of studies investigating the process whereby a motor skill transfers from a trained to an untrained hand, which is termed cross-limb transfer (Koeneke et al., 2009; Pan & Van Gemmert, 2013; Parikh & Cole, 2013; van Mier & Peterson, 2006; Stockel & Wang, 2011). It has been shown that practicing a simple motor task (ballistic finger abductions) is associated with cross-limb transfer in young adults, and there is evidence to suggest this transfer might be dependent on adaptations within bilateral motor cortices (Carroll et al., 2008; Lee et al., 2010; Parikh & Cole, 2013). Specifically, Lee and colleagues (2010) demonstrated that in young adults disrupting cortical plasticity, by applying repetitive TMS to either the left or right motor cortices immediately following training, reduced training-related gains in motor performance for the contralateral hand. This finding suggests that bilateral adaptations within the trained and untrained hemispheres in young adults specifically supports performance gains of the trained and untrained hand, respectively. Interestingly, cross-limb transfer of a simple ballistic motor task has been reported to be reduced (Parikh & Cole, 2013) or even absent (Hinder et al., 2011) in older adults, but changes in

corticospinal excitability in both the trained and the untrained hemispheres are comparable to that found in the young (Hinder et al., 2011). Given that the increase in corticospinal excitability did not manifest as a performance improvement in the untrained hand, it would seem that the mechanism underpinning cross-limb transfer in older adults is not readily explained by the cross-hemisphere hypothesis. It is plausible that the increase in excitability in the untrained corticospinal pathway had no functional impact on learning in either hand, instead occurring as a secondary consequence of adaptations outside the primary motor cortices. It is also important, however, to consider the possibility that the reduction or absence of cross-limb transfer in the presence of increased corticospinal excitability in the ipsilateral M1 might reflect a reliance in older adults of adaptations within both the trained and untrained hemisphere to support learning with the trained hand.

Although cross-limb transfer of learned tasks has been shown to be reduced in older adults, this effect appears to depend on the type of task employed. Parikh and Cole (2013) reported that older adults who demonstrated reduced cross-limb transfer following training on a simple task nonetheless exhibited comparable transfer to young adults after training on a complex grip and lift task. In contrast, task complexity does not seem a determining factor with young adults, who show transfer following training on ballistic finger abductions (Carroll et al., 2008), maze tracing (Van Mier & Petersen, 2006), dexterity and precision tasks (Pereira et al., 2011), visuomotor adaptation (Pan & Gemmert, 2013) and object weight adaptation (Parikh & Cole, 2013). It is well established that undertaking simple and complex tasks draws upon differential neural circuitry (Davare et al., 2010; Ehrsson et al., 2000, 2001; Holmstrom et al., 2011; Mima et al., 1999; Parikh & Cole, 2013; Sadato et al., 1996; Solodkin et al., 2001; Verstynen et al., 2005). For example, it has been demonstrated that, in young adults, activity is more widespread when performing a sequence of movements with multiple fingers than when performing simple repetitive tapping movements with a single finger (Verstynen et al., 2005). The same is apparent when young adults perform skilled object manipulation compared with single-joint finger movements (Davare et al., 2010; Ehrsson et al., 2000, 2001; Holmstrom et al., 2011; Mima et al., 1999; Sadato et al., 1996). Similarly, although bilateral changes in corticospinal excitability following training on complex tasks have not been investigated in older adults, data from young adults suggests that regions outside the ipsilateral motor pathway might mediate transfer of complex tasks (see Tanji, 2001 for review). Indeed, when corticospinal excitability was assessed in bilateral motor pathways after training on a complex task, changes in corticospinal excitability were limited to the targeted pathway (Pascual-Leone et al., 1995). Thus, because complex tasks recruit a more extensive and widespread network, which does not rely on the untrained motor cortex to facilitate learning of the untrained hand, age-related over-activation in the untrained motor cortex may not interfere with cross-limb transfer of complex tasks.

The influence of task complexity on cross-limb transfer in young and older adults, however, has not yet been investigated in conjunction with measures of bilateral corticospinal excitability.

The current study aimed to investigate whether cross-limb transfer and bilateral cortical excitability are altered in young and older adults after motor training on simple and complex tasks. In separate sessions, young and older participants trained on a simple (repeated ballistic thumb abduction) and a complex (finger-to-thumb opposition) task with the dominant hand. It was hypothesized that plasticity in both motor cortices would be required for unilateral motor learning in older but not young adults. Thus, whilst both groups should show bilateral increases in corticospinal excitability following training on a simple ballistic task, it was predicted that cross-limb transfer would be reduced in the older adults. With the complex task, however, it was predicted that cross-limb transfer would be maintained in older adults. Based on the notion that cross-limb transfer of a complex task is supported by more widespread neural activity, predominantly outside the primary motor cortices, it was predicted that any increase in M1 excitability after training would be limited to the trained hemisphere in young adults. In contrast, corticospinal plasticity may still manifest bilaterally in older adults to support the motoric component of learning a complex task in the trained hand.

2.3 Materials and methods

2.3.1 Participants

A total of 20 young participants between the ages of 18 and 33 years (*M* = 24.25, *SD* = 4.60, Males = 10) and 20 older participants between the ages of 65 and 77 years (*M* = 70.00, *SD* = 3.42, Males = 10) were tested. According to the Edinburgh handedness inventory (Oldfield, 1971) all but one young participant, who was classed as ambidextrous, were right-handed (Young *M* = 82.92, *SD* = 22.66, Range = 33.33-100; Older *M* = 83.30, *SD* = 16.13, Range = 44.44-100). Participants were recruited by word of mouth and through advertising in online newsletters and were reimbursed $10 per hour for their participation. Prior to commencement of testing all participants completed a TMS safety-screening questionnaire (Rossi, et al., 2009; 2011) and provided fully informed written consent. All procedures were approved by The University of Queensland Medical Research Ethics Committee. Individuals with neurological disease or damage, epilepsy, history of head injury or psychiatric disorder, or who were taking neuroactive medications were excluded from the study. All participants had normal or corrected to normal visual acuity. There were no adverse reactions to the TMS.

2.3.2 Transcranial magnetic stimulation (TMS)

TMS was administered using a figure-of-eight shaped coil with a wing diameter of 70 mm, connected to a Magstim 200^2 stimulator (Magstim Co., UK). TMS was delivered to the motor hotspot, which was defined as the optimal position on the scalp for evoking the largest and most consistent motor-evoked potential (MEP; peak-to-peak amplitude) in the target muscle, the *abductor pollicis brevis* (APB) of the left and right hands. Motor hotspots were located by placing the coil tangentially on the scalp with the handle pointing towards the back of the head, angled 45 degrees from the midline and moving it systematically in a grid-like pattern. Stimulation occurred approximately every 5 seconds at an intensity sufficient to evoke a clear MEP in the target muscle. The position of the coil for each hotspot was recorded using a frameless infrared stereotaxic neuronavigation system (Visor, ANT, Netherlands). This navigation system was used to reproduce coil angle and location within an experimental session.

Following determination of the hotspot, resting motor threshold (rMT) was obtained for the cortical representation controlling the left and right APB. The rMT was defined as the minimum TMS intensity (reported as a percentage of maximum stimulator output, % MSO), that evoked an MEP of above 50 μV in at least 3 out of 5 consecutive trials The intensity of the TMS was adjusted using a staircase (two-down, one-up) procedure until the criterion was met. Following this, TMS test intensities were established for the left and right APBs. The test intensity was defined as that required to evoke an average MEP of approximately 1 mV (range 0.5 and 1.5 mV peak-to-peak) in the resting muscles. On average this intensity equated to 126% of rMT for the right (target) APB and 125% for the left (non-target) APB. Average MEP amplitude at baseline and post training was determined using the test intensity from a block of 21 TMS pulses that were delivered every 5 ±1 seconds.

2.3.3 Recording of muscle activity

Activity from the muscles of interest was recorded using surface electromyography (EMG). Disposable 24 mm silver-silver chloride (Ag/AgCl) electrodes were used, with the active electrode placed on the belly of the APB muscle of the left and right hands and reference electrodes on the metacarpophalangeal joint of the respective thumb. MEP data were amplified (x1000), filtered (20-1000 Hz) and sampled at 2000 Hz using a NeuroLog system (Digitimer, UK). Individual sweeps were sampled from 500 ms before stimulation to 500 ms after stimulation and stored for off-line analysis using Signal software (CED, UK). Muscle activity was visually monitored throughout the

experiment using a digital oscilloscope. If activity occurred during a trial, participants were verbally prompted to relax.

2.3.4 Simple task: Repeated ballistic thumb abduction

For the simple task condition participants were required to perform a volitional ballistic thumb abduction movement. The participants' arms and hands were placed on cushioned platforms on the desk with the forearm supinated. The fingers and wrist of both hands were immobilized by straps attached to the platforms. Participants were instructed to move only the thumb as quickly as possible across the hand in a horizontal plane with the aim of maximizing peak acceleration. Acceleration data was amplified (x10), low pass filtered (1000 Hz) and sampled at 2000 Hz. Consistent with Hinder and colleagues (2011; 2013), each sweep was triggered when the abduction acceleration exceeded approximately 4.9 m/s^2. This threshold was reduced for participants whose abduction acceleration fell consistently below this point.

Prior to, and at two and 15 minutes following training, participants performed this movement for a period of 30 seconds (15 trials at 0.5 Hz), with each individual movement initiated in response to an auditory tone. Each participant was given only one practice of the movement on each hand before baseline performance was recorded. Measures of performance in the right hand always preceded the left hand, pre and post training. Performance on the simple task was quantified using an accelerometer attached to the right and left thumb. At each time point performance was defined as the peak acceleration averaged across 15 trials for each hand. Verbal encouragement, instructing participants to maximize their peak acceleration, was given prior to each block of the pre and post measures. Visual feedback (see section 2.4.1: Simple task training) and verbal encouragement were given during training but not during the pre and post measures.

2.3.4.1 Simple task training intervention

During training, participants performed the same ballistic movement with the right hand paced to the auditory tone (0.5 Hz) for one minute, followed by a 30 second rest period. This procedure was repeated ten times, resulting in a total of 10 minutes of training (300 ballistic movements). The accelerometer was fixed to the right thumb and was recording throughout the training intervention. Participants were provided visual feedback regarding their peak acceleration and were verbally encouraged to maximize peak acceleration throughout the training blocks.

2.3.5 Complex task: Finger-to-thumb opposition

In the complex condition participants were required to perform a finger-to-thumb opposition task with the right hand first, followed by the left. This paradigm was modified from that of Karni and colleagues (1995), which induced robust changes in M1 activity following training. The sequence of movements is depicted in Figure 2-1. Prior to, and at two and 15 minutes post training, participants were instructed to perform this sequence as quickly and as accurately as possible to maximize the number of sequences completed in a 30 second time period. Performance in each hand was quantified by determining the number of sequences performed correctly in the allotted time period (30 secs). Performance was recorded using a digital video camera and stored for offline analysis. Participants completed four practice sequences before baseline measures were initiated.

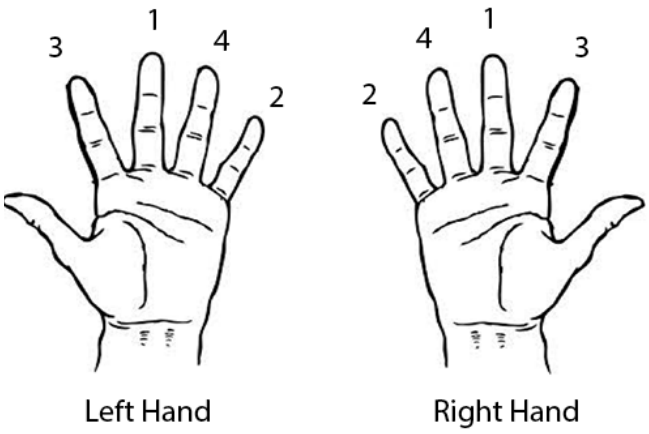

Figure 2-1 Finger-to-thumb opposition sequence for the left and right hands. Participants were instructed to move the thumb towards each of the fingers sequentially in the sequence depicted.

2.3.5.1 Complex task training intervention

During training on the complex task participants performed the same sequence as in the pre- and post-training measures, but for training the timing of each individual finger-thumb opposition movement was paced by the same auditory cue used in the simple task. That is, each individual movement in the sequence depicted for the right hand in Figure 2-1 was performed at a rate of 0.5 Hz. Only the dominant (right) hand performed the training task. As for the simple task, participants completed 10 blocks of training lasting one minute each. These blocks were separated by a 30 second rest period. Performance was recorded throughout the training intervention using a digital video camera

2.3.6 Experiment design and procedure

Participants completed two sessions at similar times of day at least 48 hours apart, with each session lasting up to two hours. During the training sessions participants were seated comfortably with both their right and left arms resting on cushioned platforms on a desk. The skin of both hands was cleaned thoroughly to minimize skin impedance and the surface electrodes were placed in position. The time course of the experiment is outlined in Figure 2-2. Participants received single pulse TMS to left and right M1 to locate the motor hotspot and to quantify cortical excitability before the motor training task. The task was then explained to the participants, and they were provided a brief chance to practice. Baseline measures of behavioral performance were then obtained from the right and left hands, followed by completion of the training task. The order of the simple and complex sessions was randomized across groups and individuals. Cortical excitability was assessed with single pulse TMS two and 15 minutes following training. MEPs were obtained from the left M1 (target hemisphere) and then the right M1 (non-target hemisphere) using the test stimulus intensity. Following assessment of cortical excitability at each time point, behavioral performance was re-measured. An eye tracker was used throughout the pre and post measures to ensure participants kept their eyes open during the experiment.

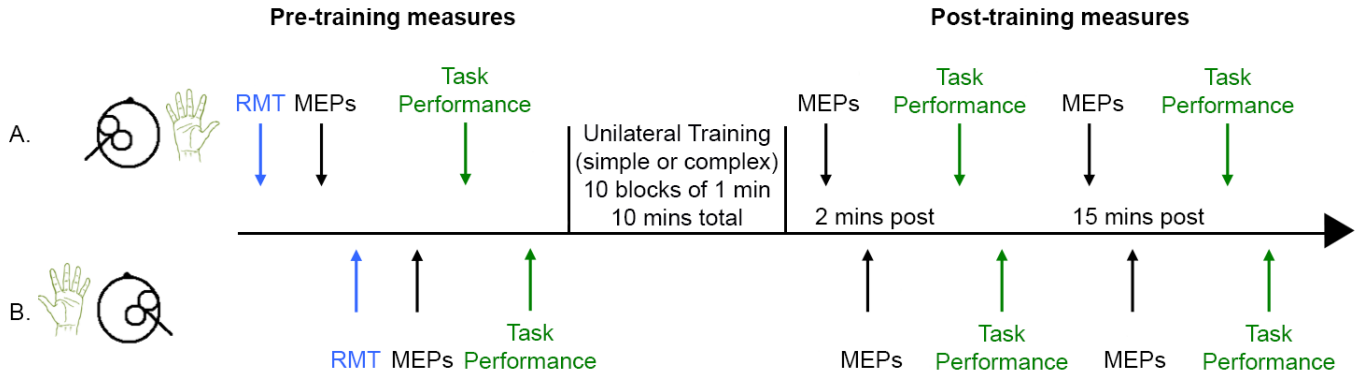

Figure 2-2 Time course of experiment. The timeline of measures is shown for the left hemisphere/right hand (trained M1 and hand) in (A) and the untrained right hemisphere/left hand in (B). Cortical excitability was quantified by measuring the amplitude of motor evoked potentials (MEPs) elicited by 21 single pulses of transcranial magnetic stimulation in the resting right and left abductor pollicis brevis muscles before and after training. Behavioral performance was also quantified before and after training.

2.3.7 Data processing & analyses

The first trial from each block of MEP data was removed and the remaining trials in each block were averaged for each participant (20 MEPs per block). Trials containing voluntary muscle activity clearly above background activity (cut off estimate ~0.03- 0.04 mV) in the 100 ms before TMS were also removed, these constituted 2.71% of all remaining trials. Baseline rMTs were analyzed using a 2 × 2 ANOVA with the between-subjects factor age (young, older) and the within-subjects factor hemisphere (trained, untrained). Mixed ANOVAs were carried out for the behavioral data with the factors of time (pre, 2 mins post, 15 mins post), hand (trained and untrained) and age (young, older). For the simple task the dependent variable was the average maximum peak acceleration, whereas for the complex task it was the average number of sequences completed correctly in the allotted time (30 seconds). As the dependent variables were not comparable in the simple and complex tasks, separate ANOVAs were conducted for each condition. The effect of training on excitability of the APB muscles was analyzed using a 2 × 2 × 3 mixed ANOVA with the between-subject factor of age (young, older) and within-subject factors muscle (rAPB, lAPB) and time (pre, 2 mins post, 15 mins post). For consistency with the behavioural analysis, separate ANOVAs were conducted for the simple and complex tasks. Bonferroni corrections were applied to all follow-up, two-tailed t-tests. Although there were no significant differences in baseline measures between the groups, additional analyses were performed with normalised behavioural and MEP data to ensure that slight differences in baseline levels did not influence the group-level effects. Normalization was achieved by calculating the difference between pre and post MEP amplitudes, dividing this value by baseline MEP amplitude, and multiplying by 100. Normalizing the data in this way did not alter the significance of any of the tests. Therefore, only the analyses with the raw data are presented. To further explore the influence of corticospinal plasticity on cross-limb transfer a transfer index was calculated, which reflected the performance gain in the untrained hand expressed as a percentage of the gain in the trained hand. The relationship between the transfer index and MEP change in the untrained corticospinal pathway was assessed using Pearson's correlation analyses. Prior to analysis the data were examined for outliers and points of high influence using studentized deleted residuals, centered leverage values, and Cook's distance. Separate analyses were conducted for young and older adults.

2.4 Results

2.4.1 Behavioral performance in the simple task

Figure 2-3 depicts average peak acceleration in the trained and untrained hands for young and older adults at each time point before and following training. As can be seen in Figure 2 -3 there was an increase in performance in both groups following training, but this effect is larger for the young than the older adults. ANOVA confirmed a training-related effect with a significant main effect of time; $F(2,76) = 37.41$, $p < .001$ $\eta_p^2 = .50$. Moreover, ANOVA also confirmed that the training-related increase in performance varied between young and older adults with a significant interaction between time and age; $F(2,76) = 9.32$, $p = .003$ $\eta_p^2 = .20$. Independent samples t-tests, which compared peak acceleration between each of the three time points in young and old adults revealed that in young adults peak acceleration increased significantly from baseline to the two ($t(19) = 5.89$, $p < .001$, $d = 1.32$) and 15 minute ($t(19) = 5.32$, $p < .001$, $d = 1.19$) post-training measures. Older adults also demonstrated significantly greater peak acceleration relative to baseline at the two ($t(19) = 3.22$, $p < .004$, $d = 0.72$) and 15 minute post-training time points ($t(19) = 2.78$, $p = .012$, $d = 0.62$), but the significant interaction indicates that the training-related effects were smaller in this group. There was no significant difference in peak acceleration between the two post-training measures in young ($t(19) = 1.29$, $p = .214$, $d = 0.29$) or older adults ($t(19) = .98$, $p = .338$, $d = 0.22$). Figure 2-3 also demonstrates that peak acceleration increased to a greater degree in the trained hand than in the untrained hand, irrespective of the participant's age. ANOVA revealed that the effect of training varied between the hands with a significant hand × time interaction; $F(2,76) = 10.08$, $p = .001$ $\eta_p^2 = .21$. Paired samples t-tests demonstrated that while peak acceleration did not differ between the hands at baseline ($t(39) = 1.56$, $p = .128$, $d = 0.25$), peak acceleration was significantly greater in the trained hand at both the two ($t(39) = 4.47$, $p < .001$, $d = 0.71$) and 15 minute ($t(39) = 3.07$, $p = .004$, $d = 0.49$) post-training time points. These effect sizes were found to meet Cohen's (1988) convention for a moderate effect ($d = 0.5$). There were no other significant main effects or interactions (ps > .436).

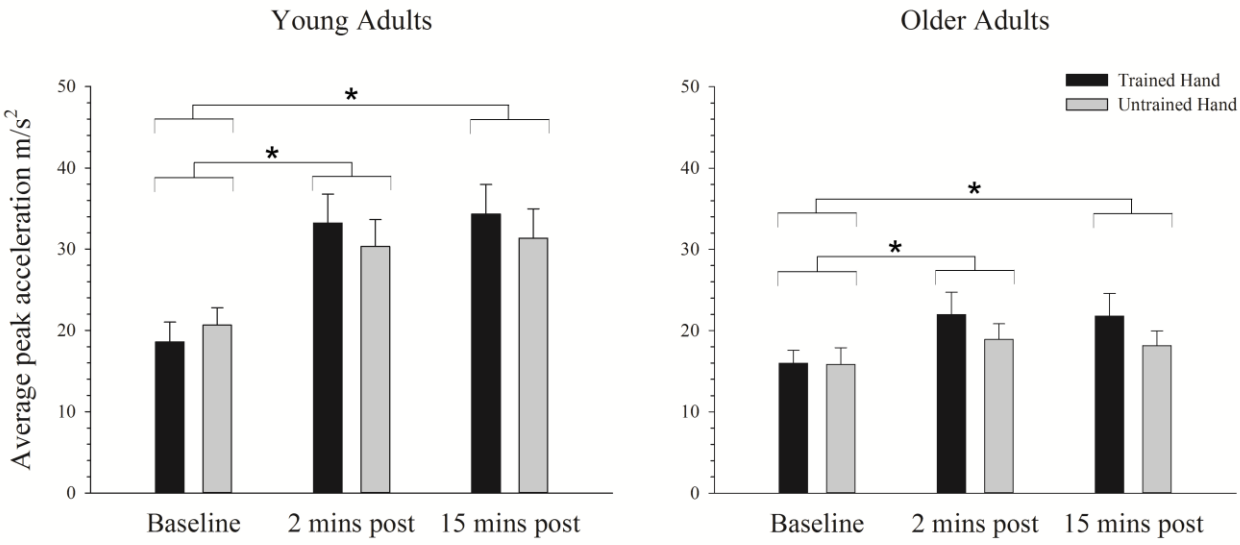

Figure 2-3 Mean peak acceleration before and after training on a simple motor task in young and older adults. Average peak acceleration increased significantly from pre to 2 and 15 minutes post training in both young and old adults (* denotes $p < .05$). The increase was significantly greater in young adults and in the trained hand, but there was no difference between the groups in the transfer of the training effects to the untrained hand. Error bars denote SEM.

2.4.2 Changes in cortical excitability in the simple task

Baseline rMTs, reported as % MSO, in the trained (M = 41.30, SE = 2.13) and untrained (M = 40.05, SE = 2.00) hemispheres of young adults did not differ from baseline rMTs in the trained (M = 40.05, SE = 1.70) or untrained (M = 41.35, SE = 1.53) hemispheres of older adults in the simple condition. ANOVA failed to reveal any differences in rMT between the hemispheres or between age groups (all $ps > .144$). Representative traces for one young and one older participant showing pre- and post-training (2 min) MEPs are depicted in Figure 2-4.

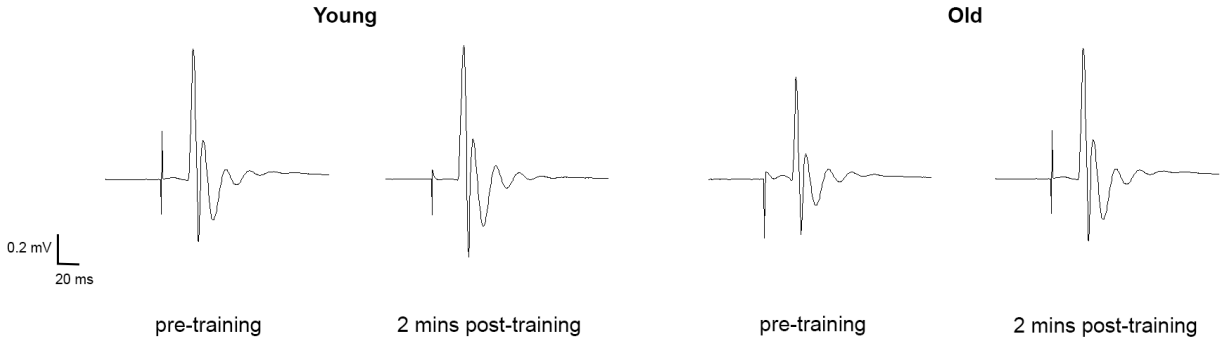

Figure 2-4 Representative MEP traces. MEPs from the right APB in two representative participants before (pre) and after (2 mins post) training. Each MEP is an average of 20 responses to TMS in the resting muscle. Training induced increases in average MEP amplitudes in both young and older adults.

The average MEP amplitude in the trained and the untrained hemispheres in young and older adults before and after training on the simple task is shown in Figure 2-5. It can be seen that MEP amplitudes increased immediately after training in both groups, with a larger increase in the older participants at the 2-min interval but a further increase at the second post-training interval in the young. ANOVA confirmed that training was associated with an increase in MEPs, as revealed by a significant main effect of time; F (2,76) = 7.66, p =.001 η_p^2 = .17. Paired samples t-tests revealed that MEP amplitude increased significantly from baseline to the 15 minute time point (t(39) = 3.59, p = .001, d = 0.56), with no difference between the two post-training measures (t(39) = 1.38, p = .177, d = 0.23). The effect size for the comparison between pre and 15 minutes post was found to meet Cohen's (1988) convention for a moderate effect (d = 0.5). Compared with baseline there was a strong trend toward an increase at the two minute post-training interval (t(39) = 2.46, p = .019; adjusted alpha level = .017, d = 0.39). The effect sizes for the change in corticospinal excitability from baseline to 2 minutes post and between 2 and 15 minutes post were found to meet the convention for a small effect (d = 0.2). There were no other significant main effects or interactions (ps > .166).

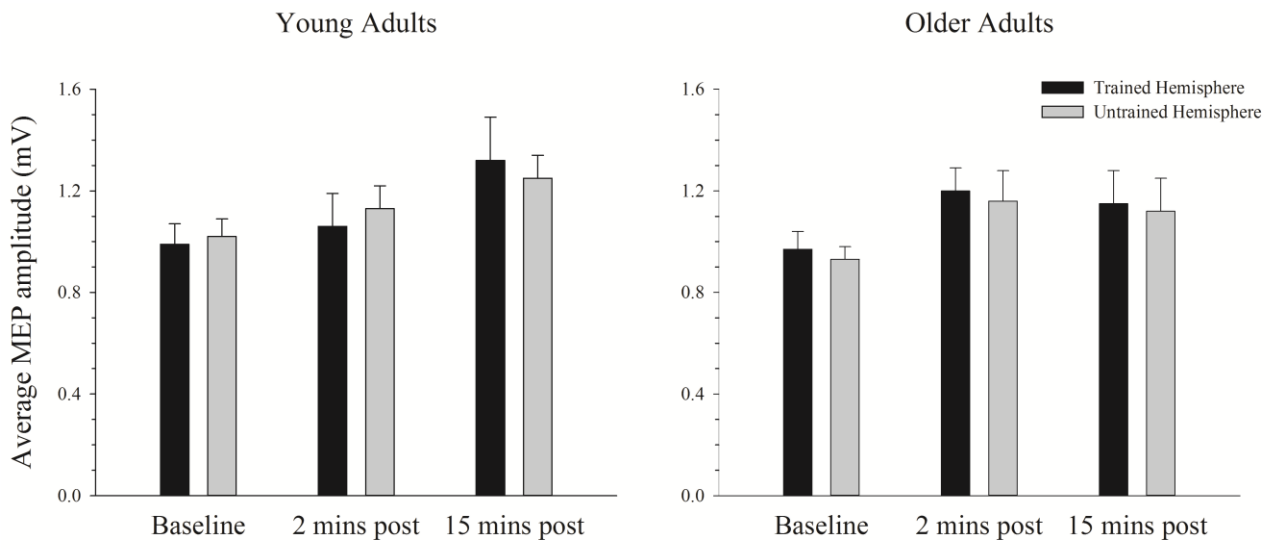

Figure 2-5 Mean MEP amplitude before and after training on a simple motor task in young and older adults. Average MEP amplitude increased from pre to post training, with the largest increase at the 15-minute time-point (p < .05). There was no difference in the MEP increase between hemispheres or the two age groups. Error bars denote SEM.

2.4.3 Behavioral performance in the complex task

Figure 2-6 depicts the average number of correct sequences performed with the trained and untrained hands of young and older adults before and after training on the complex task. As can be seen, the average number of correct sequences completed in the allotted time period increased

significantly over time in both the trained and the untrained hands. ANOVA confirmed that performance improved after training with a significant main effect of time; $F(2,76) = 102.42$, $p < .001$, $\eta_p^2 = .73$. Paired samples t-tests revealed that performance significantly increased from baseline to both the two ($t(39) = 8.70$, $p < .001$, $d = 1.37$) and 15 minute ($t(39) = 12.52$, $p < .001$, $d = 1.98$) post-training time points. A significant increase in performance was also evident from the two minute to 15 minute post-training measure; $t(39) = 5.68$, $p < .001$, $d = 0.90$. The effect sizes for these comparisons were found to exceed the convention for a large effect ($d = 0.80$). As illustrated in Figure 2-6, overall performance also varied as a function of age, whereby young adults completed significantly more correct sequences than older adults. ANOVA supported this observation with a significant main effect of age: $F(1,38) = 9.70$, $p = .003$ $\eta_p^2 = .20$. There were no other significant main effects or interactions (ps > .100).

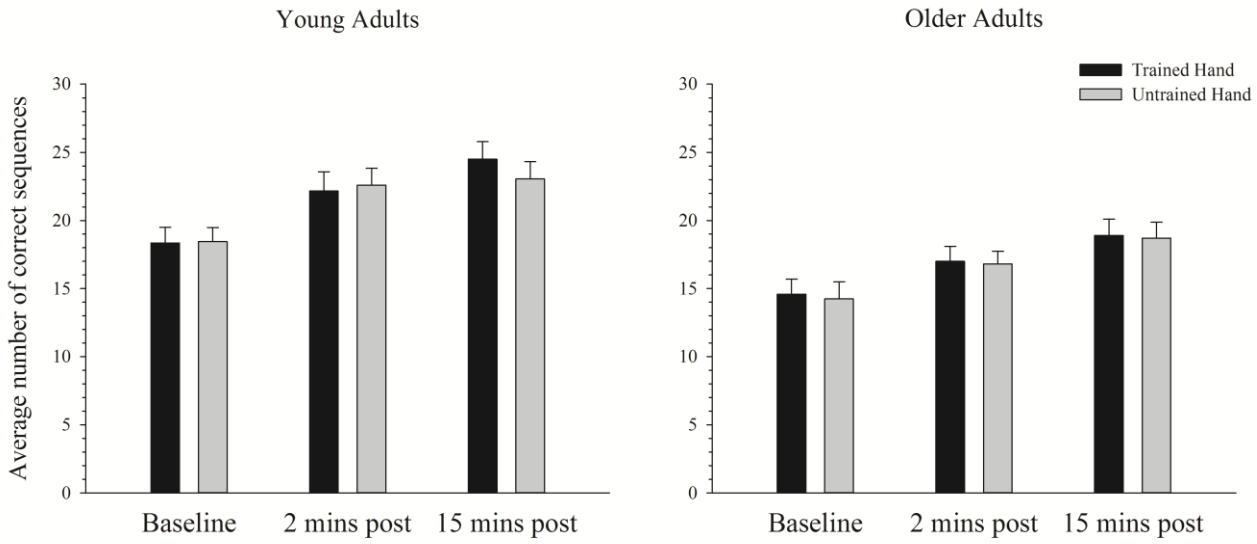

Figure 2-6 Mean correct sequences completed before and after training on a complex motor task in young and older adults. The average number of correct sequences completed increased significantly from baseline to both the 2 and 15-minute post time-point ($p < .05$). There was no difference in this training-induced effect between young and older adults or between the trained and the untrained hand ($p < .05$). Overall older adults completed fewer sequences than the younger group. Error bars denote SEM.

2.4.4 Changes in cortical excitability in the complex task

Baseline rMTs in the trained ($M = 40.40$, $SE = 2.08$) and untrained ($M = 40.30$, $SE = 1.76$) hemispheres of young adults did not differ from baseline rMTs in the trained ($M = 40.35$, $SE = 1.39$) or untrained ($M = 41.60$, $SE = 1.55$) hemispheres of older adults in the complex condition (ps > .450). The average MEP amplitude in the trained and the untrained hemispheres in young and

older adults before and after training on the complex task is shown in Figure 2-7. Overall MEP amplitude increased over time in the complex condition, with the largest increase emerging at the 15-minute post-training time point. However, ANOVA revealed only a trend toward larger MEPs post-training with a marginal main effect of time; F (2,76) = 2.92, p =.062 η_p^2 = .07. Although the greatest increase was evident at the 15 minute time point, paired sampled t-tests revealed that this increase was not significant (t(39) = 2.29, p = .028, adjusted alpha level = .017, d = 0.35). The increase evident at two minutes post-training (t(39) = .50, p = .620, d = 0.09) and between the two post-training measures (t(39) = 1.99, p = . 054, d = 0.32) was also not significant. The effect sizes for these comparisons were found to meet the convention for a small effect (d = 0.2). These effects did not differ between young and older adults or between the trained and untrained hemisphere, as indicated by the absence of any other significant main effects or interactions (ps > .644).

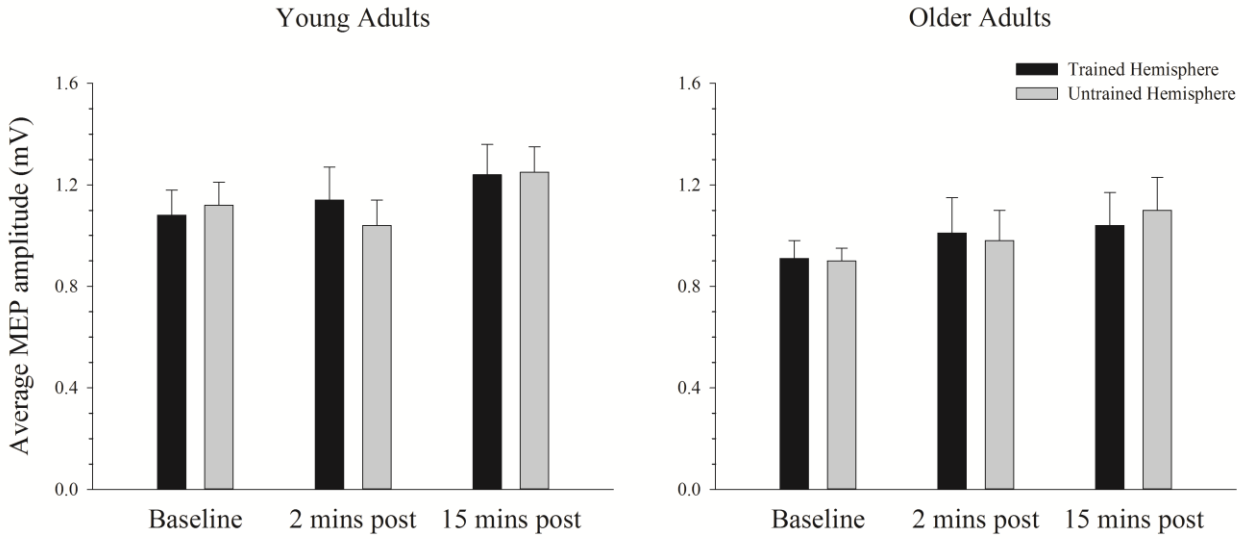

Figure 2-7 Mean MEP amplitude before and after training on a complex motor task in young and older adults. Average MEP amplitude trended toward an increase following training, with the greatest increase evident at the 15-minute post-training time point. There were no differences across hemispheres or age groups. Error bars denote SEM.

2.4.5 Relationship between corticospinal excitability in the untrained pathway to cross-limb transfer

To further explore the notion that excitability changes in the two hemispheres differentially support cross-limb transfer in young and older adults an index of transfer was created. This index represents the degree of performance gain in the untrained hand as a percentage of the performance improvement in the trained hand. Because the increase in MEP amplitudes were more reliable at the 15 min post training interval, in both the simple and complex conditions, only data for this time

point was included in the analysis. Data with Cook's distance and studentized deleted residual values above 0.2 (i.e., Cook's D >4/*n*) and 2.0, respectively, were removed from the analyses; a maximum of three data points were removed from any analysis. As can been seen in Figure 2-8, higher scores on the transfer index were associated with greater MEP change in the untrained hemisphere of young adults. However, this positive correlation was only marginally reliable: $r(16) = .428$, $p = .087$. For older adults, there was also no reliable relationship between MEP amplitude change and cross-limb transfer; $r(17)= .162$, $p = .521$. Data for the complex task are presented in Figure 2-9, which shows a positive relationship between the transfer index and MEP change in young adults, but a negative relationship in the older group. Analysis revealed, however, that there was no significant correlation between scores on the transfer index and MEP change in the untrained hemisphere in young ($r(17) = .349$, $p = .156$) or older ($r(18) = -.349$, $p = .170$) adults. Transfer index scores were also subjected to a mixed ANOVA testing the effect of age, task and time on the transfer index. The data from four participants who showed no change in the trained hand in the simple condition were removed from this analysis. Transfer to the untrained limb was somewhat greater in the complex task ($M = 129.05$, $SE = 37.11$) than in the simple task ($M = 58.197$, $SE = 14.76$), as indicated by a marginal main effect of task; $F(1,34) = 3.29$, $p = .079$ $\eta p^2 = .088$. There were no other main effects or interactions, indicating that transfer to the untrained limb did not differ between young and older adults or as a function of time (ps > .326).

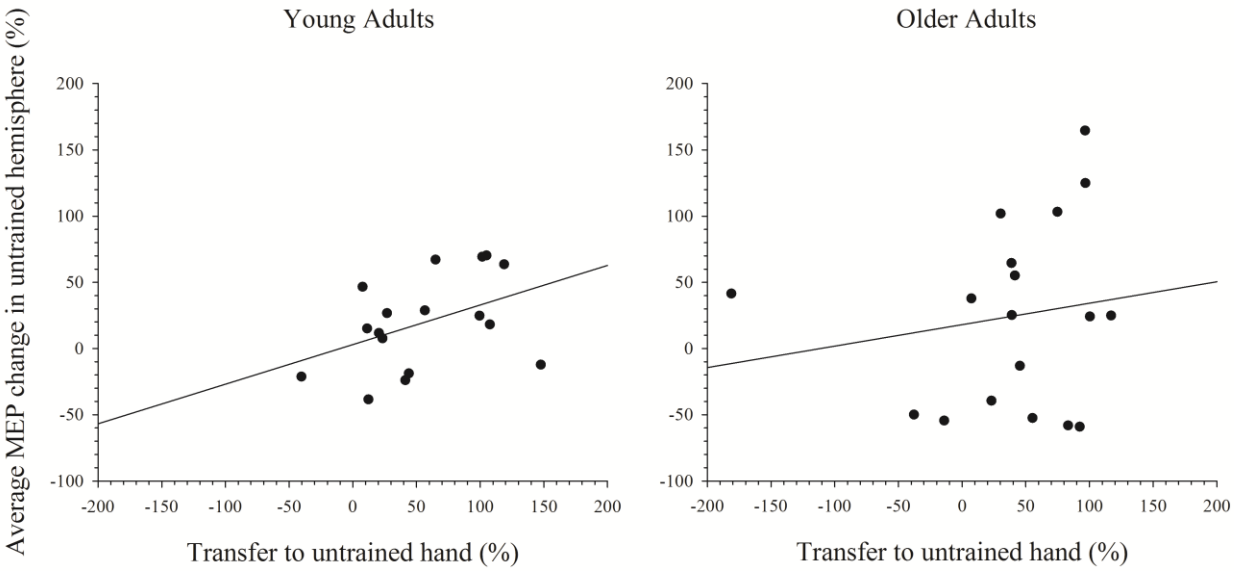

Figure 2-8 Correlation between transfer of behavioral performance gains to the untrained hand and MEP change in the untrained hemisphere after training on the simple task. Although there was a statistical trend toward an association between greater transfer and larger MEP change in the untrained hemisphere in young adults, this was not the case in older adults.

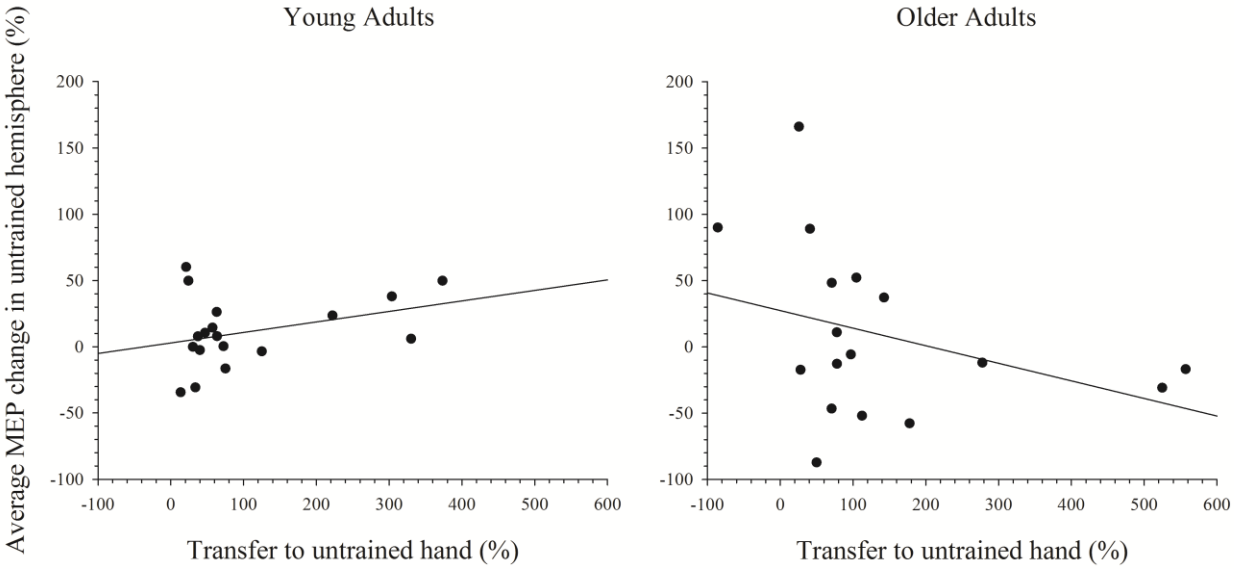

Figure 2-9 Correlation between transfer of behavioral performance gains to the untrained hand and MEP change in the untrained hemisphere after training on the complex task. Although greater transfer was non-significantly associated with greater MEP change in the untrained hemisphere in young adults, this relationship was reversed in older adults.

2.5 Discussion

The current study aimed to investigate whether cross-limb transfer and bilateral corticospinal plasticity are altered in older adults after motor training on simple and complex tasks. Both young and older adults demonstrated cross-limb transfer following training with the dominant hand on a *simple* task, but overall older adults displayed lower levels of performance gains in the trained hand. Corticospinal excitability also increased comparably across the trained and untrained hemispheres in both young and old adults following training on the simple task. After training on a *complex* task, cross-limb transfer was also evident in the young and older adults. There was also a trend towards an increase in corticospinal excitability, with the greatest increase at 15 minutes, exceeding Cohen's (1988) convention for a small effect (d = 0.20).

2.5.1 Cross-limb transfer of a simple motor skill is maintained in older adults

Previous studies have demonstrated that cross-limb transfer is reduced, or even absent in older adults (Hinder et al., 2011; Parikh & Cole, 2013), and that this effect is accompanied by bilateral increases in corticospinal excitability (Hinder et al., 2011). This observation highlights the possibility that older adults may draw upon plastic changes in more diffuse brain regions (i.e., the homologous M1) to support learning of a simple motor task with the dominant hand. In the current study, however, there was no interaction between hand, time and age, indicating that the degree of

cross-limb transfer was comparable between young and older adults. This effect is inconsistent with that found by Hinder and colleagues (2011) and by Parikh and Cole (2013), but transfer has been reported when older adults trained on a simple task with their non-dominant hand (Hinder et al., 2013). There are two key methodological differences between the current study and the previous literature that may have contributed to the discrepant findings. First, in order to maximize kinesthetic similarity between simple and complex tasks the current study focused on the APB. Previous research, however, has focused solely on the first dorsal interroseous muscle (FDI). Although it is possible that cross-limb transfer becomes increasingly muscle specific with age, the findings of Hinder and colleagues (2013), which demonstrate evidence of transfer in older adults with the FDI muscle suggest this is an unlikely explanation for the discrepancy between the findings of the current study and of Hinder and colleagues (2011). Second, in the current study the frequency and distribution of rest breaks given during training was reduced. Hinder and colleagues (2011) administered a 30 second break after every 10 trials during training, as well as a five-minute break mid-way through (after 150 trials). The current study, however, administered 30-second breaks only after every 30 trials and participants continued in this manner until all 300 movements were completed. The reduction in rest frequency may have played a role in maintaining cross-limb transfer in the older adults by allowing greater time within a block to process and respond to feedback, and to encode and refine movement kinetics for maximum acceleration. However, Hinder and colleagues (2013) implemented the same rest periods as Hinder and colleagues (2011), and found evidence of cross-limb transfer following training with the non-dominant hand in older adults. This suggests that neither alterations in the frequency of rest breaks nor hand dominance solely determine whether cross-limb transfer manifests in older adults.

In addition to the methodological difference mentioned above, variation in sample characteristics could account for the divergent findings across studies. For example, particular lifestyle characteristics, educational experience and occupational exposure can protect against age-related decline in cognition and memory. These factors can lead to differences in structural and functional neural networks or cognitive processes that allow some individuals to cope better with brain pathology, a process termed cognitive reserve (see Stern, 2009 for review). In addition, numerous factors, such as genetic polymorphisms (BDNF Val66Met; Cheeran et al., 2008), physical activity (Cirillo et al., 2009) and mirror activity (Hinder et al., 2011) have been shown to influence neuroplasticity. Importantly, there is no reason to expect systematic differences in sample characteristics between the current study and those showing an absence of transfer effects, as all employed standard eligibility requirements for TMS (e.g., no neurological or psychiatric condition, not taking neuroactive medications) and similar recruitment methods. However, such differences

cannot be ruled out. Future studies might attempt to survey a range of cognitive and lifestyle factors to determine which are associated with maintenance of transfer. Finally, it should be noted that mirror activity, which refers to the spill over of activity that can occur from an active limb to homologous muscles of a resting limb during unilateral movement, although not recorded in this experiment, does not readily explain the transfer effects. Specifically, although mirror activity is more common in older adults it has been shown that higher levels of mirror activity are associated with reduced corticospinal plasticity effects (Hinder et al., 2011), and thus does not explain the comparable bilateral corticospinal plasticity evident in young and older adults in the current study. Future studies might benefit from recording mirror activity during training in order to better understand the relationship between corticospinal plasticity and mirror activity in young and older adults.

The finding that cross-limb transfer was evident in the presence of bilateral increases in cortical excitability in the current study does not support the idea that more diffuse plasticity is needed to support learning of the trained hand in older adults. Instead, the results could be taken to suggest that cortical excitability change in each hemisphere supported learning with the contralateral hand, as per the cross-hemisphere hypothesis and as demonstrated in young adults by Lee and colleagues (2010). It is important to note, however, that MEP amplitude reflects the excitability of the corticospinal pathway, and that changes in spinal excitability were not examined in the current study. Nonetheless, evidence demonstrates that after motor training with the upper and lower limbs spinal excitability is not altered (Beck et al., 2007; Cirillo et al., 2010; Lagerquist et al., 2006; Mcdonnell & Ridding, 2006; Szecsi & Straube, 2007), suggesting that changes in MEP amplitude in the current study were driven predominately by adaptations within the brain, likely in the primary motor cortices. But it remains possible that adaptations in the spinal cord in older adults may have compensated for reductions in cortical adaptations. However, evidence suggests that neuroplasticity at the level of the spinal cord is reduced in older adults (see Papegaaij et al., 2014 for review), which argues against this possibility. This study was the first to measure training-induced changes in corticospinal excitability and behavioural performance bilaterally in young and older adults at multiple time points post training. An important question for future research is whether age-related differences manifest to a greater degree after 15 mins following training, as might be expected based on some previous research showing delayed neuroplasticity effects in the aged brain following anodal transcranial direct current stimulation in humans (Fujiyama et al., 2014) and delayed development of LTP in aged non-human animal models (Barnes, 1979; Barnes & McNaughton, 1985).

Another important consideration for future studies is whether age-related changes in neuroplasticity can be revealed using alternative measures of cortical excitability, such as paired-pulse stimulation, which provide more detailed information on changes in excitatory versus inhibitory pathways. Therefore future studies might consider restricting MEP and behavioural measurement to a single time point to allow for the inclusion of paired-pulse intracortical stimulation. As corticospinal plasticity was evident at 2 and 15 mins post in older adults, these results suggest corticospinal plasticity was not delayed in older adults, thus restricting post measures to 15 minutes might be appropriate. This would allow for the inclusion of paired-pulse intracortical stimulation, which would aid in distinguishing pure cortical effects from changes at the spinal level (see Ziemann & Rothwell, 2000 for review). It would also enable future studies to gain a more comprehensive understanding of training induced changes in the cortical microcircuitry. To improve the comprehensiveness of this investigation, future studies might also consider incorporating a post measure of rMT as an additional measure of changes in corticospinal excitability. Post rMT was not recorded in the current study due to insufficient time between the 2 and 15 minute post-training measures. As a consequence we cannot rule out the possibility that changes in membrane potentials, which are indicated by changes in rMT, added to the corticospinal plasticity effects. However, previous studies demonstrate that changes in rMT are unlikely to underpin corticospinal plasticity effects as rMT is found to remain constant following motor training (Cirillo et al., 2011; Perez et al., 2004; Tyč & Boyadjian, 2011). In addition, the added time gained from removing multiple post measures could be used to include other measures of changes in inhibition, such as silent period duration (Fuhr et al., 1991).

In the current study, to investigate further the relationship between corticospinal plasticity in the untrained hemisphere and transfer to the untrained hand correlations analyses were undertaken. The results of that analysis revealed that the relationship between greater excitability change in the untrained hemisphere and greater transfer of performance gains to the untrained hand was stronger in young, relative to older, adults. This finding not only lends support to the cross-hemisphere hypothesis of cross-limb transfer but also lends support to the argument that the role of activity (plasticity) in the untrained hemisphere in supporting learning for the trained and untrained hands differs between young and older adults. Future studies would benefit from probing the casual relationship between activity in the untrained hemisphere and transfer to the untrained hand using repetitive TMS (rTMS). Specifically, studies similar to that of Lee and colleagues (2010), in which rTMS was used to interfere with activity in M1 immediately following a training task, would assist in identifying the differential role of activity within the untrained M1 for supporting learning with the trained and untrained hands in older adults.

2.5.2 Performance gains after training on a simple motor task are reduced in older adults

Following training on the simple task, performance gains overall were lower in older relative to younger adults. This can be seen by the effect sizes, which in young adults were found to exceed Cohen's (1988) convention for a large effect (d = 0.80) but in older adults were more moderate ($0.50 < d < .08$). At baseline, however, performance on the simple task was no different between young and older adults, suggesting that the reduction in performance gains in older adults was not due to an overall (basal) decrease in motor functioning. It is unlikely that this effect can be explained by muscle fatigue or a lack of motivation as participants were given frequent breaks during training and encouragement and feedback throughout the session. Instead, the reduction in training-related performance gains might be due to a capacity limit within the peripheral musculature, downstream of the motor cortex. Comparable cortical excitability changes between young and older adults following training supports this view. Although maximal M-waves, which indicate the maximum compound action potential of a group of muscles were not obtained in this study, there is evidence to suggest an overall decline in the musculature in older adults (Rice & Cunningham, 2002, Klass et al., 2008); remodelling of muscle fibres (Larsson, 2003), reduced motor neuron numbers (Doherty et al., 1993) and/or reduced motor unit numbers and activity (Doherty et al., 1993; Gordon et al., 2004; Klein et al., 2001) are just some age-related changes in musculature that might have contributed to reduced performance gains in older adults. Thus, it is possible that age-related differences in corticospinal plasticity may have been interpreted differently if MEP data were normalized to Mmax. Therefore, due to the information maximal M-waves can provide regarding the capacity of peripheral musculature, future studies would benefit from incorporating this measure. Importantly, although older adults showed an overall smaller increase in performance following training, there was no difference between the groups in the degree of cross-limb transfer.

2.5.3 Cross-limb transfer of complex motor skills is maintained in older adults

The current study demonstrates that although overall performance was lower in older relative to younger adults, cross-limb transfer of a complex task was maintained in older adults. This result is consistent with the findings of Parikh and Cole (2013) who reported significant transfer in young and older adults after training on a grip and lift task with the dominant hand. Corticospinal excitability, however, did not change significantly in either pathway of young or older adults, in the current study, but there was a trend towards increased excitability post training. Training on a complex task has been shown to increase excitability in the pathway innervating the trained hand in young and older adults (Cirillo et al., 2011). However, there is a wealth of evidence suggesting that

regions outside M1, such as pre-motor and supplementary motor regions are predominantly involved in performance and learning of complex sequential motor tasks (Davare et al., 2010; Ehrsson et al., 2000, 2001; Holmstrom et al., 2011; Mima et al., 1999; Sadato et al., 1996; Solodkin et al., 2001; Verstynen et al., 2005). The trending increase in corticospinal plasticity in bilateral corticospinal pathways in young adults, at least as assessed with MEPs, is consistent with this evidence and further suggests that learning-related neuroplasticity induced by training on complex sequential tasks occurs outside the primary motor cortices.

Due to age-related over-activity in diffuse brain regions, which was hypothesized to support learning of the motoric components of the task in the trained hand, older adults were predicted to show a greater increase in excitability in the untrained corticospinal pathway after training on a complex task. The data from the current study demonstrated a trend towards increased corticospinal excitability in young and older adults after training on the complex task, but the effect was not significant, which consequently prevented the examination of age-related effects. The presence of cross-limb transfer in older participants following training of the simple task argues against over-activity in these individuals. Although these factors make it difficult to investigate the role of the untrained hemisphere in supporting learning with the trained hand in the current study, correlations between MEP change in the untrained hemisphere and transfer of behavioral gains to the untrained hand after training on the complex task were assessed. The results reveal a negative relationship in older adults whereby greater change in MEP amplitude in the untrained hemisphere was weakly associated with a decrease in transfer to the untrained hand. In young adults, however, a positive relationship was evident. This suggests that the increase in corticospinal excitability in the untrained pathway may reflect the increased role of the untrained hemisphere in supporting learning with the trained hand in older adults. At the very least, this result highlights the possibility that different mechanisms might mediate cross-limb transfer in young and older adults.

Future studies would benefit from using a combination of imaging techniques and TMS to probe bilateral training-related plasticity, to identify the networks contributing to learning and transfer of complex motor skills and the extent to which those networks are altered in the aged brain. As discussed previously, an important node in the motor network that has been shown to be involved in learning complex tasks is the premotor cortex (Mima et al., 1999). In addition to identifying the degree of involvement of this region in young and older adults during the performance of complex tasks with imaging techniques, excitatory or inhibitory repetitive TMS to this region could help to further establish the causative contribution of premotor regions in learning complex tasks in young and older participants. Further, future studies might also use twin coil TMS to probe changes in

intracortical inhibition (Wasserman et al., 1996) between premotor and primary motor cortex following a training intervention to investigate the connections between these regions and how these might be altered by advancing age.

2.5.4 Conclusions

The current study provides evidence to suggest that cross-limb transfer of both simple and complex tasks is maintained in older adults. The findings of the current study also suggest, however, that the extent to which cortical excitability of the untrained hemisphere supports transfer of performance gains to the untrained limb may differ in young and older adults. Future studies that interfere with activity in the motor cortices immediately following training and measure the impact of such interference on behavioral performance would help to establish the causal role of the untrained hemisphere in supporting cross-limb transfer in older adults. Nonetheless, the current study carries practical implications for rehabilitation practices involving cross-limb transfer effects. For example, it is possible that individuals experiencing limb deficits (e.g., following stroke) may benefit from training with the intact hand. However, it is important to remember that in pathological conditions, such as stroke, cortical activity can be significantly altered (Nowak et al., 2009), which could interact with the training-related changes we describe here. Finally, based on the current results it would be predicted that techniques implementing such training tasks are likely to benefit both young and older adults similarly.

2.6 Acknowledgments

This work was supported by a Project Grant from the National Health and Medical Research Council of Australia (NHMRC; APP1028210). DD was supported by an Australian Postgraduate Award scholarship. MS was supported by an NHMRC Training Fellowship (APP1012153). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding bodies.

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Chapter 3 Plasticity induced by intermittent theta burst stimulation (iTBS) in bilateral motor cortices is not altered in older adults

In the previous chapter it was found that both young and older adults demonstrated cross-limb transfer with simple and complex motor tasks. Corticospinal excitability was found to increase bilaterally in young and older adults after training on the simple task but did not change reliably in either age group after training on the complex task. This chapter uses an alternative method to induce neuroplasticity, one that does not require participants to perform a task. The aim of the second experiment of this thesis was to determine the extent to which neuroplasticity induced by TMS, specifically iTBS manifests across a more bilateral network in older adults.

This chapter is based on a paper published during candidature, which is cited below. Deviations from the published paper include the addition of summary and inferential statistics for post iTBS rMTs. Effect sizes have been incorporated and interpreted where appropriate. Section and Figure numbers have been modified for consistency with the rest of the thsesis.

Dickins, D. S. E., Sale, M. V., & Kamke, M. R. (2015). Plasticity induced by intermittent theat burst stimulation in bilateral motor coticies is not altered in older adults. *Neural Plasticity* 2015. doi: 10.1155/2015/323409.

3.1 Abstract

Numerous studies have reported that corticospinal plasticity induced in motor regions by transcranial magnetic stimulation (TMS) is attenuated in older adults. Those investigations, however, have focused solely on the stimulated corticospinal pathway. Compared with young, older adults exhibit more widespread activity across bilateral motor cortices during the performance of unilateral motor tasks, suggesting that the manifestation of corticospinal plasticity might also be altered. To address this question twenty young (<35 years old) and older adults (>65 years) underwent intermittent theta burst stimulation (iTBS) whilst attending to the hand targeted by the neuroplasticity-inducing procedure. The amplitude of motor-evoked potentials (MEPs) elicited by single pulse TMS was used to quantify corticospinal excitability before and after iTBS. Individual responses to iTBS were highly variable, with half the participants showing an unexpected decrease in corticospinal excitability. Contrary to predictions, however, there were no age-related differences in the magnitude or manifestation of corticospinal plasticity in bilateral pathways. The findings suggest that advancing age does not influence the capacity for, or manifestation of, corticospinal plasticity induced by iTBS.

3.2 Introduction

Neuroplasticity is the ability of the brain to undergo enduring morphological and functional change in response to the demands of its environment (Boroojerdi et al., 2001). In humans, transcranial magnetic stimulation (TMS) can be used to induce short term changes in corticospinal excitability (corticospinal plasticity) that are believed to be underpinned by NMDA-dependent synaptic plasticity (Hoogendam et al., 2010). Studies using TMS have reported reduced corticospinal plasticity in the older adult motor system (Fathi et al., 2010; Müller-Dahlhaus et al., 2008; Todd et al., 2010), but measures of corticospinal plasticity were limited to the representative corticospinal pathway innervating the muscle targeted by the intervention. The current study aimed to investigate whether corticospinal plasticity induced by TMS manifests more diffusely in older compared with younger adults.

In young adults TMS-induced corticospinal plasticity has been demonstrated to manifest not only in the stimulated hemisphere, but also in the opposite unstimulated pathway (Gilio et al., 2003; Gorsler et al., 2003; Heide et al., 2006; Schambra et al., 2003; Suppa et al., 2008; Stefan et al., 2008; Shin & Sohn, 2011). For example, Shin and Sohn (2011) used excitatory paired associative stimulation (PAS 25), which involved repeatedly pairing single pulse TMS to the cortical representation of the thumb with peripheral stimulation to the median nerve, to induce

neuroplasticity. The effects were probed using motor evoked potentials (MEPs), which are elicited by single-pulse TMS and provide an indirect measure of corticospinal excitability, with changes in amplitude believed to reflect synaptic plasticity (see Pascual-Leone et al., 1998 for review). Following PAS 25, which was applied only to the left cortex, MEP amplitudes were enhanced in both the muscle targeted by the procedure and in the homologous muscle of the other hand. PAS has also been used to investigate corticospinal plasticity in older adults, with results predominately showing a reduction in corticospinal plasticity (Fathi et al., 2010; Müller-Dahlhaus et al., 2008). Similar effects have been found with corticospinal plasticity induced by repetitive TMS (Todd et al., 2010), and both these observations are consistent with demonstrations of reduced corticospinal plasticity following motor training (Bickford, 1993; Churchill et al., 2003; Parikh & Cole, 2013; Sawaki et al., 2003; Curran, 1997; Howard & Howard, 2001; Feeney et al., 2002; Howard et al., 2004, 2008; Bennett et al., 2007; McNay & Willingham, 1998; Fernandez-Ruiz et al., 2000; Buch et al., 2003; Bock, 2005; Bock & Girgenrath, 2006; Heuer & Hegele, 2008; Hegele & Heuer, 2010; Anguera et al., 2011; see Voelcker-Rehage, 2008 for review), as well as with evidence of attenuated or altered neuroplasticity in aged non-human animals (Barnes, 1979; Barnes & McNaughton, 1985; Tennant et al., 2012; see Barnes, 2003 for review). Fewer studies have reported comparable levels of corticospinal plasticity and behavioural performance gains between older and younger adults (Dickins et al., 2015; Pellicciari et al., 2009; Anshel, 1978; Spirduso 1993; Fraser et al., 2009). Thus, the weight of evidence appears to suggest that neuroplasticity is likely to be altered in the aged brain. Importantly, older adults exhibit greater and more diffuse neural activity, both between and within hemispheres, when performing similar tasks to their younger counterparts; an effect attributed to age-related neurobiological change (Calautti et al., 2001; Carp et al., 2011; Heuninckx et al., 2005; Heununckx et al., 2008; Hutchinson, 2002; Inuggi et al., 2011; Naccarato et al., 2006; Mattay et al., 2002; Ward & Frackowiak, 2003). Therefore it is possible that the manifestation of corticospinal plasticity across the hemispheres is altered in older adults.

To date, only one study has investigated age-related differences in the manifestation of corticospinal plasticity induced by TMS across the stimulated and unstimulated corticospinal pathways. Di Lazzaro and colleagues (2008) administered intermittent theta burst stimulation (iTBS), which delivers high-frequency bursts of TMS at the natural theta rhythm of the hippocampus to induce long-term potentiation (LTP)-like corticospinal plasticity (Huang et al., 2005). They found preliminary evidence indicating that corticospinal plasticity induced by iTBS in the non-dominant stimulated corticospinal pathway also manifests in the contralateral unstimulated corticospinal pathway of both young and older adults. Given evidence of age-related differences in the spread of neural activity, this result suggests that any assessment of corticospinal plasticity in

older adults should consider changes in bilateral corticospinal pathways. Critically, only six older adults were tested in that study and there was no control for the potential modulation of iTBS-induced corticospinal plasticity by attention. The failure to control attention is an important limitation as older adults have been shown to experience decline in a range of cognitive abilities (Harada et al., 2013), including attention (Mahoney et al., 2010), and we have shown previously that attention alters corticospinal plasticity induced by PAS and iTBS (Kamke et al., 2012; 2014).

The current study aimed to investigate the extent to which corticospinal plasticity manifests bilaterally in young and older adults following iTBS to the dominant (left) hemisphere. It was predicted that corticospinal plasticity would be reduced in the stimulated pathway of older, relative to young, adults. It was further predicted that this reduction in older adults would be accompanied by greater corticospinal plasticity in the unstimulated pathway, relative to young adults.

3.3 Methods

3.3.1 Participants

Twenty participants aged 18-28 years (M = 22.95, SD = 2.52, Males = 10) and 20 older participants aged 65-76 (M = 70.15, SD = 3.07, Males = 10) completed the study. Two additional participants in the older group failed to complete sessions due to discomfort during the iTBS protocol. According to the Edinburgh handedness inventory (Oldfield, 1971) all participants were right-handed except one young and one older adult who were ambidextrous (Young M = 77.47, SD = 19.47, Range = 33.33-100; Older M = 84.31, SD = 17.17, Range = 33.33-100). Volunteers were recruited through advertising in online newsletters and by word of mouth and were reimbursed $10 per hour for their participation. A TMS safety-screening questionnaire (Rossi et al., 2009; Rossi et al., 2011) was used to exclude volunteers with known neurological disease or damage, epilepsy, history of head injury or psychiatric disorder, or those taking neuroactive medications. All participants had normal or corrected-to-normal visual acuity. Participants provided informed consent and all procedures were approved by The University of Queensland Medical Research Ethics Committee in accordance with the Declaration of Helsinki.

3.3.2 Transcranial Magnetic Stimulation (TMS) and Electromyography (EMG)

Changes in corticospinal excitability induced by iTBS were quantified using single pulse TMS. A figure-of-eight shaped coil with a wing diameter of 70 mm, connected to a monophasic Magstim 200^2 stimulator was used to administer monophasic TMS. The coil was angled 45 degrees from the midline with the handle pointing towards the back of the head. The coil was placed tangentially on

the scalp and moved systematically in a grid-like pattern until the motor hotspot was located. The motor hotspot was defined as the optimal position on the scalp for evoking the largest and most consistent MEP (peak-to-peak amplitude) in the target muscle, the abductor pollicis brevis (APB) of the left and right hands. Stimulation was applied at an intensity sufficient to evoke a clear motor response in the targeted muscle and occurred approximately every five seconds. The position of the coil for each hotspot was recorded using a frameless infrared stereotaxic neuronavigation system (Visor 1, ANT, Netherlands), which was used to reproduce coil angle and location within an experimental session.

Following determination of the hotspot, resting motor threshold (rMT) was obtained for the cortical representation controlling the left and right APB. The rMT was defined as the minimum TMS intensity (reported as a percentage of maximum stimulator output , % MSO), that evoked an MEP of at least 50 μV in at least 3 out of 5 consecutive trials. The intensity of the TMS was adjusted using a staircase (two-down, one-up) procedure until the criterion was met. Test TMS intensities for the left and right APBs were then established, defined as that required to evoke an average MEP of approximately 1 mV (peak-to-peak) in the resting muscles. On average this intensity equated to 126% of rMT for the right (target) APB and 125% for the left (non-target) APB (for details see Table 3-1). To determine average MEP amplitude at baseline and post iTBS, TMS pulses were delivered at the test intensity every 5 ± 1 seconds for a total of 21 pulses.

Surface electromyography (EMG) was employed to record activity from the APB muscles using disposable 24 mm silver-silver chloride (Ag/AgCl) electrodes in a belly-tendon montage. MEP data were amplified (×1000), filtered (20-1000 Hz) and sampled at 2000 Hz using a NeuroLog system (Digitimer, UK). Data were stored for off-line analysis using Signal software (CED, UK).

3.3.3 Intermittent theta-burst stimulation (iTBS)

Corticospinal plasticity was induced using the standard iTBS protocol, administered with a biphasic Magstim Super Rapid^2 stimulator. A burst of three high frequency TMS pulses (50 Hz) was repeated at a frequency of 5 Hz for two seconds (10 bursts total). This train was followed by an eight second rest period (Huang et al., 2005). Stimulation continued in this format until a total of 600 pulses were administered. iTBS was delivered to the left hemisphere at 70% of rMT. This intensity was based on the rMT measured with the Magstim Super Rapid^2 stimulator immediately prior to the iTBS intervention.

3.3.4 Attention Task

In order to control for any modulatory effects of attention on corticospinal plasticity (Kamke et al., 2012; 2014), participants overtly attended to a light-emitting diode (LED) attached just above the interphalangeal joint of the right thumb during the iTBS intervention. Participants were tasked with detecting the number of brief interruptions ("OFF" periods) to the continuously lit LED. Specifically, as presented in Figure 3-1, each trial began with the presentation of a high pitch tone, after which participants attended to the LED and silently counted the number of "OFF" periods. The presentation of a low pitch tone after iTBS signalled the end of the trial, which lasted 5.5 seconds, and prompted participants to make a verbal response as to the number of "OFF" periods detected. There could be 0, 1, or 2 "OFF" periods in a trial and these occurred between 2 and 5.5 seconds (see Figure 3-1). The number of "OFF" periods presented in a single trial was randomised and the time at which each "OFF" period occurred was jittered. When two targets were present in a trial, the second target always appeared after the iTBS burst, which occurred between 2 and 4 seconds. The task consisted of 20 trials in total.

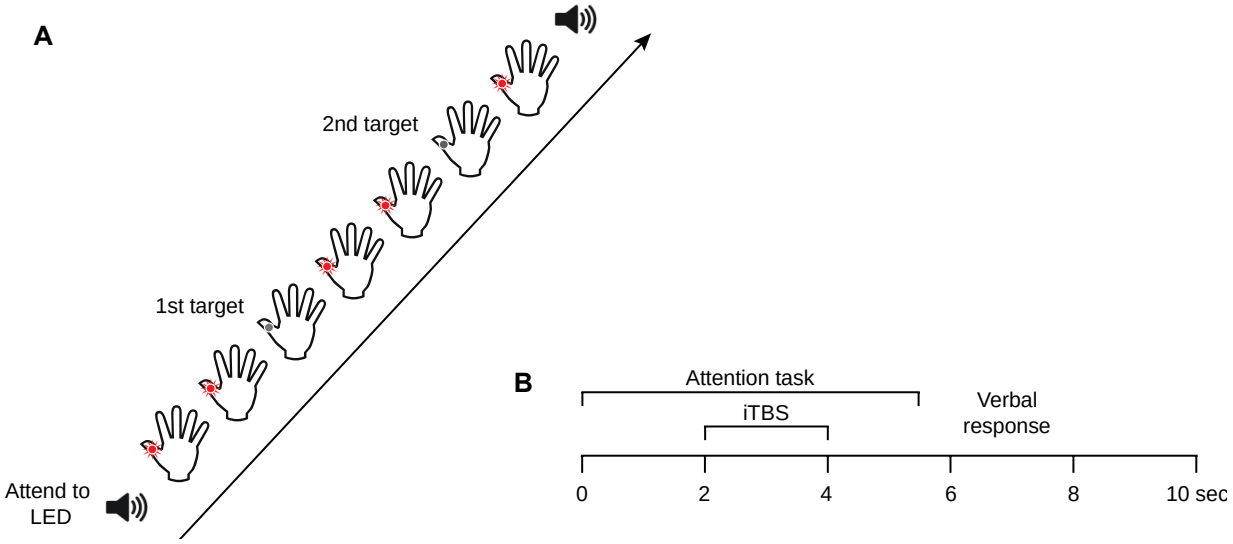

Figure 3-1 Attention task and trial timeline. A. Attention task. Participants were instructed to attend to a continuously lit LED attached to the right thumb and to count the number of interruptions ("OFF" periods). A tone indicated the start and end of each trial. B. Trial timeline. Each trial lasted a total of 10 seconds, with iTBS occurring during the attention task**.**

3.3.5 Experimental Design and Procedure

Participants were seated comfortably with both arms and hands resting on cushioned platforms on a desk. As shown in the time course for the experiment in Figure 3-2, single pulse TMS was applied to the left and right M1 to locate the motor hotspot and to quantify cortical excitability before the

iTBS intervention. Following this, the participant was provided with a brief practice of the attention task and the iTBS intervention was then administered concurrently with the spatial attention task. Cortical excitability was measured with single pulse TMS 5, 15 and 25 minutes following iTBS. At each time point MEPs were first obtained from the left (stimulated) M1 followed by the right (unstimulated) M1 using the test TMS intensity (see Figure 3-2). Participants were monitored throughout the experiment to ensure their eyes remained open and their hands and arms remained relaxed at all times.

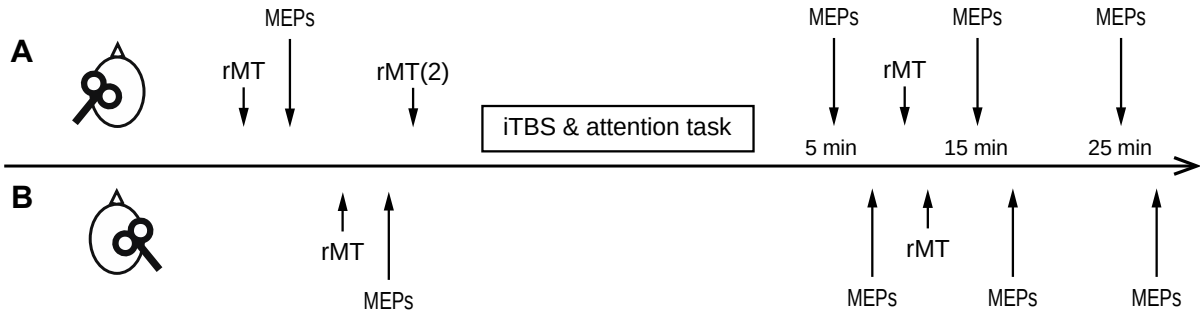

Figure 3-2 Experimental timeline. The timeline of measures is shown for the left (stimulated) hemisphere in the upper panel (A) and the right (unstimulated) hemisphere in the lower panel (B). Cortical excitability was probed before and after intermittent theta burst stimulation (iTBS) using resting motor threshold (rMT) and motor evoked potential (MEP) amplitude. Transcranial magnetic stimulation (TMS) targeted the representation in motor cortex of the right and left abductor pollicis brevis muscle. iTBS targeted the left (i.e., 'stimulated') hemisphere only. The rMT2 measure was used to establish stimulation intensity for iTBS (see section 3.3.3: intermittent theta burst stimulation).

3.3.6 Data Processing and Analyses

Performance on the spatial attention task was quantified by the number of correct trials and was compared between young and older adults using an independent samples t-test. For the corticospinal plasticity effects, the first trial from each block of MEP data (21 MEPs per block) was removed. Trials with voluntary muscle activity clearly above background activity (cut off estimate ~0.03-0.04 mV) evident in the 100 ms prior to TMS (totalling 2.3% of remaining trials) were also removed and the remaining trials were averaged. rMTs were subjected to a 2 × 2 × 2 mixed ANOVA with the repeated measures factors of time (pre, post), and hemisphere (stimulated, unstimulated), and the between subjects factor age (young, old). MEPs and test stimulus intensities were separately subjected to a 2 × 2 mixed ANOVA with the repeated measures factor of hemisphere (stimulated, unstimulated) and the between subjects factor age (young, older). Post iTBS MEP amplitudes were normalized to baseline and assessed using a mixed ANOVA with the

repeated measures factors of time (5, 15, 25 minutes post) and hemisphere (stimulated, unstimulated) and the between subjects factor of age (young, older).

Exploratory analyses were conducted separating participants into two groups; participants demonstrating increases in average MEP amplitude (average MEP change > 0%) in the right APB post iTBS (LTP-like responders) and participants demonstrating decreases in average MEP amplitude (average MEP change < 0%) in the APB post iTBS, similar characteristically to long-term depression (LTD-like responders). The factor response type (LTP-like, LTD-like) was then added into the original ANOVA. In addition to the criterion on which LTP was defined as a change > 0% and LTD < 0%, a stricter criterion of +/- 20% was also used. This larger change is more likely to exclude individuals showing normal variability in MEP amplitude, which may not be indicative of neuroplasticity (Hinder et al., 2014). Importantly, the results of the exploratory analysis did not differ as a function of the criterion used, so only data from the > 0% and < 0% split are presented. The results of the 20% criterion responder analyses are presented in Appendix C. Bonferroni corrections were applied to all follow-up, two-tailed tests.

3.4 Results

3.4.1 Performance on the spatial attention task

Both groups performed very highly on the attention task, with an average of 19.40 (±0.94; M ±SD) out of 20 correct responses for the young and 19.80 (±0.41) for the older adults. Accuracy did not differ significantly between the two age groups ($t(38) = 1.74$, $p = .093$, $d = 0.56$). This result suggests that any age differences in corticospinal plasticity following iTBS are unlikely to be due to systematic variation in the allocation of spatial attention.

3.4.2 Baseline cortical excitability

Baseline MEPs and test stimulus intensities (reported as %MSO) for the stimulated and unstimulated hemispheres in young and older adults are shown in Table 3-1. Average raw MEP amplitudes did not differ significantly at baseline (all main effects and interactions: $p > 0.147$). Although average test stimulus intensity was slightly lower in the young compared with older adults, which was confirmed by a main effect of age ($F(1,38) = 6.05$, $p = .019$, $\eta_p^2 = .137$), test stimulus intensities did not differ significantly between the hemispheres, as indicated by the absence of any additional main effects or interactions (*p*s >.732).

Table 3-1 Mean and standard error of the means for baseline cortical excitability and post-iTBS rMTs.

	Young		Older	
	Stimulated	Unstimulated	Stimulated	Unstimulated
Baseline rMT (% machine output)	38.50 (1.42)	38.40 (1.51)	40.95 (1.49)	41.95 (1.13)
Post rMT (% machine output)	38.60 (1.31)	38.50 (1.52)	41.00 (1.40)	42.00 (1.12)
Baseline MEP amplitude (mV)	1.12 (0.08)	1.03 (0.06)	1.02 (0.08)	0.93 (0.05)
Test intensity (% machine output)	**46.80** (1.78)	**47.25** (2.05)	**52.65** (1.77)	**52.90** (1.59)

Note: Test intensities (highlighted in bold) were significantly lower for young compared with older adults (p =.019). No other significant main effects or interactions were evident (ps > .125).

3.4.3 iTBS induced corticospinal plasticity in bilateral motor pathways of young and older adults

As can also be seen in Table 3-1 there was little difference in rMTs pre and post iTBS in young and older adults in the stimulated and unstimulated hemispheres. ANOVA failed to reveal any significant main effects or interactions related to rMTs (ps > .107). For MEPs, post-iTBS amplitudes varied substantially across participants, with many individuals showing and increase and numerous showing a decrease in mean amplitude. Representative MEP traces for one young and

one older participant showing such LTP-like and LTD-like responses are depicted in Figure 3-3.

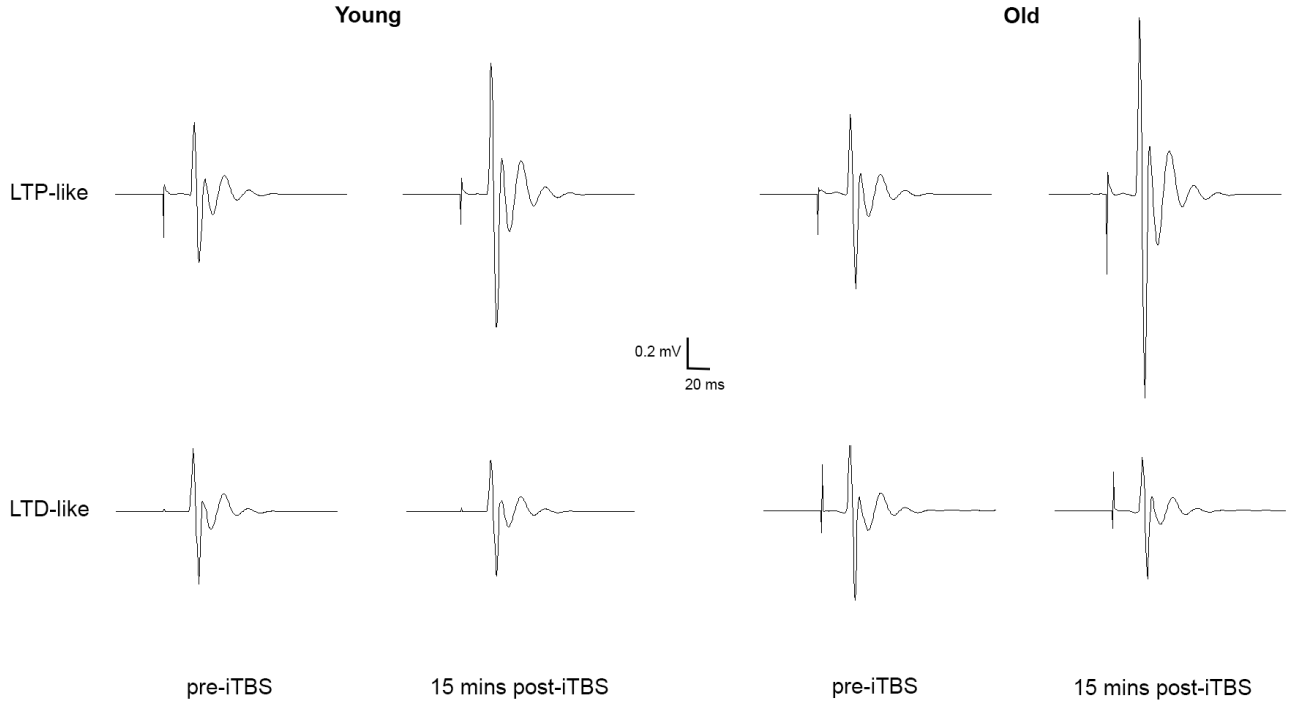

Figure 3-3 Representative MEP traces. MEPs from the right APB in four representative participants before (pre-iTBS) and after (15 mins post-iTBS) iTBS. Each MEP is an average of 20 responses to TMS in the resting muscle. iTBS induced LTP-like and LTD-like effects in both young and older adults.

The overall change in MEP amplitude at each of the three time points following iTBS (relative to each participant's baseline) is depicted in Figure 3-4. MEPs decreased immediately following iTBS in both groups, but increased at the 15-minute time point. This change in post-iTBS measures across the time points was reliable, as confirmed by a significant main effect of time; $F(2,76) = 5.80$, $p = .009$, $\eta_p^2 = .132$. The effect of time, however, did not vary significantly as a function of age or hemisphere (ps > .505). Follow-up comparisons revealed a significant difference between MEP change at 5 and 15 mins post iTBS ($t(39) = 2.78$, $p = .008$, $d = 0.44$), a marginally significant difference between 5 and 25 mins ($t(39) = 2.48$, $p = .018$, $d = 0.39$) and no significant difference between 15 and 25 mins post ($t(39) = 1.52$, $p = .136$; adjusted alpha = .017, $d = 0.24$). The effect sizes for these analyses were found to exceed Cohen's (1988) convention for a small effect ($d = 0.20$). Although the preceding analysis revealed a difference in MEP change across the post-iTBS time-points, it did not test whether any of the changes were significantly different from zero (i.e., baseline). One-sample t-tests comparing the change in MEP amplitude to zero revealed that the average change in amplitude was not significantly different from baseline at any of the post-iTBS time-points (ps > .117).

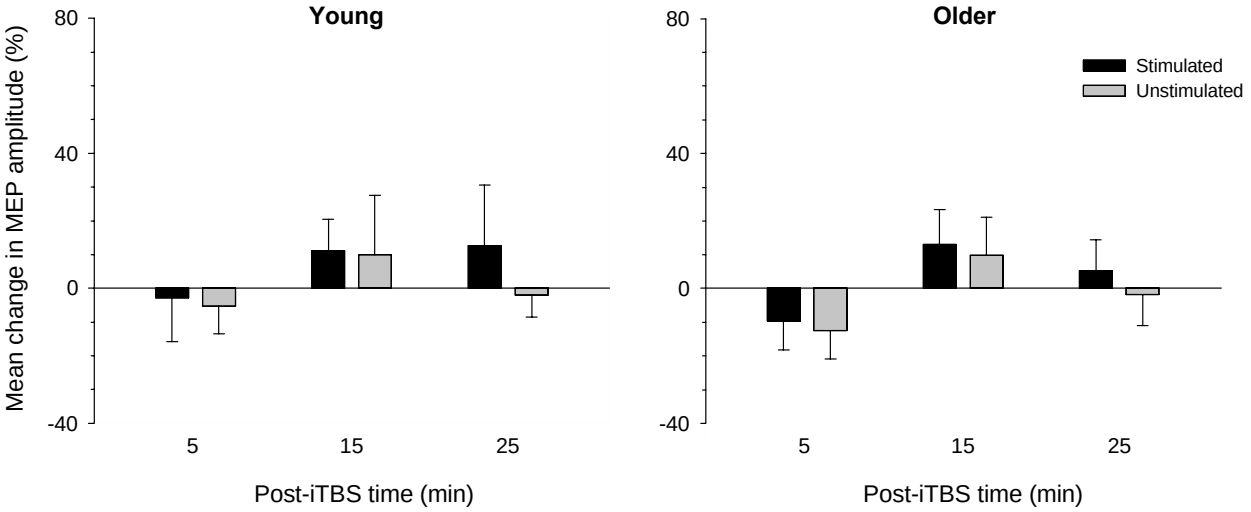

Figure 3-4 Average normalised MEP change following iTBS. Average MEP change relative to each individual's baseline varied significantly across post-iTBS measures, with the largest difference evident between the 5 and 15 minute time-points. This pattern was comparable between young and older adults, and between the hemisphere receiving iTBS (stimulated) and the hemisphere not receiving iTBS (unstimulated). Error bars denote SEM.

3.4.4 iTBS induced corticospinal plasticity in bilateral motor pathways of LTP- and LTD-like responders

As in numerous previous reports, the current study found substantial variability in individual responses to iTBS. Averaged across the time points post iTBS, 19 individuals showed an increase in MEPs (i.e., post-iTBS MEPs > 0%, Young = 9) and were classified as LTP-like responders. Twenty-one individuals showed a decrease in MEPs (MEP < 0%, Young = 11) and were classified as LTD-like responders. Data pertaining to these groups is depicted in Figure 3-5. As expected, the direction of MEP change varied as a function of response type, with LTP-like responders showing an increase in MEPs (Figure 3-5A) and LTD-like responders showing a decrease (Figure 3-5B). The difference between LTP- and LTD-like responders was confirmed by a significant main effect of response type; $F(1,36) = 22.38$, $p < .001$, $\eta_p^2 = .383$. This effect, however, was much larger in the stimulated hemisphere of both groups (see Figure 3-5), as indicated by a significant interaction between hemisphere and response type: $F(1,36) = 28.09$, $p < .001$ $\eta_p^2 = .438$. Follow-up paired comparisons demonstrated that MEP change differed significantly between the stimulated and unstimulated hemisphere in both the LTP-like ($t(18) = 3.67$, $p = .002$, $d = 0.84$) and LTD-like responders ($t(20) = 3.92$, $p = .001$, $d = 0.85$). The effect sizes of these analyses were found to exceed Cohen's (1988) convention for a large effect size ($d = 0.80$). Specifically, although MEP amplitude increased significantly relative to baseline in the stimulated hemisphere of LTP-like responders ($t(18) = 4.88$, $p < .001$), and decreased significantly relative to baseline in LTD-like

responders ($t(20) = 11.02$, $p < .001$), MEP change in the unstimulated hemisphere did not differ significantly from baseline in either LTP-like ($t(18) = .30$, $p = .766$, $d = 0.14$) nor LTD-like ($t(20) = 0.60$, $p = .557$, $d = 0.27$) groups. There was a weak trend toward an interaction between time, hemisphere and response type; $F(2,72) = 2.91$, $p = .070$, $\eta_p^2 = .075$, but no other main effects or interactions (ps > .183).

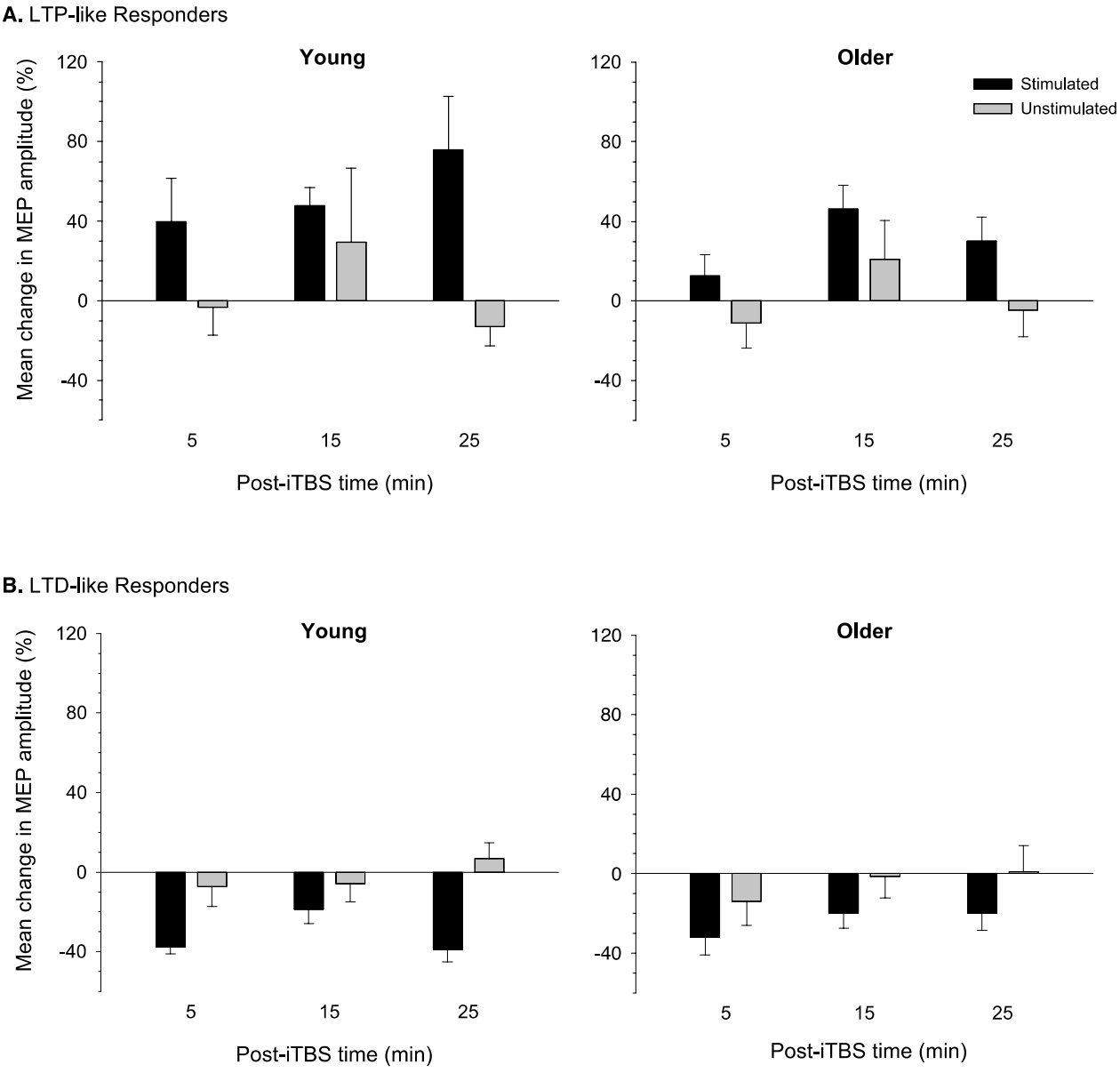

Figure 3-5 Average normalised MEP change following iTBS in young and older LTP-like (A) and LTD-like (B) responders. In both the LTP-like and LTD-like responders, MEP change was comparable between young and older adults and was greater in the hemisphere receiving iTBS (stimulated) than the hemisphere not receiving iTBS (unstimulated). Error bars denote SEM.

3.5 Discussion

The current study investigated whether corticospinal plasticity manifests differently across the stimulated and unstimulated corticospinal pathways in young and older adults following iTBS to the dominant (left) hemisphere. Contrary to our hypothesis, the effects of iTBS were found to manifest similarly in young and older adults, despite older adults exhibiting slightly attenuated cortical excitability at baseline (as indicated by an elevated TMS test intensity). Changes in MEP amplitude induced by iTBS, however, varied greatly across individuals, with only half the participants in each age group demonstrating the expected increase in cortical excitability.

3.5.1 Corticospinal plasticity induced by iTBS is subject to large individual variation but does not manifest in the non-stimulated pathway

Individual variability in iTBS effects has been demonstrated on numerous occasions (Young-Bernier et al., 2014; Vallence et al., 2013; Player et al., 2012; Hamada et al., 2013; Hinder et al., 2014; López-Alonso et al., 2014). In the current study just fewer than half the participants exhibited an increase in MEP amplitude in the target muscle (averaged across time) following iTBS. Consistent with one previous report (Young-Bernier et al., 2014), the proportion of LTP-like and LTD-like responders in the current study was similar in young (47% and 53% respectively) and older adults (53% and 47%). There was also little difference in the proportion of males and females in the LTP-like (58% and 42% respectively) and the LTD-like (43% and 57%) groups. These data suggest that neither age, gender, nor the allocation of spatial attention, which was controlled in the current study, explain the variability in our iTBS effects.

An important question in the current study was how corticospinal plasticity manifests across the stimulated and unstimulated corticospinal pathways. Previous studies suggest that iTBS induces an increase in corticospinal excitability in the stimulated pathway and a decrease in the unstimulated pathway (Suppa et al., 2008; Di Lazzaro et al., 2008; Di Lazzaro et al., 2011). In the current study, and as implemented in previous work, because corticospinal excitability at the group level did not differ reliably from baseline following iTBS, data were split into LTP-like and LTD-like responders (Müller-Dahlhaus et al., 2008; Young-Bernier et al., 2014). This analysis revealed significant corticospinal plasticity in the stimulated pathway (an increase or decrease, respectively), but there was still no reliable change evident in the unstimulated pathway. Standardised effect size calculations (Cohen's *d*) revealed that changes in corticospinal excitability on average in the simulated pathway of LTP-like and LTD-like responders were just under one standard deviation larger than changes in corticospinal excitability in the non stimulated pathway and this difference

exceeds the convention for a large effect. One explanation for the inconsistency between this result and previous research is that iTBS was applied to the left M1 in the current study but to the right M1 in previous studies (Suppa et al., 2008; Di Lazzaro et al., 2008; Di Lazzaro et al., 2011). Some evidence indicates that the change in corticospinal excitability induced by brain stimulation and motor training varies depending on the dominance of the stimulated hemisphere (Cirillo et al., 2011; Vines et al., 2008), perhaps due to stronger inhibitory projections from the dominant to the non-dominant motor cortex than vice versa (Netz et al., 1995).

Another explanation for differences between the current results and previous work relates to prior voluntary muscle activity. The intensity used for iTBS is typically set to a percentage of active motor threshold (aMT), which requires participants to maintain a voluntary contraction for a prolonged period before the iTBS intervention. Such activity can influence susceptibility to corticospinal plasticity interventions and even alter the direction of effects (Huang et al., 2008; Goldsworthy et al., 2014; see Hoogendam et al., 2010 for review). The current study minimised any influence of prior voluntary muscle activity on iTBS-induced effects by basing stimulation intensity on resting excitability (rMT), as used successfully with iTBS in previous work (Stefan et al., 2008; Goldsworthy et al., 2014; Genter et al., 2007). Importantly, in the only study to investigate bilateral changes in corticospinal excitability after iTBS in young and older adults (Di Lazzaro et al., 2008), the ipsilateral silent period was measured before and after iTBS. This measure requires participants to voluntarily contract the hand ipsilateral to the stimulation for a prolonged period, which may have altered responses to iTBS. Although the omission of the silent period measure in the current study did allow for the removal of any potential effect of prior voluntary activity on iTBS-induced responses, it also meant the removal of a probe of changes in inhibitory activity. Future studies would benefit from restricting post iTBS measures to one time point (15 mins) in order to include paired-pulse TMS measures of cortical excitability. This would give a more comprehensive view of iTBS induced changes in the balance of excitation and inhibition and how these might be altered in the aged brain. For the same reason as not measuring silent period, and to minimise discomfort, we were also unable to include a measure of maximal compound action potential (M-waves) in the muscles targeted by iTBS. Maximal M-waves reflect the function of peripheral muscles and have been found to reduce in old age (Vandervoort & McComas, 1986; McNeil et al., 2005; Christie & Kamen, 2014; Goodwill, 2015; Pitcher et al., 2003). Should corticospinal plasticity be reduced in older relative to young adults, this could be explained by capacity limits within the peripheral musculature. Although, corticospinal plasticity was equivalent across the age groups in the current study, this age difference may have been interpreted differently should MEP data have been normalised to Mmax. The finding that average test intensities were higher in older adults but that

rMTs were consistent across the age groups in the current study, suggests that greater cortical drive was necessary in older adults to induce the same size MEP as young adults, which provides further support for the suggestion that normalizing to Mmax might alter the interpretation of the findings. Future studies would benefit from recording maximal M-waves pre and post intervention in order to account for age-related changes in the peripheral muscles.

3.5.2 Manifestation of corticospinal plasticity across bilateral motor pathways is not altered in the aged brain

The results of the current study indicate that the magnitude and manifestation of corticospinal plasticity induced by TMS is not altered in older adults. It could be argued that it is not surprising that changes did not manifest in the unstimulated hemisphere or more bilaterally in older adults given the absence of any voluntary activity or sensory input from the peripheral nerves. While a unilateral motor task might involve bilateral neural activity via contralateral and ipsilateral motor pathways, and thus induce bilateral corticospinal plasticity, TMS based neuroplasticity-inducing interventions like iTBS, which typically target a cortical representation in one hemisphere, might only stimulate neural activity and trigger resultant plasticity in unilateral motor circuits. Certainly, previous literature does support this by demonstrating that plasticity effects within the same individual can vary between motor training and non-invasive brain stimulation interventions (Vallence et al., 2013). Furthermore, evidence demonstrates that bilateral plasticity is induced by PAS (Shin & Sohn, 2011), which, like motor training, involves sensory input to the peripheral nerves. Interestingly, although the current study revealed little plastic change in the unstimulated pathway, previous studies have demonstrated corticospinal plasticity in the unstimulated pathway following iTBS (Di Lazzaro et al. 2008; Di Lazzaro et al., 2011; Suppa et al., 2008). Specifically, corticospinal plasticity was found to be LTD-like in the unstimulated pathway but LTP-like in the stimulated pathway (Di Lazzaro et al. 2008; Di Lazzaro et al., 2011; Suppa et al., 2008). This finding supports the idea that different neuroplasticity inducing interventions may result in different manifestations of neuroplasticity. Interventions inducing neuroplasticity in the motor cortices via motor training or sensory stimulation may facilitate bilateral LTP-like corticospinal plasticity induction via the involvement of the sensory cortices. Should sensory information travel to both sensory cortices via contralateral and ipsilateral pathways, similar LTP-like plasticity may be induced in both motor cortices. In comparison, neuroplasticity interventions stimulating the motor cortex more focally without sensory input, thus bypassing sensory cortices, may only induce LTP-like plasticity at that location and not elsewhere in the brain. LTD-like effects in homologous regions of the opposite hemisphere might occur as a secondary consequence of the LTP-like

plasticity in the stimulated hemisphere and may be modulated by changes interhemispheric communication. The question of why LTD-like effects did not occur in the current study in the unstimulated hemisphere but did in a previous study where iTBS was used, should be investigated in future research. Very few studies have investigated bilateral changes in corticospinal excitability in young, let alone older adults. It is a question for future research to determine the extent to which different plasticity inducing interventions differentially affect excitability within the non-targeted hemisphere and the mechanisms by which this might occur.

Although a possibility, there is no reason to expect that the sample of older adults tested in the current study differed systematically from those employed in previous studies of this kind. Therefore, the discrepancy between the results of the current study and those showing a reduction in TMS-induced corticospinal plasticity in older adults may reflect methodological differences in the TMS protocol used. Specifically, most studies reporting reduced TMS-induced corticospinal plasticity in older adults have implemented PAS (Fathi et al., 2010; Müller-Dahlhaus et al., 2008), which has been shown to target similar but not identical mechanisms to iTBS (Cárdenas-Morales et al., 2010; Stefan et al., 2000). Whereas PAS is based on spike-timing dependent plasticity and is dependent upon associative pairings of TMS and peripheral nerve stimulation (Stefan et al., 2000), TBS is dependent on the rate of stimulation (Huang et al., 2005). The observation of attenuated corticospinal plasticity in older, relative to young, adults following PAS (Fathi et al., 2010; Müller-Dahlhaus et al., 2008), but maintained corticospinal plasticity following iTBS (Di Lazzaro et al., 2008; Young-Bernier et al., 2014), suggests that different mechanisms targeted by these protocols may be more or less susceptible to age-related change.

In addition to methodological differences, the influence of attention has been largely overlooked in investigations comparing corticospinal plasticity in young and older adults, despite evidence of attentional deficits with advancing age (Mahoney et al., 2010). We have shown previously that LTP-like corticospinal plasticity induced in the motor cortex by TMS is reduced under conditions of high attentional demand and when attention is directed away from the hand undergoing the neuroplasticity-inducing procedure (Kamke et al., 2012; 2014). Accordingly, the current study implemented a task that required participants to allocate their attention to the digit targeted by iTBS. An age-related reduction in corticospinal plasticity was not found under these conditions. It is possible that by directing attention to the targeted digit iTBS-induced corticospinal plasticity was restored in older adults. Future studies comparing the effect of iTBS when attention is directed to the targeted versus homologous digit of the opposite hand would clarify the degree to which neuroplasticity in older adults is influenced by attention. If attention is more critical in maintaining

neuroplasticity in older compared with young adults, greater corticospinal plasticity would be evident when attention is directed to the opposite hand. If attention does not influence corticospinal plasticity, however, there should be no difference between the two conditions.

3.5.3 Conclusions and implications

The current study demonstrates that corticospinal plasticity induced by iTBS is subject to large individual variability. The data indicate that although iTBS might be used to induce corticospinal plasticity in the stimulated pathway, it is not associated with a manifestation of plasticity in the unstimulated pathway. Importantly, irrespective of whether participants demonstrated increased or decreased corticospinal excitability following iTBS, the manifestation of corticospinal plasticity was not altered in older compared with younger adults. This finding suggests that either the mechanisms and/or pathways targeted by iTBS are not altered in the aged brain, or that corticospinal plasticity is enhanced in older adults when attention is directed to the digit targeted by the stimulation. Interestingly, the findings of the current study also suggest that strategies implementing iTBS to facilitate corticospinal plasticity with the goal of enhancing motor function (Di Lorenzo et al., 2013; Kim et al., 2004) are likely to benefit young and older adults comparably. Critically, however, the factors determining an individual's response to iTBS, including the interaction between attention and age, require further investigation.

3.6 Acknowledgments

This work was supported by a Project Grant from the National Health and Medical Research Council of Australia (NHMRC; APP1028210). DD was supported by an Australian Postgraduate Award scholarship. MS was supported by an NHMRC Training Fellowship (APP1012153). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding bodies.

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Chapter 4 Corticospinal plasticity in the human motor system induced by paired associative stimulation is highly variable across individuals but does not differ between young and older adults.

In the previous chapter it was found that although responses to iTBS were not reliable at the group level, they were highly variable across individuals. After classifying participants into groups of LTP-like (MEP change > 0%) and LTD-like (MEP change < 0%) responders the manifestation of corticospinal plasticity across the hemispheres was comparable between young and older adults. The aim of the third experiment of this thesis was to determine the extent to which corticospinal plasticity induced by a different TMS method, specifically PAS, is altered in young and older adults. Furthermore, the findings of Experiment 2 indicate that engaging in a concurrent attention task, wherein attention is allocated spatially to the muscle targeted by iTBS may protect corticospinal plasticity in the aged brain. However no studies to date had investigated the role of attention in modulating corticospinal plasticity induced by TMS in older adults. Thus, a second aim of the current experiment was to investigate how manipulating the focus of spatial attention influences PAS-induced effects. This chapter has been formatted for submission for publication.

Prior to Experiment 3 an initial experiment was conducted but was terminated following testing 12 participants, due to problems with the attention task. These issues were overcome and the protocol modified accordingly. The details and results of the initial study are provided in Appendix B.

4.1 Abstract

The adult human brain is capable of ongoing adaptation in response to the demands of its environment, a characteristic known as neuroplasticity. However, the extent to which advancing age and associated neurobiological change impacts neuroplasticity in motor regions of the brain is not well understood. Evidence suggests that neuroplasticity may manifest differently in older adults due to age-related reductions in the lateralisation of neural activity. Moreover, although evidence suggests that attention can modulate neuroplasticity, its influence has not been investigated in older adults, who demonstrate age-related change in attentional abilities. The current study investigated whether corticospinal plasticity, an index of neuroplasticity, and its modulation by attention is altered in older adults. Excitatory paired associative stimulation (PAS), a technique commonly used to induce LTP-like corticospinal plasticity non-invasively in the human motor system was implemented. PAS involved pairing peripheral nerve stimulation with single pulse transcranial magnetic stimulation (TMS) over the contralateral primary motor cortex repeatedly for 11 mins. Peripheral nerve stimulation was applied 25 ms prior to TMS (PAS 25) and each pair of stimuli were presented at a rate of 0.25- 0.17 Hz. Corticospinal excitability was measured indirectly by changes in motor-evoked potentials (MEPs), which were compared between young (19-32 years) and older (65-78 years) adults in the hemisphere targeted by PAS and the opposite hemisphere. It was hypothesised that, in comparison to young adults, older adults would demonstrate greater bilateral plasticity, such that changes in corticospinal excitability would manifest in both the targeted and the opposite non-targeted pathway. Secondly, it was predicted that in both young and older adults corticospinal plasticity would be greater when attention was directed to the spatial location of the target limb. Results showed that PAS-induced effects were highly variable across individuals, with only half of participants demonstrating the expected increase in MEP amplitude. Contrary to predictions, PAS-induced corticospinal plasticity was not more distributed in older adults. Moreover, attention to the target hand did not appear to enhance corticospinal plasticity.

4.2 Introduction

Neuroplasticity is defined as the ability of the brain to undergo enduring morphological or functional change in response to the demands of its environment (Boroojerdi et al., 2001). Synaptic plasticity, or changes in the strength of communication between neurons, is one mechanism known to contribute to the reorganisation of neural circuits and thus neuroplasticity (Malenka, 2002). Little is known about how neuroplasticity within the motor system is affected by advancing age and its associated neurobiological change, which is evident throughout the elderly brain (see Esiri, 2007 for a review). It is well established, however, that advancing age influences the structure and function

of the brain (see Seidler et al., 2010 for a review). One such age-related change that is particularly prominent is that older adults show a reduction in task-related lateralisation, and proportionately more activity in homologous regions of the contralateral hemisphere (Hutchinson, et al., 2002; Mattay et al., 2002). Although the functional relevance of reduced lateralisation has yet to be determined, it is possible that older adults recruit additional neural populations both within and across hemispheres to maintain performance on these tasks.

Synaptic plasticity in humans can be measured indirectly and non-invasively using transcranial magnetic stimulation (TMS). When applied over primary motor cortex, TMS can trigger spikes in muscle activity known as motor-evoked potentials (MEPs), which are quantified using electromyography (EMG). Because TMS actives motor output cells trans-synaptically, changes in the excitability of the synapses of cortical interneurons can be reflected by changes in MEP amplitude (Stefan et al., 2002; Wolters et al., 2003; Muellbacher at el., 2001; Di Lazzaro et al., 2005). In addition to the contribution of cortical plasticity, however, TMS induced changes in MEP amplitude might also reflect spinal contributions. Thus, changes in MEP amplitude index changes in corticospinal excitability (plasticity). In addition to measuring corticospinal plasticity, TMS can also be used to induce plasticity using a protocol known as paired associative stimulation (PAS; Stefan et al., 2000). Studies in animal models demonstrate that the efficacy of the communication between neighbouring neurons can be increased (potentiated) or decreased (depressed) depending on the synchrony and temporal order of their activity (Bliss & Lomo, 1973). Consistent with this observation synchronous and repetitive pairings of low frequency single pulse TMS with peripheral nerve stimulation, which lead to coincident inputs to motor cortex, can also induce long-term potentiation (LTP)-like increases in corticospinal excitability in humans (Stefan et al., 2000; Wolters et al., 2003). Long-term depression (LTD)-like decreases in corticospinal excitability have also been demonstrated when PAS pairings are asynchronous and do not lead to temporally coincident inputs to M1 (Wolters et al., 2003).

Studies using PAS to induce changes in corticospinal excitability suggest that plasticity is reduced in old relative to young adults (Fathi et al., 2010, Müller -Dahlhaus et al., 2008). This finding is consistent with results obtained from animal models (Barnes et al., 1996; Landfield et al., 1978, Moore et al., 1993). An important caveat in interpreting these results in the context of understanding age-related changes in neuroplasticity is that in the aforementioned studies corticospinal plasticity was only measured in the pathway targeted by PAS. Previous research in young adults has demonstrated that PAS-induced effects manifest not only in the pathway receiving TMS (target), but also in the pathway not receiving TMS (non-target; Shin & Sohn, 2011). Bilateral

corticospinal plasticity effects are also evident after alternative TMS neuroplasticity-inducing protocols (Suppa et al., 2008; Ishikawa et al 2007, Stefan et al., 2008; Bashir et al., 2014). The fact that older adults show reductions in the laterality of neural activity relative to young adults during the performance of motor tasks (Hutchinson, et al., 2002; Mattay et al., 2002) suggests that the circuits involved in motor performance may be more distributed in older adults. It is therefore possible that the reduction in TMS-induced corticospinal plasticity evident in the target pathway of older adults is coupled with greater change in the non-target pathway, relative to young adults. The current study tested this hypothesis. Measures of cognitive and motor ability, physical activity, and psychological factors were also included to examine associations between these characteristics and corticospinal plasticity in the two age groups.

Although corticospinal plasticity induced by PAS can occur in the absence of any involvement from the participant, previous research indicates that TMS-induced effects in the motor cortices of young adults can be impacted by concurrently undertaking a cognitive task (Kamke et al., 2012; Rosenkranz & Rothwell, 2004; 2006; Stefan et al., 2004). For example, allocating spatial attention towards the hand targeted by the TMS intervention results in increased excitability in the coticospinal pathway innervating the target muscle relative to conditions in which attention was directed elsewhere (Rosenkranz & Rothwell, 2004; 2006; Stefan et al., 2004). Older adults are more susceptible to structural and chemical change in the frontal and parietal regions (Cabeza, 2002), which are known to play a critical role in controlling attentional processes (Corbetta & Shulman, 2002). To date, the impact of age-related change on the influence that attention exerts on TMS-induced corticospinal plasticity has not been investigated. The current study also tested the hypothesis that attention to the target hand enhances PAS-induced corticospinal plasticity in the target pathway of both young and older adults.

4.3 Methods

4.3.1 Participants

A total of 40 volunteers, 20 between the ages of 19 and 32 years (*M*= 24.40, *SD*= 3.86, Males= 10) and 20 between the ages of 65 and 78 years (*M*= 69.55, *SD*= 3.99, Males= 10) were tested. Participants were recruited through advertising in online newsletters and by word of mouth, and were reimbursed $10 per hour for their involvement. All participants were right-handed handed (Young *M* = 81.34, *SD* = 18.08, Older *M* = 84.54, *SD* = 19.12), as determined by The Edinburgh Handedness Inventory (Oldfield, 1971). Prior to commencement of testing all participants completed a TMS safety-screening questionnaire (Rossi, et al., 2009; 2011) and provided fully

informed written consent. All procedures were approved by The University of Queensland's Medical Research Ethics Committee. Individuals with neurological disease or damage, epilepsy, history of head injury or psychiatric disorder, or taking neuroactive medications were excluded from the study. All participants had normal or corrected to normal visual acuity. There were no adverse reactions to TMS.

4.3.2 Cognitive and psychological assessment

Participants were administered a battery of cognitive tests measuring attention, perceptual speed, cognitive flexibility, executive control, response inhibition, working memory, and executive capacity. These were the Stroop test (Stroop, 1935), two components of the fourth edition Wechsler Adult Intelligence Scale (WAIS-IV; Wechsler, 2008), The Digit Span Test and the Logical Memory Test. In addition, each participant's psychological wellbeing and connectedness to social groups was measured using the Satisfaction with life scale (Diener et al., 1985) and the Multiple Identities Scale (Haslam et al., 2008). The General Physical Activity Questionnaire (GPAQ; Dept of Health, 2006) was used to measure the average physical activity undertaken by each participant in a typical week. Participants also completed a demographic questionnaire denoting the occupation in which they spent most time and the highest level of formal education completed. Educational attainment was recorded on an ordinal scale from 0 'no schooling completed' to 11 'doctorate degree'.

4.3.3 Motor assessment

Participants were required to perform six different timed tapping tasks, unilateral and bilateral, ranging from a single key press to a complex sequence. Participants were instructed to make as many accurate key presses as possible within 15 seconds. All tapping tasks were completed on a standard computer keyboard and were repeated three times in separate blocks. The six tasks were as follows; *Task 1. Right Index*- tap the 'o' key with the right index finger, *Task 2. Left Index*- tap the 'w' key with the left index finger, *Task 3. Right Alternate*- tap the 'o' and 'p' keys alternately using the index and middle finger of the right hand, *Task 4. Left Alternate*- tap the 'w' and 'q' keys alternately using the index and middle finger of the left hand, *Task 5. Index Alternate*- tap the 'o' and 'w' keys alternately using the index fingers of the right and left hands, *Task 6. Bilateral Alternate*- tap the 'o' 'q' 'w' and 'p' keys in that sequence using the right index finger, left middle finger, left index finger and right middle finger respectively. Performance was quantified based on the number of accurate sequences completed in the allocated time period (Hausmann et al., 2004).

4.3.4 Transcranial Magnetic Stimulation (TMS)

TMS was administered using a figure-of-eight shaped coil with a wing diameter of 70 mm, connected to a Magstim 200^2 stimulator (Magstim Co., UK). The coil was placed tangentially on the scalp with the handle pointing towards the back of the head, angled 45 degrees from the midline and was moved systematically in a grid-like pattern until the motor hotspot was located. The motor hotspot was defined as the optimal position on the scalp for evoking the largest and most consistent motor-evoked potential (MEP; peak-to-peak amplitude) in the target muscle, the *abductor pollicis brevis* (APB) of the left and right hands. Stimulation occurred approximately every 5 seconds at an intensity sufficient to evoke a clear MEP in the target muscle. A frameless infrared stereotaxic neuronavigation system (Visor 1, ANT, Netherlands) was used to record the location and angle of the coil for each hotspot, enabling these to be reproduced within an experimental session.

Following determination of the hotspot, resting motor threshold (rMT) was obtained for the cortical representation controlling the left and right APB. The rMT was defined as the minimum TMS intensity (reported as a percentage of maximum stimulator output, % MSO), that evoked an MEP of at least 50 μV in at least 3 out of 5 consecutive trials. The intensity of the TMS was adjusted using a staircase (two-down, one-up) procedure until the criterion was met. Following this, TMS test intensities were established for the left and right APBs. The test intensity was defined as that required to evoke an average MEP of approximately 1 mV (peak-to-peak) in the resting muscles. On average this intensity equated to 121% of rMT for the right (target) APB and 123% of rMT for the left (non-target) APB (see Table 4-1 for details). MEP amplitude was quantified by averaging responses to 21 TMS pulses delivered at test intensity every 5 ±1 seconds. MEP amplitude was quantified at baseline and at four time-points post PAS.

4.3.5 Electromyography EMG

Activity from the right and left APB was recorded using surface electromyography (EMG). Disposable 24 mm silver-silver chloride (Ag/AgCl) electrodes were used, with the active electrode placed on the belly of the APB muscle of the left and right hands and reference electrodes on the metacarpophalangeal joint of the respective thumb. MEP data were amplified (x1000), filtered (20-2000 Hz) and sampled at 2000 Hz using a NeuroLog system (Digitimer, UK), National Instruments Data Acquisition Interface (BNC-2110, National Instruments, USA) and custom Matlab software (Mathworks, USA). Individual sweeps were sampled from 500 ms before stimulation to 500 ms after stimulation and stored for off-line analysis. Muscle activity was visually monitored throughout

the experiment using a digital oscilloscope. If activity occurred during a trial, participants were verbally prompted to relax.

4.3.6 PAS procedure

To avoid potential problems associated with fatigue from having to undertake the attention task for a long period of time an 11-minute PAS protocol, which is shorter than that used more widely in the literature (15-30 mins), was implemented in the current study. This duration, although short relative to that originally used with PAS (i.e., 30 min in Stefan et al., 2000) has been shown to induce corticospinal plasticity in young adults (Sale et al., 2007). One hundred and thirty two pulses of TMS were administered to the left hemisphere ('target M1') at the predetermined test intensity, spaced 4 to 6 seconds apart, for a period of 11 mins. Each pulse was paired with peripheral nerve stimulation, which occurred 25 ms prior to each TMS pulse. This interval was chosen as it has been shown previously to induce LTP-like increases in excitability (Wolters et al., 2003). Peripheral nerve stimulation was presented to the median nerve using a constant current stimulator (Digitimer DS7A) and bar electrode (200 μs pulse width; cathode proximal). The intensity of the stimulation was adjusted to be at the threshold to produce a m-wave response in the right APB.

4.3.7 Visual-spatial attention manipulation

Sustained spatial attention was manipulated across the two PAS sessions. Participants were instructed to overtly attend to a light emitting diode (LED) placed on the thumb, just above the interphalangeal joint. Attention was directed to the target and non-target thumb in separate sessions, the order of which was counterbalanced across individuals. Participants were tasked with making a verbal response each time they detected two brief, consecutive interruptions ("OFF" periods) to the continuously lit LED. Targets appeared on average every 10 seconds, but were jittered randomly, such that the interval between targets varied between 5 and 30 seconds. Targets were timed such that participants did not respond during a PAS pulse. Targets were also intermingled with non-targets (single "OFF periods") that were also jittered randomly. The temporal presentation of targets remained constant across the attend right and attend left conditions. The attention task was performed for the duration of the PAS intervention (11 mins).

4.3.8 Experiment Design and Procedure

Participants completed two PAS sessions that took place at similar times of day at least 24 hours apart, with each session lasting up to 2 hours. Cognitive and motor assessments took place in an additional session. During the PAS sessions participants were seated comfortably with their arms

resting on cushions on a desk. The skin of both hands was cleaned thoroughly to minimise skin impedance and electrodes were placed in position. An eye tracker was used throughout the pre and post measures to ensure participants eyes remained open. Figure 4-1 depicts the timeline of the PAS sessions. Single pulse TMS was applied to the target and non-target M1s to locate the motor hotspot and to quantify corticospinal excitability before the neuroplasticity intervention. This was followed by a brief practice of the attention task, which was then undertaken during PAS. Following the intervention, single pulse TMS was administered at the test intensity, first to the target followed by the non-target hemisphere, to measure post PAS MEP amplitude at 5, 15, 25, and 35 minutes. Between the 5 and 15 min post-test MEP measures rMTs were remeasured.

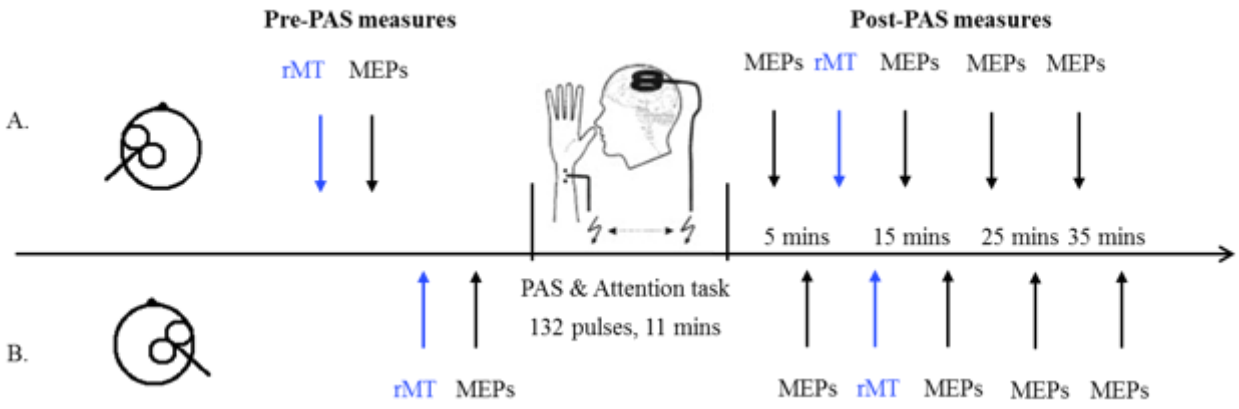

Figure 4-1 Time course of experiment. Row A denotes the time course for the left hemisphere (target M1), while row B denotes the time course for non-target (right) hemisphere. MEPs were recorded from the left M1 followed by the right M1 at baseline and 5, 15, 25, and 35 minutes following the completion of PAS. At baseline, transcranial magnetic stimulation (TMS) intensity was adjusted to produce MEPs of, on average, 1 mV peak-to-peak amplitude. Paired associative stimulation (PAS) was applied with an interstimulus interval of 25 ms. Participants attended toward the right and left hands in separate sessions. MEP amplitudes post PAS were recorded using the same TMS intensity as baseline. Resting motor threshold (rMT) was recorded at two time points, once before and once post PAS.

4.3.9 Data processing and analyses

To identify age differences in cognition, psychological well-being and physical activity, scores on these measures were subjected to an independent samples t-test. Motor performance was assessed across age groups and tasks with a 2 × 6 mixed ANOVA testing the factors of age (young, older) and task (right index, left index, index alternate, right alternate, left alternate, bilateral alternate). Performance on the spatial attention task, undertaken during PAS, was quantified using the total number of errors made during the task. Total error was calculated by summating the number of

targets missed (misses) with the number of false positives for each participant. Performance was compared between age groups and attention conditions using a 2 × 2 mixed ANOVA testing the factors attention (attend right, attend left) and age (young, older).

Corticospinal plasticity was assessed using EMG data that were analysed offline using custom Matlab software. The first pulse from each block of 21 MEPs was removed, as were trials containing muscle activity cleary above background activity (cut off estimate ~0.03- 0.04mV) in the 100 ms prior to TMS. The remaining trials in each block were averaged for each participant. Baseline MEPs and test intensities were subjected to a 2 × 2 × 2 mixed ANOVA with the factors of hemisphere (target, non-target), attention (attend right, attend left) and age (young, older). Baseline and post-PAS rMTs were subjected to a 2 × 2 × 2 × 2 ANOVA with the between subject factor of age (young, older) and the within subjects factors of hemisphere (target, non-target), time (pre, post) and attention (attend right, attend left). Post-PAS MEP amplitudes were expressed as the average percentage change from each participant's baseline. Post-PAS MEP change in the APB muscles were compared using a 2 × 2 × 2 × 4 mixed ANOVA with factors of age (young, old), hemisphere (target, non-target), attention (right, left) and time (5, 15, 25, and 35 mins post-PAS). One young participant failed to complete the 35 minute post measure in the attend right condition. This missing data was replaced with the overall average of the 5, 15 and 25 minute post-PAS measures in the attend right condition for that individual. Because no PAS-induced effects were evident at the group level, and individuals showed large variability in their responses to PAS, participants were classified into two groups based on the direction of MEP change induced separately in the attend right and attend left conditions. Individuals demonstrating an increase in MEP amplitude (> 0% change) averaged over all post-PAS time points in the target muscle were classified as LTP-like responders. Individuals exhibiting a decrease in MEP amplitude (< 0%) in the target muscle post PAS were classified as LTD-like responders. Responder classification was included as an additional factor in the exploratory 2 × 2 × 4 ANOVA for the attend right and attend left conditions separately. Similar to the previous experiment, LTP-like and LTD-like responders were classified using two criteria, MEP change above and below 0%, and above and below 20% in order to assess differences where the changes in MEP amplitude might be deemed more indicative of plasticity (Hinder et al., 2014). The results of the analysis based on a 20% criterion were not different from the 0% criterion, and are presented in Appendix C. All main effects and interactions were followed up with paired comparisons using Bonferroni corrections and two-tailed t-tests.

4.4 Results

4.4.1 Age differences in cognitive, psychological, and physical activity assessment

Table 4-1 displays the means, standard deviations and t-test results for the cognitive, motor and psychological results. Significant effects are shown with an asterisk. As can be seen in Table 4-1, older adults reported significantly less sedentary activity and more physical activity per week than did young adults. Young adults demonstrated greater accuracy in the logical memory immediate recall task than older adults. In addition, in comparison to young adults, older adults experienced significantly greater cognitive interference between the two components of the Stroop task. The highest level of educational attainment was similar for both young and older adults, with the most common level of education being a bachelor's degree. There were no other significant differences in the performance of young and old adults across the different cognitive and psychological measures.

Table 4-1 Mean, standard error of the mean, and t-test results for the cognitive, motor and psychological data of young and older adults.

	Young	Older	T-Test
Stroop interference	-41.06 (3.30)	-49.95 (2.38)	$t(36) = 2.22$, $p = .033$*, $d = 0.74$
Digit span test	20.61 (1.35)	20.15 (1.14)	$t(36) = .26$, $p = .794$, $d = 0.09$
Logical memory immediate recall	14.83 (0.87)	11.55 (0.79)	$t(36) = 2.83$, $p = .008$*, $d = 0.94$
Logical memory delayed recall	13.28 (1.14)	10.10 (0.75)	$t(36) = 2.37$, $p = .023$*, $d = 0.79$
Satisfaction with life scale	27.83 (0.94)	27.60 (0.77)	$t(36) = .19$, $p = .848$, $d = 0.06$
Multiple Identities	18.94 (1.00)	15.75 (1.44)	$t(36) = 1.79$, $p = .083$, $d = 0.60$
GPAQ- Average minutes per week	494.03 (66.25)	984.25 (206.73)	$t(36) = 2.26$, $p = .034$*, $d = 0.75$
Sedentary behavior (mins per week)	513.33 (58.81)	378.00(34.80)	$t(36) = 2.16$, $p = .038$*, $d = 0.72$
Edinburgh Handedness Inventory	81.19 (4.50)	84.54 (4.28)	$t(33) = .52$, $p = .608$, $d = 0.17$
Level of educational attainment	7.28 (0.49)	6.10 (0.55)	$t(36) = 1.58$, $p = .123$, $d = 0.53$

* significant at $p < .05$.

4.4.2 Age differences in motor assessment

Figure 4-2 displays the performance of young and older adults on the six tapping tasks testing unilateral and bilateral motor functioning. As can been seen performance decreased with increasing task complexity for both young and older adults, an effect which proved reliable with a significant main effect of task; $F(5,180) = 435.73$, $p < .001$, $\eta_p^2 = .92$. Overall performance was poorer in older relative to young adults, as indicated by a main effect of age; $F(1,36) = 41.23$, $p < .001$, $\eta_p^2 = .53$. Also, it can be seen that performance across the tasks varied as a function of age. This effect was found to be reliable with a significant task × age interaction; $F(5,180) = 5.89$, $p < .001$, $\eta_p^2 = .14$. Follow-up t-tests compared performance on each of the different tasks separately in young and older adults. Although performance decreased significantly as the tasks became increasing more complex, there was no difference between the index alternate and the right alternate for young adults ($t(17) = 2.53$, $p = .022$, $d = 0.60$), whereas there was a significant difference between these tasks for older adults ($t(19) = 4.86$, $p < .001$, $d = 1.09$), when adjusting for multiple comparisons.

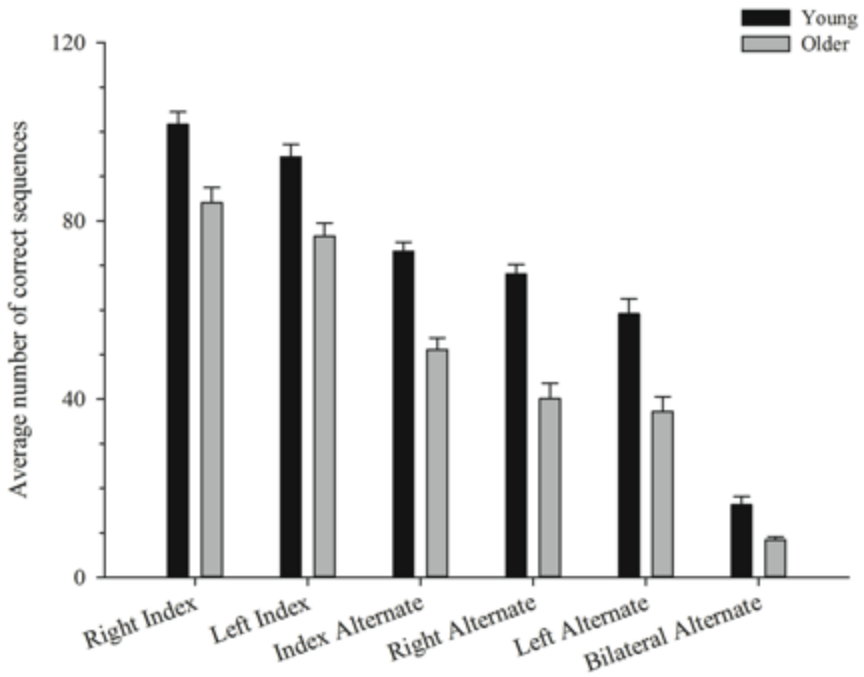

Figure 4-2 Motor performance on the six different tapping tasks in young and older adults. The number of correct motor sequences completed was significantly reduced in older compared with younger adults. Overall performance, averaged across the two age groups, declined with tasks involving greater complexity. Error bars denote SEM.

4.4.3 Spatial attention task accuracy

For the spatial attention task undertaken during PAS overall accuracy was high and few errors were made. There was no significant difference between the attend right (M = 1.10, SE = 0.05) and the attend left condition (M = .65, SE = 0.03); $F(1,38) = 3.62$, $p = .065$, $\eta_p^2 = .087$. There was also no significant difference in the number of errors made by young (M = .95, SE = 0.05) and older adults (M = .80, SE = 0.05) and no interaction between age and attention (ps > .530).

4.4.4 Baseline Cortical Excitability

Table 4-2 displays the means and standard deviations for baseline cortical excitability. As can be seen in Table 4-2 baseline MEPs did not differ across conditions. ANOVA did reveal a weak trend toward an Attention × Hemisphere × Age interaction; $F(1,38) = 3.62$, $p = .065$, $\eta_p^2 = .09$, driven by slightly larger baseline MEPs in the target hemisphere of young adults, but there were no other significant main effects or interactions (ps >.132). There were no differences in test stimulus intensities across all conditions (ps > .138). Also shown in the table, there was little difference in post rMT (reported as % MSO) across the conditions, but ANOVA revealed a significant Attention × Hemisphere × Age interaction; $F(1,38) = 6.89$, $p = .012$, $\eta_p^2 = .15$. This effect was driven by slightly higher rMTs in the target hemisphere in the attend left condition in older adults. Follow up 2 × 2 ANOVAs with the factors of Attention (right, left) and Hemisphere (target, non-target) were conducted separately for young and older adults. It was found that rMTs in young adults did not differ between the conditions, as indicated by the absence of any main effects of interactions (ps > .179). In older adults however, rMTs in the target hemisphere varied across the two sessions, as indicated by a significant interaction between attention and hemisphere ($F(1,19) = 5.33$, $p = .032$, $\eta_p^2 = .219$). Specifically, in older adults rMT did not differ between the attend left and attend right sessions in the non-target hemisphere ($t(19) = .110$, $p = .914$, $d = 0.03$), but the target hemisphere rMT was significantly greater in the attend left than in the attend right session ($t(19) = 2.50$, $p = .022$; after correcting for multiple comparisons, $d = 0.56$). This difference, however, was less than 2% machine output. Importantly, post-PAS rMTs did not differ from baseline rMTs (all other main effects and interactions, ps >.144).

Table 4-2 Means and standard error of the means for baseline cortical excitability and post-PAS rMTs

	Young				Older			
	Target Hem		Non-target Hem		Target Hem		Non-target Hem	
	Attend Right	Attend Left	Attend Right	Attend Left	Attend Right	Attend Left	Attend Right	Attend Left
Baseline MEP amplitude (mV)	0.94 *(0.07)*	0.96 *(0.06)*	0.88 *(0.07)*	0.79 *(0.07)*	0.86 *(0.06)*	0.83 *(0.06)*	0.84 *(0.05)*	0.88 *(0.05)*
Test intensity (% machine output)	48.15 *(2.02)*	49.15 *(2.02)*	50.15 *(2.24)*	50.05 *(2.18)*	53.30 *(2.32)*	54.60 *(2.40)*	53.75 *(2.44)*	53.55 *(2.48)*
Baseline rMT (% machine output)	40.70 *(1.62)*	40.45 *(1.49)*	40.60 *(1.68)*	41.60 *(1.50)*	43.20 *(1.72)*	45.15 *(1.93)*	43.45 *(1.72)*	43.45 *(1.89)*
Post rMT (% machine output)	41.00 *(0.35)*	40.55 *(0.37)*	40.75 *(0.36)*	41.30 *(0.38)*	43.05 *(0.35)*	44.75 *(0.37)*	43.75 *(0.36)*	43.60 *(0.38)*

4.4.5 PAS-Induced Corticospinal Plasticity

As in the previous (iTBS) experiment, individuals showed large variability in responses to the plasticity intervention. Young adults tended to show more frequent LTP-like effects than older adults. Figure 4-3 depicts representative MEP traces for one young and one older adult.

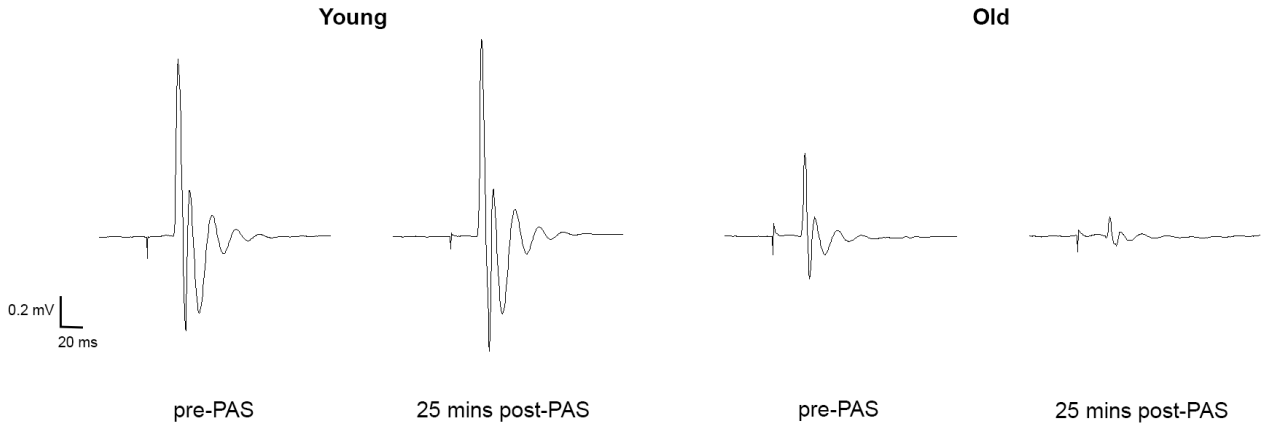

Figure 4-3 Representative MEP traces. MEPs from the right APB in two representative participants before (pre-PAS) and after (2 mins post-PAS) PAS. Each MEP is an average of 20 responses to TMS in the resting muscle.

Figure 4-4 illustrates the average MEP change in the target and non-target hemispheres, normalized to each individual's baseline response. Separate plots are presented for young and older adults and for the attend right (Row A) and attend left (Row B) conditions. Overall young adults appear to show an increase and older adults a decrease in MEP amplitude following PAS. The results of the ANOVA, however, fail to find a reliable difference and only show a weak trend toward a main effect of age; $F(1,38) = 3.20$, $p = .080$, $\eta_p^2 = .078$. Moreover, follow-up analysis revealed that MEP change in each group was not significantly different from zero (*p*s > .134). It is evident from Figure 4-4 that there was a difference in MEP change across the post-PAS measures, which was confirmed by a marginally significant main effect of time; $F(3,144) = 2.65$, $p = .052$, $\eta_p^2 = .065$. The greatest difference in MEP change across time was found between 5 and 25 mins post PAS. This difference, however, was not statistically reliable ($t(39) = 2.58$, $p = .014$, $d = 0.41$, all other $p > .097$; adjusted alpha = .008). Furthermore, MEP change was not significantly different from baseline at any of the time-points post PAS (*p*s >.100). There were no other significant main effects or interactions (*p*s >.148).

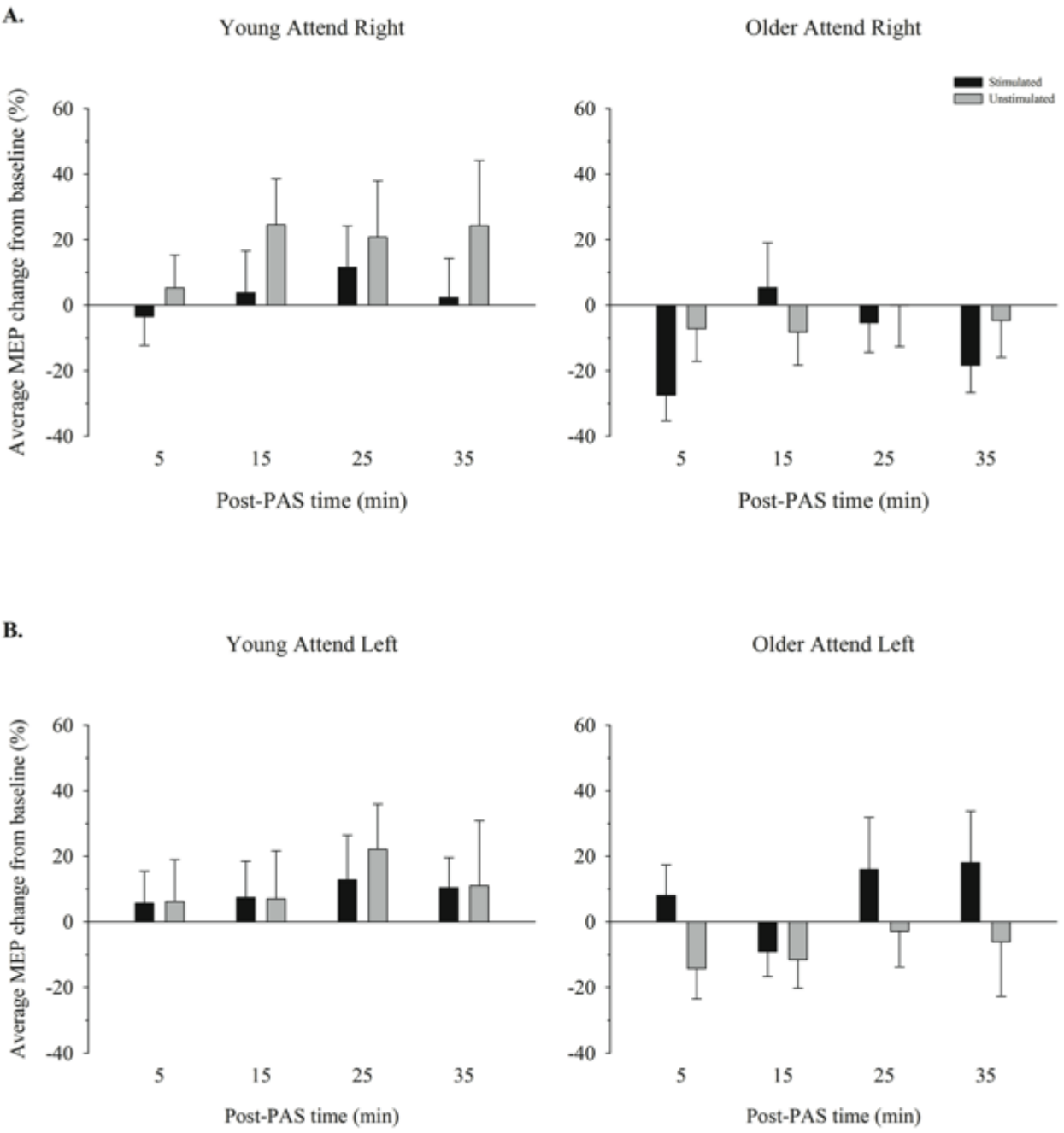

Figure 4-4 Mean MEP change relative to each individuals baseline. Following PAS the amplitude of MEPs increased from baseline in young adults and decreased in older adults, but these effects were not statistically reliable. The percentage of MEP change post PAS relative to baseline did not differ significantly between the stimulated and unstimulated hemispheres nor between the attend left and attend right conditions. Error bars denote SEM**.**

Although there were no PAS-induced effects at the group level, it can be seen from the large error bars in Figure 4-4 that there was great individual variability in responses. Analysis of individual responses revealed that only half of all participants demonstrated the expected increase in MEP amplitude averaged across all post PAS time-points in the target muscle; this was true for both the

attend right (20 total: 12 young, 8 older; 9 female, 11 male) and attend left (22 total: 11 young, 11 older, 7 female, 15 male) sessions. The other half of participants demonstrated a decrease in MEP amplitude. To explore these effects, participants were classified into two groups; individuals demonstrating an increase in MEP amplitude (> 0% change) and those showing a decrease (< 0%). The former are referred to as LTP-like responders and the latter LTD-like responders. Participants were classified into these two groups based on responses in the target hemisphere, allowing examination of corticospinal plasticity in the non-target pathway.

Figure 4-5 shows the average MEP change relative to each individuals baseline in the attend right condition separately for young and older adults (columns) and for the LTP- (Row A) and LTD-like (Row B) responders. It is evident that, as expected, MEPs increased in LTP-like responders but decreased in LTD-like responders, a difference which proved reliable with a main effect of response type; $F(1, 36) = 8.00$, $p = .008$, $\eta_p^2 = .182$. Looking at Figure 4-5B it seems there is a difference across hemispheres in LTD-like responders, where young adults show opposite effects in each hemisphere but older adults show effects in the same direction. ANOVA revealed that MEP change in the LTP- and LTD-like groups did vary across the target and non-target hemispheres, which was supported by a significant interaction between hemisphere and responder type; $F(1, 36) = 10.42$, $p = .003$, $\eta_p^2 = .224$, but there was no difference between the young and older groups (all other main effects and interactions non-significant; ps > .125). Specifically, MEP change did not differ between the hemispheres in LTP-like responders ($t(19) = 1.12$, $p = .277$, $d = 0.25$), but was significantly larger in the target hemisphere than in the non-target hemisphere in LTD-like responders ($t(19) = 3.39$, $p = .003$, $d = 0.76$). Although the preceding analysis indicates the difference in corticospinal plasticity induced bilaterally, it does not indicate whether the change in MEP amplitude was significant. In order to test this, one-sample t-tests compared MEP change in each of these conditions to zero. It was found that MEP change was significant only in the target and not the non-target hemisphere for both LTP-like (target $t(19) = 3.28$, $p = .004$, $d = 1.50$, non-target $t(19) = .97$, $p = .342$, $d = 0.45$) and LTD-like (target $t(19) = 6.18$, $p < .001$, $d = 2.84$, non-target $t(19) = .29$, $p = .774$, $d = 0.13$) responders. Finally, although all other main effects and interactions did not vary as a function of the criterion used for the responder analysis, there was a marginal main effect of age with a criterion of ±20% change (F (1, 17) = 4.14, $p = .058$, $\eta_p^2 = .196$), with younger adults showing slightly greater average MEP change than older adults in the attend right condition. The results of that analyses are presented in Appendix C.

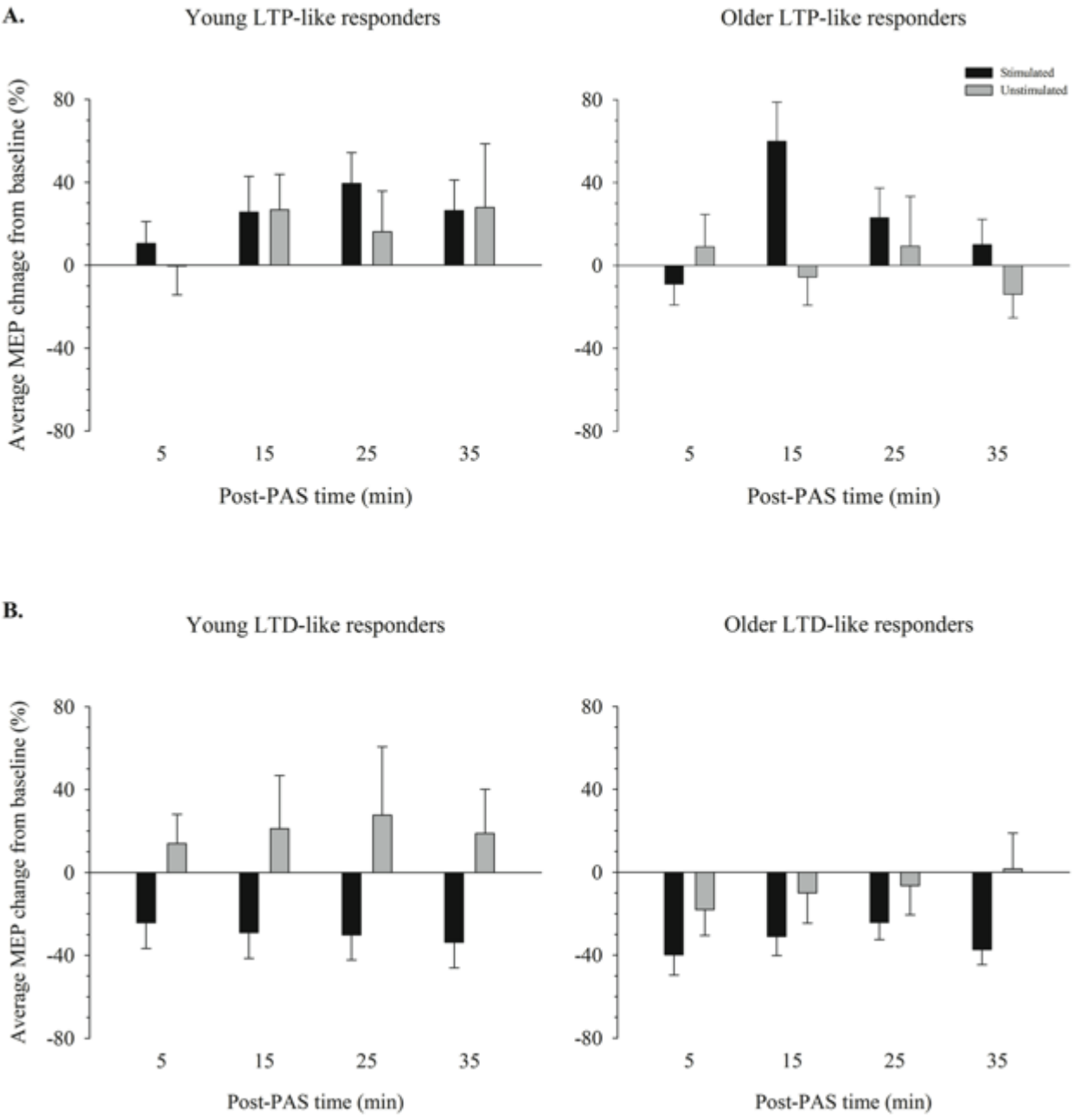

Figure 4-5 Mean MEP change in young and older LTP- and LTD- like responders in the attend right condition. MEP amplitudes increased significantly in LTP-like responders and decreased significantly in LTD-like responders. Although there was no difference in the corticospinal plasticity induced in the target and non-target pathways in LTP-like responders, there was a significant difference between the hemispheres in LTD-like responders. However, PAS-induced corticospinal plasticity was significant (different from zero) only in the target (stimulated) pathway in both LTP- and LTD-like responders. Error bars denote SEM.

The bilateral distribution of corticospinal plasticity effects was also examined in young and older LTP- and LTD-like responders for the attend left session. This data is depicted in Figure 4-6. As expected, and similar to the effects evident in the attend right session, LTP-like responders

demonstrated an increase in MEP amplitude, whereas LTD-like responders demonstrate a decrease. The effects in each responder group were reliably different from one another, as indicated by a significant main effect of response type; $F(1, 36) = 8.65$, $p = .006$, $\eta_p^2 = .194$. It is also evident from Figure 4-6 that MEP change in the LTP- and LTD-like groups varied across the hemispheres, and that these hemispheric differences don't vary across age groups for LTP-like responders but do vary across age groups in LTD-like responders. ANOVA revealed a significant interaction between hemisphere and responder type, $F(1, 36) = 11.12$, $p = .002$, $\eta_p^2 = .236$, but there were no other significant main effects or interactions (ps > .132). Follow up analysis showed that MEP change was greater in the target than in the non-target hemisphere in LTP-like responders ($t(21) = 2.83$, $p = .010$, $d = 0.60$), but did not differ between the hemispheres in LTD-like responders ($t(17) = 1.93$, $p = .071$, $d = 0.46$). To assess whether the MEP change in the two hemispheres was statistically reliable, one-sample t-tests compared MEP change in each of these conditions to zero. It was found that MEP change was significant only in the target and not the non-target hemisphere for both LTP-like (target $t(21) = 4.59$, $p < .001$, $d = 2.00$, non-target $t(21) = .15$, $p = .880$, $d = 0.07$) and LTD-like (target $t(17) = 8.12$, $p < .001$, $d = 3.94$, non-target $t(17) = 0.09$, $p = .933$, $d = .04$) responders. Similar to the attend right session, although all other main effects and interactions did not vary as a function of the criterion for the responder analysis, there was a weak trend whereby younger adults showed greater MEP change than older adults with a responder criterion of ±20% change in the attend left condition; F (1, 19) = 3.60, $p = .073$, $\eta_p^2 = .159$. The results of the 20% criterion responder analyses are presented in Appendix C.

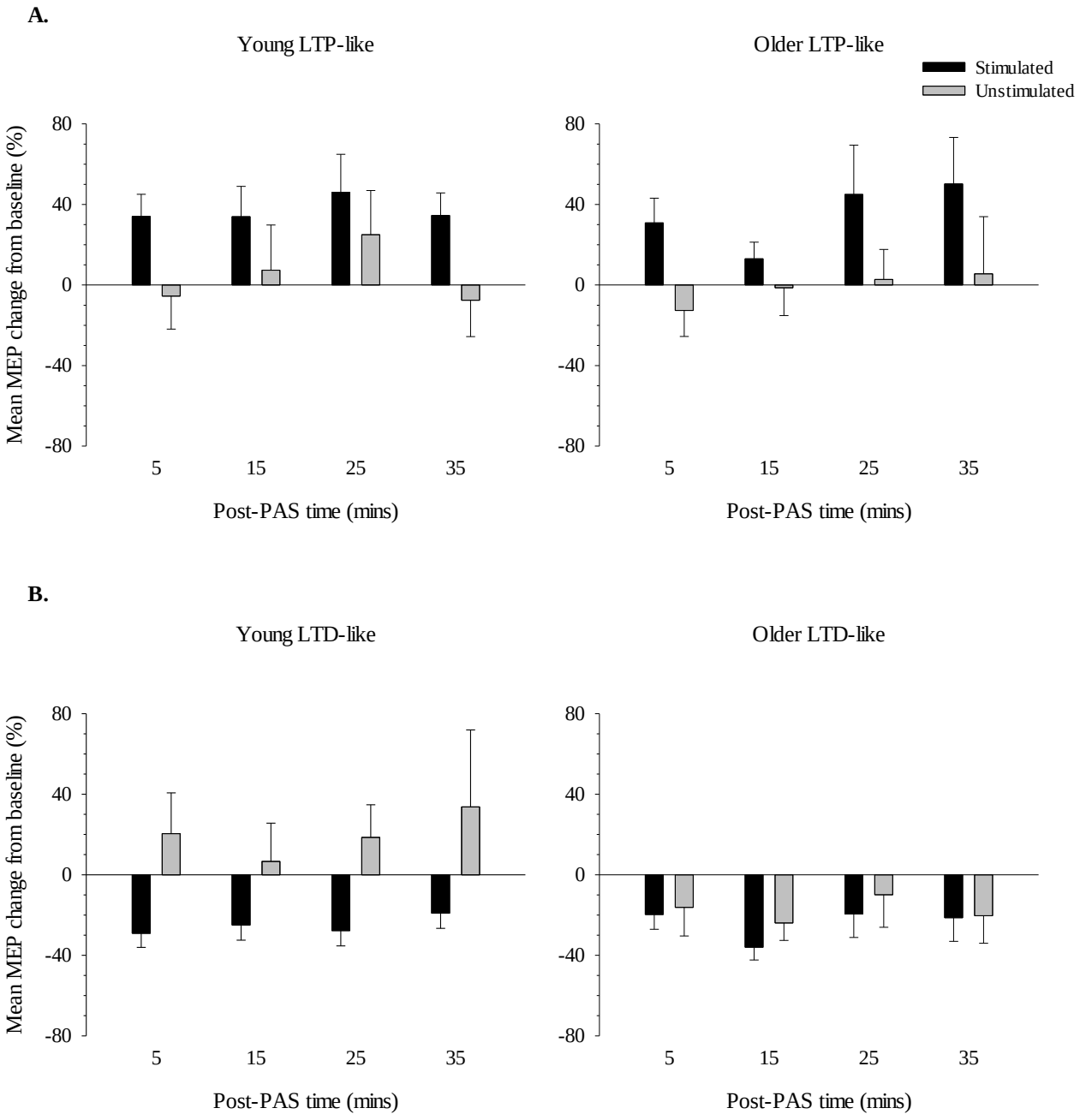

Figure 4-6 Mean MEP change relative to each individuals baseline for young and older LTP-and LTD-like responders in the attend left condition. MEP amplitudes increased significantly in LTP-like responders and decreased significantly in LTD-like responders. Although there was no difference in the corticospinal plasticity induced in the target (stimulated) and non-target (unstimulated) pathways in LTD-like responders, there was a significant difference between the pathways in LTP-like responders. PAS-induced corticospinal plasticity was significant only in the target pathway, however, in both LTP- and LTD-like responders. Error bars denote SEM.

4.5 Discussion

The aim of this study was to compare the effects of a TMS plasticity-inducing procedure on corticospinal excitability within the target and the non-target motor pathways of young and older adults. We hypothesised that compared with young adults, older adults would show greater bilateral PAS-induced corticospinal plasticity, due to age-related reductions in the lateralisation of neural activity. It was demonstrated that older adults performed worse relative to young adults on the Stroop, Logical memory recall and motor performance tasks. These effects are typical of adults around 69 years of age with an average to high level of education (Van der Elst et al., 2006; Abikoff et al., 1987; Aoki & Fukuoka, 2010). The magnitude of PAS-induced corticospinal plasticity was not altered with advancing age, but there was a trend whereby older adults tended to demonstrate decreases as opposed to increases in MEP amplitude. At the group level, however, reliable PAS-induced corticospinal plasticity effects were not evident. This effect was due to substantial individual variability in the PAS-induced effects, with only half of all participants demonstrating the expected increase in MEP amplitude. Moreover, rather than showing no change, individuals demonstrated robust corticospinal plasticity in opposite directions, with half the participants demonstrating positive (> 0%) and half negative (< 0%) MEP change post PAS. It was also found that significant changes in corticospinal excitability were limited to the target pathway. Importantly, this pattern of effects did not differ between young and older adults. Contrary to prediction, this result suggests that PAS-induced corticospinal plasticity does not manifest more bilaterally with advancing age. With regards to the modulation of PAS-induced corticospinal plasticity by attention, attending to the target hand did not significantly enhance PAS-induced corticospinal plasticity in comparison to when attention was directed to the opposite hand.

4.5.1 Individual variability in PAS-induced corticospinal plasticity

As noted above, there was considerable interindividual variability in the magnitude of PAS-induced corticospinal plasticity in the current study, with only half of all participants demonstrating the expected increase in MEP amplitude. Importantly, the proportion of young and older adults demonstrating LTP-like or LTD-like responses was approximately equivalent (40-60%), therefore age does not account for the variability in the direction of PAS induced effects in the target hemisphere. In addition, the number of male and female LTP- and LTD-like responders was similar, suggesting that gender also does not account for differences in the direction of corticospinal plasticity effects. Not only were responses more variable across individuals than in previous reports (Fratello et al. 2006; Müller -Dahlhaus et al., 2008) but also, when looking only at the individuals who demonstrated the expected increase in MEP amplitude in the current study, corticospinal

plasticity effects in the target pathway were smaller in comparison to previous reports (~30% increase in MEP amplitude). One methodological factor that may have contributed to this variation could be the duration of the PAS intervention. To avoid potential problems associated with fatigue from having to undertake the attention task for a long period of time an 11-minute PAS protocol, which is shorter than that used more widely in the literature (15-30 mins), was implemented in the current study. This duration, although short relative to that originally used with PAS (i.e., 30 min in Stefan et al., 2000) has been shown to induce corticospinal plasticity in young adults (Sale et al., 2007). The study by Sale and colleagues (2007) demonstrated that the shorter PAS protocol used in the current study facilitated MEP amplitudes on average by 51%. Few studies, however, have utilised this shorter protocol, therefore its reliability for inducing PAS effects is not well established. It is possible that this shorter PAS protocol may not only result in more variable PAS-induced effects across individuals but also differentially affect responses in young and older adults. Specifically, in aged animal models, longer stimulation protocols are required to induce LTP and this has been attributed to age-related reductions in intracellular calcium levels (Foster, 1999; Foster & Norris, 1997). Based on this evidence and reports of attenuated and sometimes non-existent TMS-induced corticospinal plasticity in older adults (Todd et al, 2010; Bashir et al., 2014; Fathi et al, 2010; Müller-Dahlhaus et al., 2008; Tecchio et al., 2008; Freitas et al., 2011), it is possible that longer stimulation protocols are necessary to reach the threshold for LTP-like effects in older adults. However, in the current study similar numbers of young and older adults responded with the expected increase in cortical excitability following PAS. Therefore, it seems likely that the magnitude and manifestation of corticospinal plasticity induced by PAS is more dependent on factors other than the stimulation protocol.

It is also possible that the use of a standard interval between the presentation of the peripheral nerve stimulation and the TMS pulse (25 ms) contributed to the variation evident between individuals and age groups in the current study. The signal generated by the peripheral electrical stimulation takes approximately 21-23 ms to reach M1. Hence, PAS 25 usually results in arrival of the afferent signal prior to the presentation of the TMS pulse, and thereby induces LTP-like effects. In individuals where the afferent signal takes longer to reach M1, the TMS pulse might be delivered prior to the arrival of the afferent signal from the peripheral nerve, which may be more likely to induce LTD-like effects (Wolters et al., 2003; but see Thickbroom et al., 2007). This could be a particular problem in older adults where conduction time may be slowed by age-related degeneration in the peripheral somatosensory afferents (Tanosaki et al., 1999; Zumsteg & Wiser, 2002), but the evidence regarding this is mixed (Fathi et al., 2008; Pellicciari et al., 2009). Interestingly, evidence demonstrates that PAS effects might not be reversed due to small changes in the timing of inputs to

M1. Specifically, it has been shown that intervals of 21.5 ms can also induce reliable increases in excitability in young adults (Weise et al., 2006; Hamada et al., 2014). With an interval of 21.5 ms the same temporal reversal of inputs to M1 as might be expected in older adults is likely to occur in young individuals, yet LTP was induced. Although effects induced by PAS 21.5 and PAS 25 may be mediated by different interneuron circuits (Hamada et al., 2014), as both intervals appear to induce similar responses it remains possible that small systematic differences in conduction time between young and older adults or LTP-and LTD-like responders might not significantly alter the direction of corticospinal plasticity effects. This contention is further supported by Müller-Dahlhaus and colleagues (2008) who demonstrated similar variability in corticospinal plasticity effects when the PAS interval was tailored to each individual's peripheral conduction time for young and older adults. Importantly, that result suggests that the use of a standard interstimulus PAS interval is unlikely to account for the substantial individual variability in PAS-induced corticospinal plasticity in the current study. Instead, it is likely that differences in additional individual characteristics contributed to the observed variability in responses to PAS (Ridding & Ziemann, 2010). Importantly, we controlled for the potential influence of time of day (Sale et al., 2007) and attention (Kamke et al., 2012; 2014), but additional factors such as genetic polymorphisms or nicotine consumption may have played a role (see Ridding & Ziemann, 2010 for review). Future studies would benefit from investigating the additional factors that mediate PAS-induced corticospinal plasticity, and whether these vary between young and older adults.

4.5.2 Distribution of PAS-induced effects across bilateral motor cortices

A key question in the current study was whether the distribution of PAS-induced corticospinal plasticity across the hemispheres varied between young and older adults. Although there were no differences in PAS-induced corticospinal plasticity between the hemispheres at the group level, there was a reliable difference in PAS-induced corticospinal plasticity between the target and non-target hemispheres when examined in LTP- and LTD-like responders separately. Specifically, reliable PAS-induced corticospinal plasticity effects were evident only in the target pathway and not in the non-target pathway. This was the case for both LTP-like and LTD-like responders and was an effect that did not differ between young and older adults. Although this finding is inconsistent with the few studies that have demonstrated reliable bilateral TMS-induced corticospinal plasticity effects (Suppa et al., 2008; Ishikawa et al 2007, Stefan et al., 2008; Bashir et al., 2014), these studies used alternative TMS protocols that are likely to have induced corticospinal plasticity by similar but slightly different mechanisms. Only one study has investigated bilateral effects induced by PAS and those authors found bilateral LTP-like effects at the group level that did not differ

between the hemispheres in young adults (Shin & Sohn, 2011). The results of the current study are similar in that the degree of MEP change did not differ reliably between the target and non-target hemispheres, but this effect was found only in the LTP-like responders in the attend right condition and not at the overall group level. In the LTD-like responders in the attend right condition, MEP change in the target pathway was reliably larger than MEP change in the non-target pathway, suggesting that LTD-like corticospinal plasticity induced by excitatory PAS when attention is allocated to the target hand is isolated to the targeted pathway. For the attend left condition the opposite effect was observed: PAS-induced effects did differ across the pathways in LTP-like responders but were comparable across the pathways in LTD-like responders.

Although there was a trend toward a difference in PAS-induced effects in older and young adults, this difference was not reliable. Importantly, when looking at the LTP-and LTD-like responders separately, there was little evidence of altered corticospinal plasticity in older adults. This result, demonstrating comparable corticospinal plasticity across the age groups, is consistent with other studies using TMS to investigate corticospinal plasticity in the motor cortices of young and older adults (Di Lazzaro et al., 2008; Pellicciari et al., 2009; Young-Bernier et al., 2014). However, this result does not fit with the wider body of literature from human (Fathi et al., 2010; Müller-Dahlhaus et al., 2008; Todd et al., 2010) and non-human animal models (Barnes, 1979; Barnes & McNaughton, 1985; Deupree et al., 1993; Sankar et al., 2000) demonstrating attenuated neuroplasticity in the aged brain.

It is possible that the current sample of healthy, self-selecting older adults did not show declines in corticospinal plasticity as they were not experiencing age-related neurobiological change. Based on the similar recruitment and screening criteria to previous studies of this kind (Fathi et al., 2010; Müller-Dahlhaus et al., 2008; Todd et al., 2010), however, it would not be expected that the current sample of older adults were systematically different from those of previous studies demonstrating a decline in corticospinal plasticity and neuroplasticity in general. In addition, it was found that older adults performed worse on the cognitive and motor assessments compared with their younger counterparts in the current study, suggesting this sample was experiencing some level of age-related neurobiological decline. Another explanation for the failure to find a reduction in corticospinal plasticity in older adults is that they were more physically active than the younger group. Based on the finding that physical activity can facilitate corticospinal plasticity (Cirillo et al., 2009), it is possible that plasticity was boosted in our sample of older adults and possibly even attenuated in the young group, due to inactivity. To explore the relationship between cognitive and motor performance and levels of corticospinal plasticity a series of correlations were performed. No

relationship was found between performance on the cognitive, motor and psychological measures with average MEP change post PAS (data not shown). Although this suggests that variability in these factors does not account for variability in PAS-induced corticospinal plasticity, it must be acknowledged that it seems unlikely that any one factor alone is likely to explain individual differences in PAS-induced effects. To better understand corticospinal plasticity effects in young and older adults and to account for some of the variability in responses, future studies would benefit from including an additional measure of age-related change in the peripheral musculature, such as maximal M-waves. These have been found to be reduced in older adults (Vandervoort & McComas, 1986; McNeil et al., 2005; Christie & Kamen, 2014; Goodwill, 2015; Pitcher et al., 2003), suggesting that the interpretation of the results may have been altered should MEP data have been normalised to Mmax. Therefore, future studies would benefit from measuring the capacity of the peripheral musculature using maximal M-waves, in order to assess the degree to which this influences corticospinal plasticity. It is also an interesting question whether there are more discrete changes in cortical microcircuitry that were not detected with the single pulse MEP measure used in the experiments of this thesis. This might be particularly informative when comparing between LTP-like and LTD-like responders. It was beyond the scope of the current study to include a comprehensive battery of measures probing changes in the microcircuitry as there was insufficient time between the post PAS time points to include additional measures. Future studies, however, would benefit from using paired pulse TMS techniques to better understand age-related differences in the balance of excitation and inhibition in these circuits.

4.5.3 The effect of spatial attention

It was hypothesised that corticospinal plasticity would be enhanced in conditions in which attention was directed to the hand targeted with PAS. Importantly, although there were greater errors made in the attend right condition, accuracy was high and few errors were made overall, indicating that participants were engaged in the task. Error rates did not differ significantly between young and older adults. Our results indicate that there was no differences in post-PAS MEP change between attend right and attend left conditions. This finding does not lend support to our hypothesis and is not consistent with the findings of studies investigating the modulatory role of attention in TMS-induced corticospinal plasticity (Johansen-Berg & Matthews, 2002; Kamke et al., 2012; Kamke et al., 2014; Rosenkranz & Rothwell, 2004; 2006; Stefan et al., 2004). However, this null effect is difficult to interpret, as there were no PAS-induced effects at the group level. Furthermore, it was also not possible to look at the role of attention in LTP and LTD-like responders as these were classified separately for the different testing sessions, in which attention was directed to one or

other hand. This was done because there was inter-session variability in PAS-induced responses within individuals (see Ziemann & Siebner, 2015). Specifically, many participants who were classified as an LTP-like responder in one condition were the opposite in the other condition, and *vice versa*. An additional 'quadrant' analysis was performed on this data to better investigate the manisfeatation of plasticity across the hemispheres in individuals who responded consistently and inconsistently across the different attention conditions. The results and interpretation of this analysis can be found in Appendix D.

The failure to find an effect of attention in the current study may be explained by variation in the attention manipulation across the different studies. Some previous studies have demonstrated the facilitatory role of attention on corticospinal plasticity by manipulating attention with tactile stimulation (Stefan et al., 2004). It is possible that studies involving tactile stimulation have a greater attentional effect on corticospinal plasticity due to the interaction between the TMS stimulation and heightened excitability in sensorimotor cortices triggered by attending to the tactile stimulation. However, other studies have used manipulations of attention that are more external to the motor system. For example, Kamke and colleagues (2014) demonstrated that PAS-induced corticospinal plasticity could be enhanced by allocating attention toward the target hand using a visual spatial attention task. This suggests that corticospinal plasticity can be modulated by attention, even in the absence of tactile stimulation. In difference to Kamke and colleagues (2014), the current study required participants to perform an overt visual attention task located on the thumb, wherein participants verbally responded each time they detected a target. Kamke and colleagues (2014) instead required participants to silently count the number of targets and report this number at the end of each trial, during which PAS occurred. It is possible that the modified version used in the current experiment was too easy and did not engage sufficient attentional resources to alter PAS-induced corticospinal plasticity. More specifically, by using a trial-by-trial design the previous work may have ensured attention was directed toward the hand every time PAS occurred, which is not guaranteed in the present study. Alternatively, previous research also demonstrates that corticospinal plasticity is attenuated under conditions of high cognitive and attentional load (Kamke et al., 2012; Stefan et al., 2004). Thus it is also possible that the current attention task was too difficult, thereby reducing corticospinal plasticity. Based on the low number of errors made in the current study, however, it seems unlikely that the task was too difficult. Future research might consider varying the complexity of concurrent attention manipulations to assess the degree to which the modulation of corticospinal plasticity by attention is dependent on the attention task in attempt to identify the ideal attentional/task conditions in which LTP-like corticospinal plasticity is enhanced by attention.

It is interesting to note that the distribution of corticospinal plasticity across bilateral corticospinal pathways in LTP-like and LTD-like responders differed depending on the (attention) session. More specifically, LTP-like responders demonstrated comparable corticospinal plasticity bilaterally in the attend right condition but not in the attend left condition. Moreover, LTD-like responders demonstrated comparable corticospinal plasticity bilaterally in the attend left condition but not in the attend right condition. At the very least the current results together with the wider literature highlighting attention effects (Stefan et al., 2004; Kamke et al., 2012; 2014) suggest that attention should be controlled in future studies investigating PAS-induced corticospinal plasticity in young and older adults.

4.5.4 Conclusions

The current study investigated how PAS-induced corticospinal plasticity manifests differentially in the target and non-target corticospinal pathways of young and older adults and how attention might influence this effect. PAS-induced corticospinal plasticity in both young and older adults was not significant overall, but there was great individual variability in responses. Examining LTP-and LTD-like responders separately revealed robust PAS-induced effects occurring in opposite directions. These effects were more pronounced in the target corticospinal pathway in both young and older adults. Contrary to predictions this result suggests that the distribution of PAS-induced corticospinal plasticity across bilateral corticospinal pathways is not altered in the aged brain. There was no evidence to suggest that corticospinal plasticity is significantly modulated by the focus of spatial attention. However, this null result is difficult to interpret due to the lack of corticospinal plasticity effects at the group level. Based on previous work and evidence that attention may be altered in older adults the role of attention in modulating corticospinal plasticity remains an important question.

The implications of this research are particularly important in light of our current ageing population. With advancing age comes increased risk of experiencing brain injury such as stroke, as well as age-related decline in motor functioning (Buckley et al., 2009; Donnan et al., 2008; Falkenstein et al., 2006). A greater understanding of how neuroplasticity is affected by advancing age, and how factors such as attention might modulate neuroplasticity, will have implications for the development of age-appropriate strategies to enhance motor learning in older adults. Moreover, this understanding will be important for informing motor rehabilitation in older adults suffering a brain injury.

4.6 Acknowledgements

This work was supported by a Project Grant from the National Health and Medical Research Council of Australia (NHMRC; APP1028210). DD was supported by an Australian Postgraduate Award scholarship. MS was supported by an NHMRC Training Fellowship (APP1012153). The funders had no role in study design, data collection and analysis, or preparation of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding bodies.

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Chapter 5 General Discussion

5.1 Summary of aims and results

The aim of this thesis was to determine the extent to which neuroplasticity in the motor system is altered in neurologically healthy older adults. This research utilised three different methods of inducing neuroplasticity in the motor system; motor training, iTBS and PAS. Neuroplasticity was quantified in bilateral corticospinal pathways following each intervention by measuring MEPs. Neuroplasticity was also quantified behaviourally in Experiment 1. Experiment 1 aimed to investigate whether transfer of a unilateral motor skill to the opposite, untrained hand and associated changes in bilateral corticospinal excitability are altered in older adults. Moreover, Experiment 1 investigated whether these effects vary between simple and complex tasks. It was hypothesised that young adults would demonstrate transfer with simple and complex tasks, but that older adults would demonstrate transfer with only the complex task. Contrary to the experimental hypothesis and to previous literature, it was demonstrated that older adults transfered performance gains to the opposite, untrained hand, irrespective of the complexity of the task. It was also hypothesised that corticospinal excitability would increase bilaterally in young adults with the simple task, but only in the corticospinal pathway innervating the trained hand with the complex task. In older adults corticospinal excitability was predicted to increase bilaterally with both simple and complex tasks. Contrary to the hypotheses young and older adults demonstrated comparable corticospinal plasticity with the simple and complex tasks. Specifically, MEPs increased bilaterally with both the simple and complex tasks, although changes in MEPs in the complex task were statistically marginal. It was also found that the performance improvements were smaller in older, relative to young adults in the simple condition.

Experiment 2 aimed to investigate how the manifestation of corticospinal plasticity is altered following iTBS. It was hypothesised that older adults would show greater MEP change in the unstimulated pathway relative to younger adults, which would be accompanied by a reduction in MEP change in the stimulated pathway. There were no reliable corticospinal plasticity effects evident at the group level due to substantial inter-individual variability. After categorising participants into LTP-like and LTD-like responders based on average responses in the target muscle, iTBS was found to induce strong increases and decreases in MEP amplitude in these groups, respectively. Importantly, iTBS was equally effective at inducing corticospinal plasticity in young and older adults, and the distribution of the effects across bilateral corticospinal pathways did not vary as a function of age. Specifically, iTBS effects were restricted to the stimulated pathway in young and older adults. Experiment 3 aimed to further investigate whether corticospinal plasticity is altered in the ageing brain, while also examining the role of attention in modulating corticospinal

plasticity in young and older adults. It was hypothesised that older adults would demonstrate greater MEP change in the unstimulated pathway relative to young adults, which would be accompanied by reduced corticospinal plasticity in the stimulated pathway. Like iTBS-induced effects, PAS responses were also highly variable across individuals. At the group level it was found that the magnitude of corticospinal plasticity effects did not differ between the groups, but there was a weak trend wherein young adults were more likely to demonstrate increases and older adults demonstrate decreases in MEP amplitude. To examine the distribution of corticospinal plasticity across the targeted and non-targeted pathways individuals were classified as LTP-like and LTD-like responders, as per Experiment 2. Similar to the effects of iTBS, PAS was shown to induce significant increases and decreases in MEP amplitude in these groups, respectively. There was some evidence for changes in excitability in both the targeted and non-targeted pathways, but PAS was found to be equally effective at inducing corticospinal plasticity in young and older adults and importantly advancing age did not alter the distribution of corticospinal plasticity across the targeted and non-targeted corticospinal pathways.

Together, the results from the three studies using different methods to induce corticospinal plasticity suggest that neuroplasticity is not necessarily reduced in the aged brain. In each of the previous experimental chapters, explanations for, and the implications of these findings were discussed. These ideas are expanded upon in the following sections, and directions for future research are presented.

5.2 Individual variability in responses to plasticity-inducing interventions

Although PAS and iTBS did induce robust changes in MEP amplitude in the target M1, half the participants demonstrated the expected increase in MEP amplitude while the other half demonstrated a decrease. As a result of this variability there were no reliable corticospinal plasticity effects at the group level with PAS or iTBS. Variability in responses to TMS-based neuroplasticity interventions has been demonstrated on several occasions (Japyassú et al., 2014; Müller-Dahlhaus et al., 2008; Lopez-Alonso et al., 2014; Hamada et al., 2013; Sale et al., 2007; Fratello et al., 2006). TMS-induced corticospinal plasticity responses are influenced by many of the same factors that modulate synaptic plasticity in animal models. Indeed, such effects further support the idea that TMS-induced corticospinal plasticity and synaptic plasticity involve similar mechanisms. The problem, however, is that individual differences in the factors that influence neuroplasticity increase the variability in TMS-induced corticospinal plasticity, thereby reducing the reliability of TMS interventions at the group level. The following paragraphs highlight some of the factors known to contribute to TMS-induced corticospinal plasticity (see Ridding & Ziemann, 2010 for review) and

discuss the degree to which these factors may have contributed to the variability evident in the current experiments.

The factor at the centre of the investigations of this thesis was age. While age has been shown to influence corticospinal plasticity induced by TMS interventions previously (Fathi et al., 2010; Müller-Dahlhaus et al., 2008; Todd et al., 2010), the findings from the experiments of this thesis are mixed. Experiment 2 showed no difference in responses between young and older adults, whereas Experiment 3 indicated a marginally different direction of effects in young and older adults, with similar absolute magnitude. Age differences in corticospinal plasticity are discussed in more detail later in this chapter. For now, as there were equal numbers of young and older adults in each of the (LTP- and LTD-like) responder groups, these findings suggest that factors other than age might be more important in modulating responses to different TMS interventions. Gender has also been shown to influence neuroplasticity (McEwen, 1999; Galea et al., 2006). Although there were equal numbers of males and females in each age group, gender effects may depend on circulating hormone levels and menstrual cycles (Inghilleri et al., 2004), which could produce a systematic difference between young and older adults. As discussed in Chapter 3 and 4, however, corticospinal plasticity did not vary reliably as a function of gender and there were equivalent proportions of males and females in each of the responder groups. Attention is also a factor that has been shown to modulate corticospinal plasticity in response to different TMS interventions (Stefan et al., 2004; Kamke et al., 2012; 2014). Importantly, attention was controlled in Experiment 2 and 3, so there were likely no systematic differences in the allocation of attention. The role of attention is also discussed later in this chapter. Another source of variability with TMS interventions is time of day (Sale et al., 2008). In each of the experiments of this thesis participants completed sessions at the same time of day, which was counterbalanced across individuals to eliminate variability due to diurnal fluctuations in cortisol (Sale et al., 2008). Moreover, time of day has been shown to modulate the magnitude of corticospinal plasticity but not the direction, which was the main driver of variability in Experiment 2 and 3. Therefore, systematic differences in time of day would not be expected to explain these findings.

An additional factor shown to be critical in determining neuroplasticity induction is the history of synaptic activity. Specifically, responses to neuroplasticity inducing protocols are dependent on whether the cells targeted by that intervention have been previously potentiated or suppressed (Muller et al., 2007; Siebner et al., 2004; Lang et al., 2004; Nitsche et al., 2007; Ragert et al., 2009; Hamada et al., 2009; Ziemann et al., 2004; Gentner et al., 2008; Antal et al., 2007). As discussed in Chapter 1, priming with prior voluntary activity influences the direction (LTP-like *vs.* LTD-like),

magnitude, and variability of plastic responses (Gentner et al., 2008; Iezzi et al., 2008; Kujirai et al., 2006; Stefan et al., 2006; Ziemann et al., 2004; Rosenkranz et al., 2007). For example, Goldsworthy and colleagues (2014) demonstrated that when participants voluntarily activated the target muscle activity prior to TBS, corticospinal plasticity became more variable across subjects than when administered without prior voluntary contraction. While individual variability in responses to neuroplasticity interventions may have been increased by prior voluntary activity in previous research, there was no prior activity involved in either the current PAS or TBS studies. Prior activity was minimised in these experiments by using rMT as opposed to aMT, as the former does not require voluntary muscle activity. Thus, prior voluntary activity shortly before the intervention should not account for the variability evident in the PAS and TBS studies of this thesis.

Although activity immediately before the neuroplasticity-inducing interventions was controlled, activity occurring before the testing sessions was not. Evidence indicates that prior voluntary activity can influence neuroplasticity induction, even when occurring days before a neuroplasticity inducing intervention (Buschler, 2012). For example, evidence from studies in animal models demonstrates that exposure to an enriched environment under social conditions facilitates robust LTP synaptic plasticity (Buschler, 2012). Participants in the experiments of this thesis came to the lab from many different backgrounds, with different expertise. Based on the evidence from animal models it might be assumed that variability in participants' life experience might contribute to the variability in the direction of corticospinal plasticity effects evident in Experiment 2 and 3. Evidence from studies in humans certainly suggests that prior experience influences brain function (Gindrat et al., 2015). For example, somatosensory cortical activity is modulated by touch screen mobile phone use (Gindrat et al., 2015) and cortical representations of relevant fingers are enlarged in string players (Elbert et al., 1995; Pantev et al., 2001) and Braille readers (Pascual-Leone & Torres, 1993). Not only might prior experiences differ between individuals but they might also systematically differ between young and older adults due to cohort effects. Following from the above example, older adults may be less likely to use touch-screen mobile phones and thereby not experience the same change in brain function as younger adults, which could systematically alter responses to TMS neuroplasticity-inducing interventions targeting small hand muscles. Studies investigating metaplasticity typically do not take into account prior activity occurring before the testing session. Instead, these studies investigate the effects of priming with voluntary activity immediately prior to a neuroplasticity intervention (see Todd & Ridding, 2010 for review). Evidence does demonstrate, however, that metaplasticity may differ as a function of prior experience (Reuter et al., 2014; Rosenkranz et al., 2007). For example, experts with high occupational use of the hands and fingers are found to experience stronger intervention-related

improvement than non-experts after training on a tactile discrimination task (Reuter et al., 2014), supporting the idea that activity prior to the testing session influences responses to neuroplasticity inducing interventions. Future studies might try to account for the effects of prior experience and voluntary activity by manipulating the initial activity state of cells and synapses prior to neuroplasticity induction. These cells can be primed immediately prior to a neuroplasticity intervention by applying non-invasive brain stimulation (Silvanto & Pascual-Leone, 2008). Although it is still possible that prior experience influences additional factors during neuroplasticity induction, such as neuromodulators, which may result in differential neuroplasticity, variability in responses due to differences in initial cell states at baseline may be reduced.

One factor that may potentially influence PAS responses is peripheral conduction time. Peripheral conduction time refers to the time it takes somatosensory signals to travel from a peripheral nerve to the somatosensory and motor cortices. In order to reduce the time demands on older adult participants and to avoid altering the activity state of the target representation and corticospinal pathway prior to the PAS intervention, somatosensory conduction time was not measured. As a result, the interstimulus interval (ISI) between the peripheral nerve stimulation and TMS pulse was not individualised to each participant's conduction time in Experiment 3. Instead, the standard 25 ms ISI was used. This interval has been shown to induce robust corticospinal plasticity effects in young adults on many occasions (Stefan et al., 2000; 2004; Wolters et al., 2003; Fathi et al., 2010; Di Lazzaro et al., 2011; Rosenkranz et al., 2007; Player et al., 2012; Shin & Sohn, 2011; Kamke et al., 2012). However, there were no reliable PAS effects at the group level evident in Experiment 3 of this thesis, as individuals showed either LTP- or LTD-like effects. If conduction time were prolonged in some individuals, presentation of the TMS pulse would occur prior to the arrival of the somatosensory afferent signal in M1. If PAS effects reflect spike-timing synaptic plasticity this change in the order of events may explain LTD-like decreases in MEP amplitude. This could be a particular problem when comparing young and older adults, as the latter group might be more likely to show LTD-like effects because conduction time is slowed with advancing age. Whether conduction time is altered by advancing age is not well understood. Evidence suggests that somatosensory pathways might be altered by advancing age (Tanosaki et al., 1999; Zumsteg & Wiser, 2002), but the evidence is mixed regarding age-related differences in peripheral conduction time (Fathi et al., 2008; Pellicciari et al., 2009). The equal number of young and older adults in both LTP-and LTD-like responders in Experiments 2 and 3 suggests that there was no systematic age difference in conduction time. Moreover, a significant proportion of participants responded with LTP-like effects in one session, but LTD-like effects in the other session, which suggests conduction time does not explain the individual difference in responses as conduction time was held

constant across sessions. In addition, evidence suggests that a reversal of inputs does not necessarily result in LTD (Weise et al., 2006). Therefore, a small systematic difference in conduction time between young and older adults or LTP-and LTD-like responders might not significantly alter corticospinal plasticity effects. More specifically, intervals of 21.5 ms can also induce reliable increases in excitability in young adults (Weise et al., 2006). With an interval of 21.5 ms the same temporal reversal of inputs to M1 is likely to occur in some individuals, yet LTP was induced in that study. Thickbroom (2007) suggests that PAS is not a spike-time dependent intervention, as the temporal resolution is not sufficiently precise. Instead, it is suggested that PAS triggers converging inputs that increase network activity. Therefore, PAS may be more mechanistically similar to rate-dependent models of synaptic plasticity. This contention might explain why the two stimuli must occur within a specific time window (between 21.5-25 ms) for LTP-induction (Wolters et al., 2003) and also why LTP-like effects are not critically dependent on the afferent signal arriving immediately prior to the TMS pulse (Weise et al., 2006). This explanation, suggesting that the precise order of the inputs might not be so important for LTP-induction is consistent with evidence that demonstrates that even after basing the ISI on each individual's latency of somatosensory electrical potentials evoked by peripheral nerve stimulation, considerable variability in the direction of PAS effects remains (Müller-Dahlhaus et al., 2008; Kriváneková et al., 2011). Therefore, factors other than conduction time may be driving individual variability in the direction of PAS effects.

One factor that is well documented for its facilitatory effects on neuroplasticity induction is participation in regular aerobic exercise (see Kramer & Erickson 2007 for review; Cirillo et al., 2009). Physical activity is therefore an additional factor that may have varied systematically between participants showing LTP-like increases and LTD-like decreases in MEP amplitude. Moreover, physical activity might be expected to vary systematically between young and older adults and thereby explain marginal age differences in the direction of PAS induced effects. Physical and sedentary activity was quantified in Experiment 3, but contrary to previous literature neither physical nor sedentary activity correlated with MEP change. It could be argued that the score obtained from the self report measures was not a reliable reflection of participant's actual physical and sedentary activity. However, similar self report measures have been used in previous studies (Cirillo et al., 2009) to distinguish highly active and less active individuals, who demonstrated significantly different responses to a TMS neuroplasticity intervention. Therefore, it seems that variability in the frequency of physical activity did not explain variability in corticospinal plasticity responses in Experiment 3. Moreover, with regards to any potential systematic difference between young and older adults, contrary to what might be expected, older adults reported greater levels of physical activity than did young adults in Experiment 3. Based on

the evidence indicating the facilitatory effect of physical exercise, this group of highly active older adults might be expected to show enhanced corticospinal plasticity relative to the less active (younger) group. Instead, there were no differences between young and older adults. It is possible, of course, that corticospinal plasticity was increased in this highly active group of older individuals and/or that corticospinal plasticity was reduced in the young adults relative to the normal population due to inactivity. This may have led to no difference at the group level in this particular sample, where one does in fact exist in the wider population. It is also possible that there was a systematic difference in demand characteristics between young and older adults, such that older adults dishonestly reported higher levels of physical activity. Future studies may benefit from using more objective measures to ascertain levels of physical activity, such as body mass index (BMI, World Health Organisation, 2006) or implement the use of personal activity monitors in the weeks prior to and/or between testing sessions to more thoroughly establish the link between physical activity and corticospinal plasticity.

There are numerous additional variables that may have contributed to the individual variability observed in coticospinal plasticity effects in the studies of this thesis. For example, individual differences in the amount of sleep participants had before each session (Marshall et al., 2006), whether they were regular consumers of nicotine (Swayne et al., 2009) or alcohol (Zorumski et al., 2014), or carriers of the single-nucleotide polymorphism of the BDNF gene (Kleim et al., 2006; Cheeran et al., 2008; but see Chaieb et al., 2014 for review) may have made some participants more likely to express LTD than LTP. There may also be a systematic difference in these factors between young and older adults, which might account for the marginal age difference in the direction of PAS-induced responses in Experiment 3, as well as the age-related declines in cognitive and motor performance (Experiment 3) and motor learning (Experiment 1). For example, it is well established that sleep is altered in old age (e.g., Moraes et al., 2014). Slow wave sleep plays a critical role in maintaining homeostasis at a synapse (see Tononi & Cirelli, 2006 for review) and a reduction in the amount of slow wave sleep may result in insufficient normalising of synapses, which may make them less likely to undergo further potentiation in response to a neuroplasticity intervention. This highlights the need for future studies to better characterise participants by quantifying lifestyle and genetic factors or by targeting specific groups of characteristically similar individuals. The former would be better done with more objective measures to avoid systematic differences in demand characteristics between young and older adults.

Interestingly, although there was great variability in responses to the TMS neuroplasticity protocols, the direction of corticospinal plasticity was more consistent when induced by motor training. More

specifically, participants demonstrated more consistent LTP-like as opposed to LTD-like MEP change. Identical recruitment and screening strategies were used for all three experiments, suggesting this difference was not due to systematic variability in participant characteristics. Although it is assumed that each neuroplasticity intervention targets a similar neural network, motor training involves several components over and above those involved with TBS and PAS, which may have resulted in more consistent LTP-like effects across individuals. During motor training, participants must monitor and adjust their behaviour in accordance with the requirements of the tasks. This goal-directed behaviour involves cognitive as well as motor regions (see Gallivan & Culham, 2015 for review). In addition, there is much sensory input and sensorimotor integration occurring during motor training, which is potentially absent with TBS and reduced with PAS. Moreover, motor training presumably engages the network in a systematic manner, activating task-relevant excitatory and inhibitory cells in specific temporal sequences, whereas TMS immediately excites all cell types within a circumscribed region. Arguably, PAS is likely to stimulate the sensori-motor network in a way that is more similar to motor training than with iTBS, but both techniques are non-focal and stimulate many cell types within M1, which might be irrelevant and have counteractive effects on one another that do not occur with goal-directed motor training. The non-specific nature of activity may increase variability in the direction of iTBS- and PAS-induced effects across individuals. These factors together may promote LTP-like plasticity more so in response to motor training than TMS interventions. It is also possible that the factors that contribute to individual variation discussed above, for example prior voluntary activity or the BDNF polymorphism, may have differential effects on neuroplasticity induced by motor training and TMS (see Chaieb et al., 2014 for review of the effects of the BDNF polymorphism on TMS-induced and training-induced plasticity).

Because each of the techniques used to induce neuroplasticity in this thesis likely involves similar but slightly different mechanisms, implementing the different protocols within the same individual may be beneficial. However, Vallence and colleagues (2013) investigated the relationship between participants' responses to different TMS neuroplasticity protocols (PAS, cTBS, iTBS) and whether these responses correlated with performance improvements following training on a simple ballistic task. These authors found decreases in MEP amplitude with cTBS but no reliable change in responses to any other protocol, suggesting no association between responses to different protocols. Time of day and gender were controlled in each of these experiments, but attention was not. Therefore, it is possible that individual differences in attention contributed to the variability in responses. Future studies would benefit from investigating the effects of different neuroplasticity-inducing protocols within subjects while controlling for attention. In addition, these authors suggest

that large intra- and inter- group variability makes it difficult to demonstrate associations between responses to different protocols. Future studies should investigate the influence of different neuroplasticity-inducing protocols within subjects, but also include between groups factors thought to be important for neuroplasticity, such as genetic polymorphism or level of physical activity. Studies of this kind would develop a better understanding of the factors that facilitate neuroplasticity, which might inform the development of better strategies to facilitate neuroplasticity in the aged brain.

5.3 Age differences in the manifestation of corticospinal plasticity

As discussed in the introduction of this thesis, evidence demonstrates that synaptic plasticity is altered with age in non-human animal models (Barnes, 1979; Barnes & McNaughton, 1985). Studies so far, examining neuroplasticity within the corticospinal pathways of the motor system, suggest that neuroplasticity, specifically corticospinal plasticity, is also reduced in older humans (Todd et al, 2010; Fathi et al, 2010; Müller-Dahlhaus et al., 2008; Tecchio et al., 2008; Freitas et al., 2011). But the focus of that work has been on the stimulated or trained corticospinal pathway. It is well established that older adults exhibit greater and more widespread activity within and across hemispheres during the performance of cognitive and motor tasks (Cabeza, 2002; Reuter-Lorenz et al., 2000; Grady et al., 1996; Calautti et al., 2001; Carp et al., 2011; Heuninckx et al., 2005; Heuninckx et al., 2008; Hutchinson, 2002; Inuggi et al., 2011). Consistent with this evidence, it was predicted that corticospinal plasticity would become less lateralised to the stimulated pathway, and would instead manifest over a more bilateral network in older relative to younger adults. Contrary to previous literature (Todd et al, 2010; Fathi et al, 2010; Müller-Dahlhaus et al., 2008; Tecchio et al., 2008; Todd et al., 2010; Freitas et al., 2011) support for this hypothesis was not found. Instead, the current findings suggest that the manifestation of corticospinal plasticity is not reduced in the target hemisphere and does not become less lateralised in old compared with young adults. Consistent with previous literature (Hinder et al., 2011), and in line with the spill over or cross activation model of cross limb transfer, whereby motor engrams are stored in bilateral M1s during unilateral movement or training, it was also expected that the age-related reductions in the specificity and laterality of neural activity would interfere with the spill over mechanism governing cross limb transfer and thus the transfer of a unilateral motor skill to the opposite, untrained hand would be reduced in older adults. This hypothesis was also not supported. Instead, young and older adults demonstrated comparable cross-limb transfer, with both simple and complex tasks. Together these results suggest that neuroplasticity is not reduced in the aged brain and that it does not manifest over a more bilateral motor network in older adults.

5.3.1 Why was corticospinal plasticity not reduced in the target hemisphere?

Several methodological differences exist between the current work and previous literature, which may account for the differences in the degree of corticospinal plasticity observed across studies. A comparison between the previous literature and the studies of this thesis, however, suggests that the lack of an age difference in the magnitude of corticospinal plasticity effects in the target hemisphere is not due to stimulation of the dominant as opposed to the non-dominant hemisphere, nor to any prior activity undertaken immediately before the intervention. Specifically, studies have demonstrated reduced corticospinal plasticity in the target pathway of old compared with young adults after interventions targeting the cortical representations of the dominant (Todd et al., 2010; Fathi et al., 2010; Müller-Dahlhaus et al., 2008; Tecchio et al., 2008; Freitas et al., 2011) and the non-dominant target muscles (Bashir et al., 2014; Di Lazzaro et al., 2008; 2011). In addition, corticospinal plasticity has been reported to be reduced in older adults both when the intervention is administered with (Todd et al., 2010; Freitas et al., 2011) and without (Fathi et al., 2010; Müller-Dahlhaus et al., 2008; Tecchio et al., 2008) prior voluntary activity. Furthermore, as discussed in Chapter 2, although measures of changes in spinal excitability were not undertaken in this thesis it is unlikely that such effects compensated for reduced cortical plasticity. Specifically, plasticity induced by TMS has been shown to involve very little adaptation at the spinal level in young adults (Stefan et al., 2000; Wolters et al., 2003; Muellbacher et al., 2001; Di Lazzaro et al., 2005). Moreover, plasticity within the spinal cord appears to be reduced with advancing age (see Papegaaij, 2014 for review). Therefore, any contribution of spinal adaptations to corticospinal plasticity is likely to be attenuated in older adults.

Given the apparently small age-related differences in corticospinal plasticity induced in the experiments involved in this thesis, it could be argued that the older adults participating in the experiments of this thesis were not experiencing substantial age-related neurobiological change. These older adults were healthy self-selecting participants and neuroimaging was not used to quantify age-related neurobiological structural change. However, it is noteworthy that older adults in Experiment 1 demonstrated significantly smaller performance improvements relative to the younger participants. In addition, Experiment 3 showed that the older adults in that study performed worse on several cognitive and motor tasks. Although declines in motor performance and learning may be due to age-related change in the peripheral nervous system, such as in the musculature (Rice & Cunningham, 2002, Klass et al., 2008), changes in cognitive performance reflect age-related change in the brain. Previous studies using similar recruitment strategies and screening criteria have also found evidence of age-related reductions in motor performance (Seidler et al., 2002; Hinder et

al., 2011; Parikh & Cole, 2013; Rogasch et al., 2009). Therefore, there is no reason to expect that the current sample of older adults was not experiencing some degree of age-related brain change or that they differed systematically from those recruited in previous studies.

It is important to note that although corticospinal plasticity effects were comparable in young and older adults, there were no reliable corticospinal plasticity effects evident at the group level. Robust effects emerged only after classifying participants into LTP-like and LTD-like responders. Investigating effects separately in LTP-and LTD-like responders has been done previously in young (Lopex-Alonso, 2014; Müller-Dahlhaus et al., 2008; Player et al., 2012; Kamke et al., 2012) but not older adults. It is therefore possible that in previous studies reporting non-significant corticospinal plasticity effects in the elderly (Todd et al., 2010; Fathi et al., 2010; Tecchio et al., 2008), that corticospinal plasticity was potentially induced in the older adults but that the variability in the direction of effects across individuals masked those effects at the group level.

Another factor that may have contributed to the apparent maintenance of corticospinal plasticity in the target pathway in older adults in this thesis is attention. It has been demonstrated that undertaking a concurrent attention task during a neuroplasticity intervention can modulate corticospinal plasticity effects in young adults (Kamke et al., 2012; 2014). No studies to date have investigated how corticospinal plasticity is altered by task complexity or the involvement of cognitive processes in the aged brain. Thus, it is possible that the involvement of attention and its allocation to the target limb may have facilitated corticospinal plasticity in older adults. The potential mechanism underlying this effect is discussed in section 5.4.

5.3.2 Why did corticospinal plasticity not manifest over a more bilateral motor network in older adults?

Contrary to the hypothesis that corticospinal plasticity would manifest more diffusely in older adults, with iTBS and PAS, changes in MEPs were only evident in the target pathway and this effect was reliable only after classifying participants as LTP- and LTD-like responders. In this context it is possible that the older adults in the current study were not experiencing age-related reductions in the lateralisation of neural activity. Without such change corticospinal plasticity may not be expected to manifest more diffusely. As well as the absence of any neuroimaging measure of age-related change in brain structure at baseline, there was also no measure of age-related change in functional task-related activity. Therefore, although reductions in motor learning in Experiment 1 and reductions in motor performance in Experiment 3 indicate that the samples of older adults tested in the studies of this thesis were experiencing age-related neurological change, the degree to

which these individuals were experiencing changes in the lateralisation of task-related functional neural activity remains unknown. Nonetheless, there were a number of similarities between the sample characteristics of the current studies and those of previous literature demonstrating age-related change in the lateralisation of neural activity. For example, there were similar gender distributions in all samples, the majority of participants were right-handed, had no history of neurological conditions, were English speakers and were of similar age (Naccarato et al., 2006; Carp et al., 2011). Based on the similarities in participants' characteristics, it could be expected that the current sample of older adults were likely to experience age-related change in the lateralisation of neural networks. Future research may benefit from using neuroimaging methods to quantify age-related changes in the lateralisation of functional task-related activity at baseline

Another reason for attenuated corticospinal plasticity in the non-targeted pathway in Experiment 2 and 3 might be that corticospinal plasticity manifested outside the representation of the target limb, in the surrounding premotor or non-motor regions. TMS is limited in that it can only quantify corticospinal plasticity within the stimulated corticospinal pathway and not in surrounding areas. It is possible that age-related reductions in the lateralisation of neural activity resulted in cortical adaptations in the opposite hemisphere that occurred across pre-motor or sensorimotor regions. This might be the case especially in Experiment 2 and 3, wherein corticospinal plasticity was induced with TMS interventions, which as discussed earlier may stimulate slightly different cells to those involved in motor training. If there were few cortical adaptations occurring in the homologous representation in the hemisphere opposite to that stimulated during the intervention, but greater adaptations outside this representation in pre motor regions, which drive performance improvements in the untrained hand, a reliable change in MEP amplitude may not be detected from stimulation of the corticospinal pathway ipsilateral to the trained limb. Therefore, although no reliable effects were evident in the non-target pathway in Experiment 2 and 3, the possibility that cortical adaptations did occur in the hemisphere ipsilateral to the trained hand, especially in older adults who characteristically show reductions in the specificity of neural circuits, cannot be ruled out. To address this limitation, in addition to the use of TMS, future studies might implement neuroimaging techniques that can assist in probing neuroplasticity in locations outside the representation targeted with TMS. Moreover, future studies might use paired pulse TMS methods to quantify changes in inhibition and facilitation between M1 and extra- or non-motor regions within and across the hemispheres, which may occur in the absence of any change in MEP amplitude. More specifically, paired pulse techniques using two coils placed over different cortical sites might be used to assess changes in interactions between premotor and motor regions in young and older adults after neuroplasticity interventions. Cortical plasticity in regions outside the target

representation can be identified by investigating changes in the communication between regions. For example the reponse evoked by single pulse TMS the left M1, can be inhibited, if proceeded a few milliseconds by a single pulse of TMS to another cortical region, for example the right M1. This example is known as interhemispheric inhibition. Changes in the degree of this inhibition may signify the presence plasticity in the the right M1.

Interestingly, contrary to previous research (Stefan et al., 2008; Shin & Sohn, 2011; Suppa et al., 2008; Di Lazzaro et al., 2008; 2011; Bashir et al., 2014) bilateral corticospinal plasticity was also not present in young adults in Experiment 2 or 3. Evidence suggests that targeting different hemispheres with the TMS protocol does not explain this finding: bilateral corticospinal plasticity in young adults has been demonstrated after interventions targeting both the dominant (Stefan et al., 2008; Shin & Sohn, 2011; Suppa et al., 2008;) and non-dominant hemispheres (Suppa et al., 2008; Di Lazzaro et al., 2008; 2011; Bashir et al., 2014). Instead, it is possible that the absence of prior voluntary activity played some role in attenuating corticospinal plasticity effects in the non-target/unstimulated hemisphere. Bilateral corticospinal plasticity has been demonstrated mostly in young adults in the presence of prior voluntary activity (Di Lazzaro et al., 2008; 2010; Stefan et al., 2008; Ishikawa et al., 2007; Suppa et al., 2008; Bashir et al., 2014). Only one study has shown bilateral corticospinal plasticity in young adults without prior activity and only nine participants were tested (Shin and Sohn, 2011). It is possible that prior activity may be necessary to trigger reliable corticospinal plasticity effects in the non-target/unstimulated pathway. As discussed in Chapter 1 and earlier in this chapter, priming triggers a change in the activity state of cells and synapses, which can alter subsequent synaptic plasticity induction at that location (Abraham & Bear, 1996). More specifically, evidence demonstrates that cells and synapses transition through structured states in the following order; silent, recently silent, active, potentiated and depressed (Montgomery & Madison, 2004). The proportion of synapses in the 'active state' at the time of a given intervention can influence synaptic plasticity effects (see Thickbroom, 2007). Prior activity may have increased the proportion of cells in the active state in the unstimulated pathway, thus resulting in bilateral corticospinal plasticity. The route by which prior voluntary activity might influence the unstimulated pathway is not well understood but could occur due to cortical adaptations within M1 driven directly by ipsilateral inputs, or indirectly as a consequence of activity in the representation contralateral to the target muscle (see Ruddy & Carson, 2013 for review). In either case, the removal of prior activity in Experiment 2 and 3 may have attenuated corticospinal plasticity effects in the non-target/unstimulated pathway.

It is also possible that the inclusion of the attention task altered the manifestation of corticospinal plasticity across the bilateral motor system. Previous research investigating the manifestation of corticospinal plasticity across M1's (Stefan et al., 2008; Shin & Sohn, 2011; Suppa et al., 2008; Di Lazzaro et al., 2008; 2011; Bashir et al., 2014; Di Lazzaro et al., 2008; 2010; Ishikawa et al., 2007) has not included an attention task during the interventions. The mechanism by which attention might alter the distribution of neuroplasticity is discussed in the following section.

5.4 Attention as a potential modulator of plasticity in young and older adults

As discussed previously, attention has been shown to modulate corticospinal plasticity induced by TMS methods (Kamke et al., 2012; 2014; Stefan et al., 2004), yet no studies have investigated this in older adults. As discussed in Chapter 1, older adults demonstrate declines in several attentional processes (Mahoney et al., 2010), suggesting that the modulation of corticospinal plasticity by attention could be altered. Experiment 1 aimed to investigate the influence of task complexity on the phenomenon of cross-limb transfer in young and older adults. Evidence suggests that increasing the complexity of a task requires increasing cognitive involvement (Davare et al., 2010; Ehrsson et al., 2000, 2001; Holmstrom et al., 2011; Mima et al., 1999; Parikh & Cole, 2013; Sadato et al., 1996; Solodkin et al., 2001; Verstynen et al., 2005; Halsband & Lange, 2006). Contrary to predictions, there was no age difference in the transfer of a simple motor task. This result is inconsistent with the previous literature highlighting an age difference in transfer, whereby older adults are able to transfer complex but not simple motor skills (Parikh & Cole, 2013; Hinder et al., 2011). Consistent with previous literature and the hypothesis that greater cognitive involvement may protect transfer in the aged brain, transfer was evident in both young and older adults in the complex task.

One explanation for the transfer of training-related improvements in performance to the untrained hand in older adults is that cognitive involvement was increased in the simple condition relative to previous studies. Specifically, participants were administered with longer training periods and less frequent rest breaks than used in previous studies. This meant that participants had longer to refine their movements to better maximise acceleration. It is well established that feedback improves motor performance (see Lauber & Keller, 2014 for review). The extra time to process and adapt to this feedback may have motivated participants to try harder or increased their cognitive involvement or attention to the task. Such cognitive involvement may have facilitated the mechanism responsible for cross-limb transfer, be it via a bilateral access or cross activation model, and thereby maintained transfer in older adults.

Together the findings of this thesis support the notion that the involvement of cognitive processes, specifically attention, may play some role in modulating neuroplasticity in young and older adults. Experiment 1, which demonstrated that transfer was maintained in older adults with simple and complex tasks, suggests that increased cognitive involvement or attention may facilitate neuroplasticity in the aged brain, assuming that the change in the training protocol did engage participants to a greater extent with the simple task, relative to previous studies. Attention was controlled in Experiment 2, and it was found that there was no difference between young and older adults in the manifestation of corticospinal plasticity following iTBS and that corticospinal plasticity was more prominent in the pathway targeted by the intervention. As there was no bilateral effect of iTBS in the young or older adults, which is contrary to previous literature (Suppa et al., 2008; Di Lazzaro et al., 2008), it is possible that controlling for attention may have influenced the distribution of corticospinal plasticity responses to iTBS. Lastly, attention was systematically manipulated in Experiment 3 to examine its influence on corticospinal plasticity in young and older adults. Corticospinal plasticity effects in Experiment 3 differed marginally between young and older adults and occurred in the corticospinal pathway from the stimulated representation only, similar to responses following iTBS. The manifestation of corticospinal plasticity did not differ reliably between the attention conditions, but the change in MEP amplitude increased over time to a greater degree with attention allocated to the target hand.

Research suggests that the involvement of additional cognitive networks or processes (such as attention) may function to facilitate (or gate) neuroplasticity through the release of neuromodulators and their influence on inhibitory interneurons. Specifically, findings from animal models suggest that changes in activity-dependent modulation of inhibition may account for reduced neuroplasticity in old age (Liguz-Lecznar et al., 2015). In animal models, neuroplasticity is facilitated by a general increase in excitation, which is shaped by the activity of the inhibitory GABAergic system (Liguz-Lecznar et al.,2015). Neuroplasticity is attenuated if GABAergic neurons fail to respond to increases in excitation (Liguz-Lecznar et al., 2015; Ouellet & Villers-Sidani, 2014; Heise et al., 2013). In humans, intracortical inhibition (ICI) is probed with paired-pulse TMS protocols (Kujirai et al., 1993; Valls-Sole et al., 1992). During an inhibitory paired-pulse protocol, two pulses are presented. When the first pulse, the conditioning stimulus is presented just milliseconds prior to a secondary pulse, the test stimulus, an MEP is evoked that is reduced in amplitude relative to when the test stimulus is presented alone. Evidence suggests that the reduction in MEP amplitude is mediated by intracortical GABAergic inhibition (Ziemann et al., 1995; Ziemann, 2003; McDonnell et al., 2006; Florian et al., 2008). A handful of studies have shown variable age-related changes in ICI at rest: ICI has been shown to be reduced (Peinemann et al., 2001; Papegaaji et al., 2014;

Marneweck et al., 2011; Beynel et al., 2014; Opie & Semmler, 2014 Opie et al., In press), increased (Smith et al., 2009; Mc Ginley et al., 2010) and even comparable in the aged brain relative to young adults (Oliviero et al., 2006; Opie & Semmler, 2014; Stevens-Lapsley et al., 2013; Rogasch et al., 2009; Opie et al., In Press; Smith et al., 2011). With regards to activity-dependent changes in inhibition, studies demonstrate that older adults fail to show changes in inhibition during cutaneous afferent input (Kossev et al., 2002; Smith et al., 2011). Older adults do, however, show comparable changes in inhibition to young adults during voluntary contraction (McGinley et al., 2010), cognitive tasks (Beynel et al., 2014) and motor training (Cirillo et al., 2011; Hinder et al., 2011). Although there is a motor component in two of these examples, these findings suggest that age-related declines in inhibitory modulation may be reduced with greater task complexity, potentially due to the presence of greater cognitive involvement/attention. Therefore, undertaking an attention task during TBS and PAS may have facilitated neuroplasticity induction in older adults by rectifying age-related declines in inhibitory modulation.

A potential mechanism by which greater cognitive involvement or attention might rectify inhibitory modulation in older adults is by influencing the activity of neuromodulators (Pauls et al., 2012). Evidence indicates that neuromodulators play a critical role in cognitive processes, especially attention (Noudoost & Moore, 2011). For example, methylphenidate, which inhibits the reuptake of dopamine and noradrenalin, improves performance on memory tasks in animal models and in healthy adults (Tian et al., 2009; Linssen et al., 2012). In addition, pharmacological manipulation of dopaminergic circuits enhances neural responses to attended visual stimuli and reduces variability in responses across trials in animal models (Noudoost & Moore, 2011). Evidence suggests that neuromodulators such as dopamine might alter the activity of inhibitory interneurons (Moore, 2006; Muly et al., 1998). Therefore, it is plausible that attention might influence inhibitory activity in older adults via neuromodulators. Future research is needed to identify exactly how the involvement of cognition and attention might alter inhibition in the motor cortex in young and older adults. One approach would be to probe inhibitory circuits with paired-pulse TMS protocols under conditions varying in attentional involvement.

The involvement of additional cognitive process, such as attention, may have also altered the manifestation of neuroplasticity across the hemispheres in Experiment 2. There was no evidence of bilateral corticospinal plasticity in Experiment 2, not even in young adults. This finding is inconsistent with previous literature demonstrating bilateral changes in MEP amplitudes after TMS interventions (Shin & Sohn, 2011; Suppa et al., 2008; Ishikawa et al 2007, Stefan et al., 2008; Di Lazzaro et al., 2008; Di Lazzaro et al., 2011). Evidence has been presented to suggest that LTP-like

effects are enhanced and LTD-like effects suppressed at neural connections that fall within attentional focus (Kamke et al., 2014). As a consequence it follows that attention acts to enhance LTD-like effects while suppressing LTP-like effects at neural connections that do not fall within the focus of attention or are irrelevant to the current task. Suppression of LTP-like effects at unattended locations may explain why corticospinal plasticity manifested in the target but not the non-target corticospinal pathway in young and older LTP-like responders. However, this idea does not account for the large proportion of individuals who demonstrated unexpected LTD-like effects in the target pathway when allocating attention to the target hand. Only one study has demonstrated that attention has opposite effects on LTP-like and LTD-like corticospinal plasticity induction (Kamke et al., 2014), so the reliability of this effect needs to be established. As a number of individuals demonstrated LTD-like effects when attending to the target hand, the findings of the current study instead provide support for the alternative explanation that attention acts simply to gate neuroplasticity, boosting synaptic plasticity mechanisms with additional factors determining the direction of the response. In this case, LTD-effects in the target pathway in the attend target condition could be explained by attention failing to boost corticospinal plasticity mechanisms enough to reach the threshold for LTP-like induction. Assuming attention boosts neuroplasticity in this manner, it would be predicted that directing attention to the non-target hand during the plasticity intervention will suppress LTP-like effects in the target pathway relative to when attention is allocated to the target hand. Experiment 3 manipulated the location of spatial attention during PAS to test this hypothesis and to examine age differences in this effect. There were no reliable effects of attention in either young or older adults.

Several possible explanations may account for why, contrary to previous literature, corticospinal plasticity was not modulated by attention in Experiment 3. First, methodological differences between studies may have played a role. Previous studies targeted cortical representations in the non-dominant hemisphere (Stefan et al., 2004; Kamke et al., 2012; 2014), whereas PAS targeted a cortical representation in the dominant hemisphere in Experiment 3. Some evidence demonstrates hemispheric differences in TMS-induced corticospinal plasticity effects (Vines et al., 2008), which are shown to be due to differences in interhemispheric inhibition from the dominant to the non-dominant hemisphere and *vice versa* (Ziemann & Hallet, 2001). Although no studies have investigated hemispheric differences in the modulation of corticospinal plasticity by attention, hemispheric differences in inhibition suggest that it is possible that attention modulates corticospinal plasticity in the dominant and non-dominant hemispheres differently. Future studies would benefit from investigating the role of hemisphere dominance in influencing the modulation of corticospinal plasticity by attention. In addition, the attention task used in Experiment 3 was

modified from that used previously (Kamke et al., 2014). Instead of counting the number of targets and holding these in memory until the end of a trial, participants were instructed to make a verbal response as soon as they detected each target. This adaptation may have reduced the complexity of the task, allowing participants to disengage or recruit less attentional resources which resulted in less attentional modulation of corticospinal plasticity. Contrary to predictions older adults did not perform worse on the attention task or exhibit less modulation of corticospinal plasticity by attention. Future studies would benefit from using a task more similar to the one used by Kamke and colleagues (2012; 2014) to increase the level of task complexity and engagement in the task. Under these conditions one might better establish the effect of attention on neuroplasticity in older adults.

5.5 Conclusions and significance of the findings

Although experimentally-induced synaptic plasticity may be altered in aged animal models, the findings of this thesis demonstrate that the capacity for change resembling synaptic modification within the human motor system does not necessarily decrease with advancing age. As discussed, compensatory mechanisms at the network level within and outside the motor cortices may take place under conditions of increased cognitive involvement/attention. These mechanisms may function to protect neuroplasticity in older humans and govern the distribution of neuroplasticity in young and older adults. The nature and location of these mechanisms require further investigation.

Together the findings of the experiments within this thesis develop our understanding of neuroplasticity in the older adult brain. The finding that older adults can transfer simple and complex motor skills from a trained to untrained hand may inform the development of rehabilitation strategies promoting neuroplasticity in older adults recovering from brain injury. However, the findings of Experiment 1 demonstrate that while motor training can induce changes in bilateral corticospinal excitability, the increases in the untrained hemisphere appear to be negatively associated with transfer to the untrained hand in older adults. At the very least, this suggests that the mechanism governing cross-limb transfer might be altered in older adults, suggesting that unilateral training may result in differential rehabilitative effects in young and older adults. The absence of any age difference in the corticospinal plasticity effect induced by iTBS in Experiment 2 demonstrates the potential for using non-invasive brain stimulation methods to facilitate neuroplasticity in the aged brain. It is possible that advancing age influences training- and stimulation-induced neuroplasticity differentially, suggesting that while old and young adults may require tailored motor training rehabilitative techniques, the same brain stimulation protocols may be used in young and older adults to assist in facilitating neuroplasticity. Furthermore, a recurring

theme of Experiment 1 and 2 is the potential role of cognition and attention in modulating the magnitude and distribution of neuroplasticity. It is possible that differences in motivation and attention help explain differences in corticospinal plasticity induced by training versus TMS in young and older humans, as training is presumably more influenced by cognitive factors. This has implications for older adults experiencing decline in cognition and attention due to disease or brain injury, who may suffer secondary decays in neuroplasticity in motor regions as a result. It also highlights the potential for enhancing neuroplasticity in motor regions by cognitive training. A critical theme throughout the thesis was that of individual variability. Although TMS-based procedures for inducing neuroplasticity were equally effective in young and older adults at the group level, individual variability in the magnitude of effects is problematic for using the techniques in targeted rehabilitation. Future research must attempt to identify the factors that can best predict individual outcomes in response to TMS-based neuroplasticity interventions and behavioural training. Developing ways of manipulating these factors may potentially assist in protecting against declines in neuroplasticity in old age.

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Appendix A Cross-limb Transfer Pilot Experiment

As noted in Chapter 2, a pilot study was conducted to test whether the training protocol led to the expected increase in performance in young and older adults in the trained hand. The details of this pilot are described below, with implications for the final design of the study reported in Chapter 2 presented at the end of the appendix.

A.1 Methods

The methods of this pilot are largely the same to those reported in Chapter 2. Therefore, only alterations to the already discussed methods are detailed below.

A.1.1 Participants

Nineteen right-handed volunteers participated, 13 aged between 18 and 35 years (M = 22.83, SD = 1.75, Male = 6) and six above 65 years (M= 69.67, SD = 1.86, Male = 6). One participant did not complete the simple session and therefore was removed from the analyses for this condition.

A.1.2 Simple task

Participants practiced the task approximately 10 times before baseline performance was measured. During training participants performed the cued movement for two minutes and rested for 30 seconds after each block. There were four blocks, totalling 10 minutes of training. There was no online feedback presented on the computer screen, but verbal encouragement was given throughout training and at the start of each pre and post measure. No restraints were placed on the fingers or the wrist.

A.1.3 Complex task

Participants practiced the task until they were comfortable they could remember the sequence prior to baseline measures of performance. Participants were not presented with a diagram of the sequence on the computer screen during training.

A.1.4 Procedure

Behavioural performance in the two hands and cortical excitability in the two hemispheres were measured post training at only one time point (2 mins post). There was no 15 mins post measure. Only the behavioural results for the trained hand are reported in this appendix, as demonstrating a training-related improvement in that hand is critical to the study design.

A.1.5 Data Analyses

Paired-samples t-tests were conducted comparing performance between pre and post measures separately for young and older adults in the simple and complex conditions.

A.2 Results

A.2.1 Cross-limb transfer after training on a simple ballistic task

As can be seen in Figure A-1 peak acceleration remained unchanged in young adults ($t(11) = .530$, $p = .607$), but reduced significantly in older adults ($t(5) = 4.03$, $p = .010$).

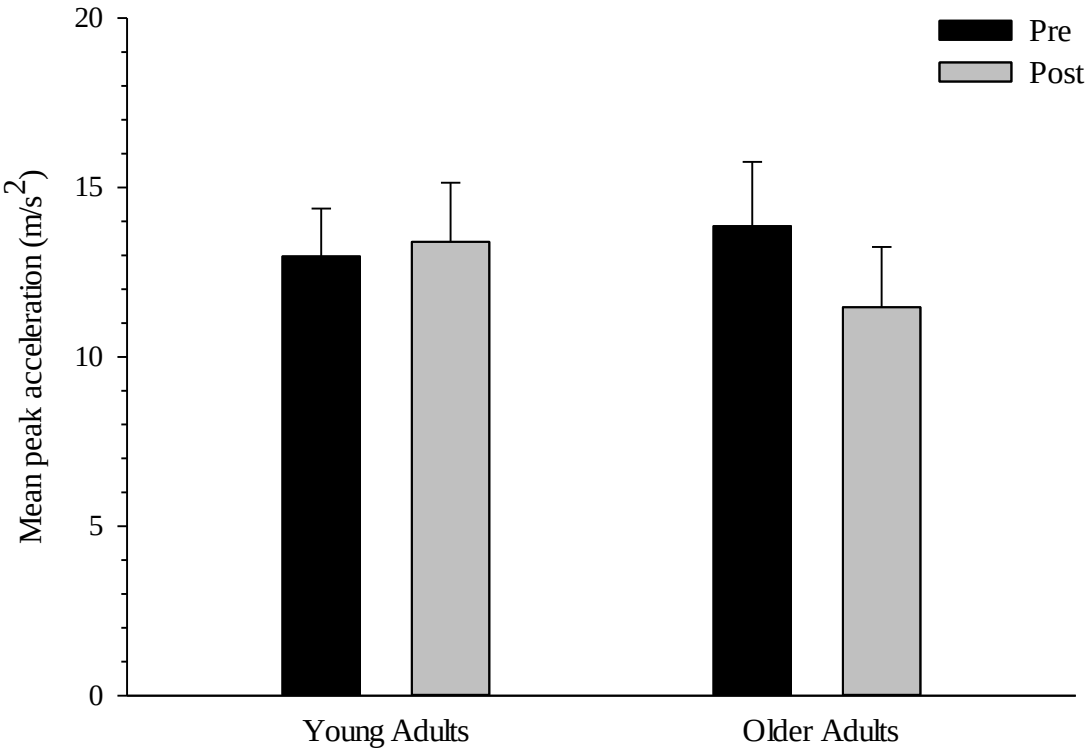

Figure A-1 Behavioural performance in the simple condition. On average maximum peak acceleration in the thumb of the trained hand, following the ballistic training task remained unchanged in young adults but decreased in older adults. Error bars denote SEM.

A.2.2 Cross-limb transfer after training on a complex motor sequence

As can be seen in Figure A-2 the average number of correct sequences performed did increase reliably from pre to post training in young ($t(12) = 5.52$, $p < .001$) but not older adults ($t(5) = .00$, $p = 1.000$.

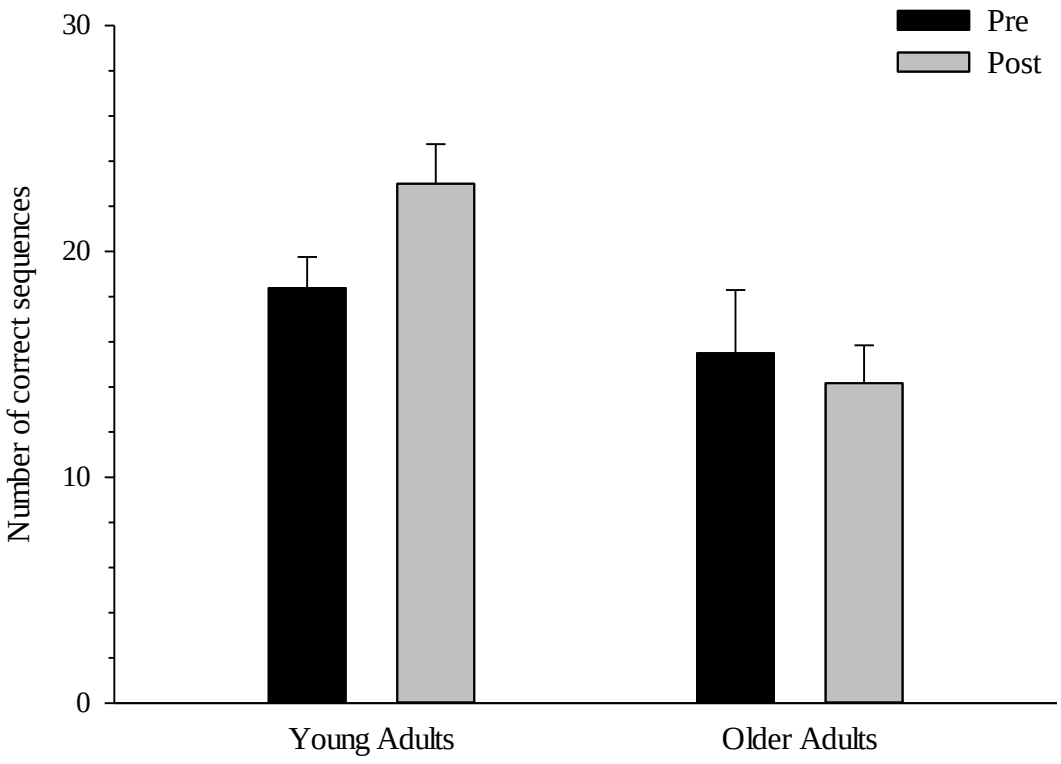

Figure A-2 Behavioural performance in the complex condition. The number of sequences completed increased significantly pre to post training in young but not older adults. Error bars denote SEM.

A.3 Implications for Experiment 1

Both the ballistic thumb movement and the finger-to-thumb sequence task are well-established methods of inducing training-related change in behavioural performance and cortical excitability in young adults (Carroll et al., 2008; Hinder et al., 2011; Lee at al., 2010; Karni et al., 1998). In this pilot study, although there was a performance increase in the young in the complex condition, there was no significant change in behaviour in the simple task. Moreover, older adults did not demonstrate any improvements in performance, instead showing a decrease in performance in the simple condition. A number of factors could account for these effects.

In contrast to some previous studies mentioned (Hinder et al., 2011), but consistent with others (Muellbacher, 2001) we did not present feedback to participants regarding their behavioural performance during training. Although participants were verbally encouraged to perform each task to the best of their ability throughout the sessions, decreases in motivation over time may have contributed to the small performance gains evident in this pilot study. Therefore, to maximise

training-related performance gains, participants were provided with visual feedback of their peak acceleration throughout training in Experiment 1 (Chapter 2). In addition, in order to reduce the involvement of other muscle groups and thereby increase training related changes in performance in the thumb, in Experiment 1 restraints were placed on the fingers and the forearm.

In this pilot experiment the performance of older adults deteriorated from pre to post training during the simple task and failed to improve over time during the complex task. This effect might be explained by muscle fatigue. To reduce the likelihood of muscle fatigue in Experiment 1 the frequency of rest periods during training is increased to allow sufficient recovery within the peripheral muscles. In order to ensure participants remembered the sequence during training, the sequence was presented on screen during the training protocol in Experiment 1. In addition, in order to examine the time course of training effects in young and older adults, Experiment 1 also incorporates a second measure of performance and cortical excitability at 15 minutes post training.

A.4 References

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Appendix B Pilot PAS Experiment

As discussed in Chapter 4 an initial study was conducted to test the hypothesis outlined in that chapter. However, this study was terminated after testing 12 participants as numerous participants reported having trouble detecting the LED targets. The attention task was subsequently modified. The details and results of this initial study are presented here for completeness, with implications for the final design of the study reported in Chapter 4 presented at the end of the appendix.

B.1 Methods

The methods of this pilot are largely the same as those reported previously in the manuscript of Chapter 4. Therefore, only alterations to the already discussed methods are detailed below.

B.1.1 Participants

Twelve volunteers between the ages of 18 and 35 years participated (M = 23.92, SD = 3.78, Male = 6).

B.1.2 Materials

B.1.2.1 Attention task

Participants were instructed to overtly attend to the activity of an LED positioned on the inside of the thumb on the right and left hands, in separate sessions. Participants responded verbally each time they detected an interruption to the continuously lit LED ("OFF period"). Targets appeared approximately every 30 seconds and were jittered randomly. Participants performed this task for the duration of the neuroplasticity intervention (11 mins).

B.1.3 Analysis

Accuracy data for the attention task were compared between the attend right and attend left sessions with paired-samples t-tests. Baseline MEP data for the APBs was subjected to a 2 × 2 within subjects ANOVA with the factors of attention (right, left) and hemisphere (stimulated, unstimulated). Mean PAS-induced changes in MEP amplitude, relative to each individual's baseline, were subjected to a 2 × 3 × 2 within subjects ANOVA with the factors attention (right, left), time (5, 15, 25 mins post PAS), and hemisphere (stimulated, unstimulated).

B.2 Results

Accuracy during the attention task was high overall and the number of errors made did not differ significantly between the attend right (M = 1.33, SD = 1.44) and attend left (M = 1.67, SD = 3.17)

conditions ($t(11) = .346$, $p = .736$). Baseline MEPs in the stimulated and non-stimulated hemispheres varied as a function of attention, as shown by a significant interaction between attention and hemisphere $F(1,11) = 8.42$, $p = .014$, $\eta_p^2 = .433$. There were no other significant main effects or interactions (*ps* >.495). Follow up comparisons trended towards greater MEP amplitude in the stimulated hemisphere in the attend right condition ($M = .77$, $SD = .22$) than in the attend left condition ($M = .69$, $SD = .24$; $t(11) = 1.92$, $p = .081$), but this difference was not reliable. There was also no significant differences between the attend right ($M = .69$, $SD = .14$) and attend left ($M = .72$, $SD = .20$) conditions in the unstimulated hemisphere, or between the stimulated and unstimulated hemispheres (*p*s > .197).

Figure B-1 shows mean MEP change at each of the time points post PAS, in the stimulated/target hemisphere and the unstimulated/non-target hemisphere for the attend right and attend left conditions. It can be seen that MEP change was greater in the stimulated hemisphere than in the unstimulated hemisphere, which was supported by a marginal effect of hemisphere $F(1,11) = 4.77$, $p = .052$, $\eta_p^2 = .30$. Although MEP change in the stimulated hemisphere was significantly increased from baseline ($t(11) = 3.51$, $p = .005$), change in the unstimulated hemisphere was not different from baseline ($t(11) = 1.64$, $p = .130$). There were no other main effects or interactions (*p*s > .135).

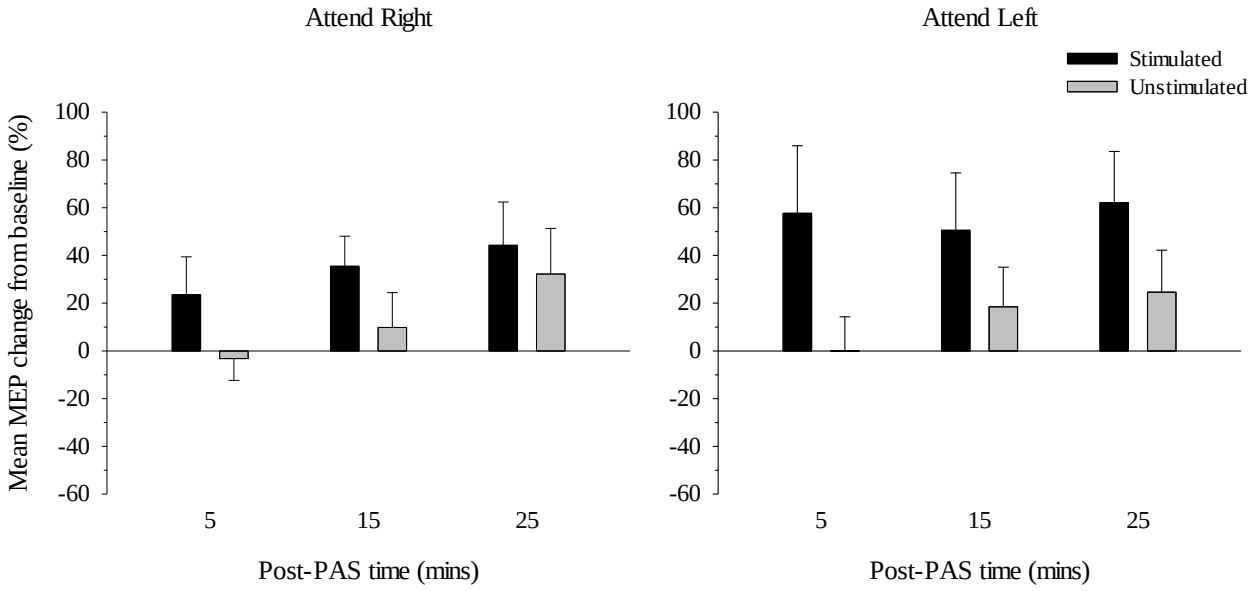

Figure B-1 Mean MEP change from baseline in the target and non-target hemispheres as a function of post-PAS time in the attend right and attend left conditions. Statistically reliable MEP change was evident in the stimulated but not the unstimulated hemisphere. The difference between the stimulated and unstimulated hemispheres was also significant. Error bars denote SEM

B.3 Implications for Experiment 3

The results of this initial study were inconsistent with previous literature, which suggests that attention to the target hand facilitates corticospinal plasticity effects (Kamke et al., 2014). Several concerns were highlighted during this experiment that are addressed in Experiment 3. First, participants reported difficulty seeing the LED as the task progressed. Although it is not clear whether such perceptual difficulty can influence corticospinal plasticity induced by non-invasive brain stimulation, increased task difficulty and cognitive demand has been shown to do reduce it (Kamke et al., 2012, Stefan et al., 2004; Antal et al., 2007). In order to improve the visibility a brighter LED was used, which was relocated to the interphalangeal joint as opposed to the metacarpophalangeal joint of the thumb. A number of participants also reported being drawn to the LED on the opposite hand, which was also flickering spontaneously in peripheral vision. This was likely to disrupt the participants' focus on the desired hand, potentially drawing attention to the opposite non-target hand, which Kamke and colleagues (2014) showed could reduce the level of corticospinal plasticity induced in the corticospinal pathway innervating the target hand. To overcome this problem in Experiment 3 only one LED, which was placed on either the target or non-target hand, was used in any one session. In addition it could be speculated that the attention task may have been too easy and required very little engagement/attention to perform highly. More specifically, it is possible that the target (OFF period) may have popped out from the static ON display, such that participants were drawn to it. Consequently participants may not have had to continuously monitor the LED, which means that attention may not have been in the desired location for the majority of PAS pulses. In order to increase participants' engagement in the task, the frequency of the targets was increased and targets were made more difficult to identify by the addition of distractors. In Experiment 3, instead of participants identifying a single "OFF" period, participants now respond verbally each time the LED blinked off and on again twice in quick succession. Single "OFF" periods/blinks became distractors. Experiment 3 also included an additional post PAS MEP measure at 35 mins in order to better quantify the time course of PAS effects. All other methods remained the same.

B.4 References

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Appendix C ANOVA results for the 20% MEP change responder analyses (iTBS & PAS)

Table C-1 Significant main effects and interactions found with a 20% MEP change cut off for LTP-like and LTD-like responders

Experiment	Condition	Main effects & Interactions	ANOVA statistics
Exp. 2 iTBS		Time	$F(2,46) = 8.12$, $p = .003$, $\eta_p^2 = .261$
		Response	$F(1,23) = 25.95$, $p < .001$, $\eta_p^2 = .530$
		Hemisphere x Response group	$F(1,23) = 25.33$, $p < .001$, $\eta_p^2 = .524$
		Time x Hemisphere x Response group	$F(2,46) = 3.49$, $p = .054$, $\eta_p^2 = .132$
		All other	Fs < 2.13, ps > .145
Exp. 3 PAS	Attend Right	Response group	$F(1,17) = 24.41$, $p < .001$, $\eta_p^2 = .590$
		Age	$F(1,17) = 4.14$, $p = .058$, $\eta_p^2 = .196$
		Hemisphere x Response group	$F(1,17) = 12.80$, $p = .002$, $\eta_p^2 = .429$
		All other	Fs < 2.23, ps > .154
	Attend Left	Response group	$F(1,19) = 11.28$, $p = .003$, $\eta_p^2 = .372$
		Age	$F(1,19) = 3.60$, $p = .073$, $\eta_p^2 = .159$
		Hemisphere x Response group	$F(1,19) = 7.40$, $p = .014$, $\eta_p^2 = .280$
		Hemisphere x Age	$F(1,19) = 2.96$, $p = .101$ $\eta_p^2 = .135$
		All other	Fs < 1.42, ps > .252

Individuals with a mean MEP change >20% were classed as LTP-like, those with < -20% were classed as LTD-like

Appendix D Additional PAS responder analyses

As discussed in Chapter 4 to give a better idea of how plasticity manifested across the hemispheres in individuals who responded more or less consistently to PAS across sessions, an additional exploratory analysis was conducted. As can be seen in Figure D-1 participants could be classified into four categories based on plasticity responses across the two separate sessions. Participants either demonstrated LTP-like effects in both sessions, LTD -like effects in both sessions, LTP-like effects in the attend right condition with LTD-like effects in the attend left condition, or LTD-like effects in the attend right condition with LTP-like effects in the attend left condition (with the criterion >/< 0% change).

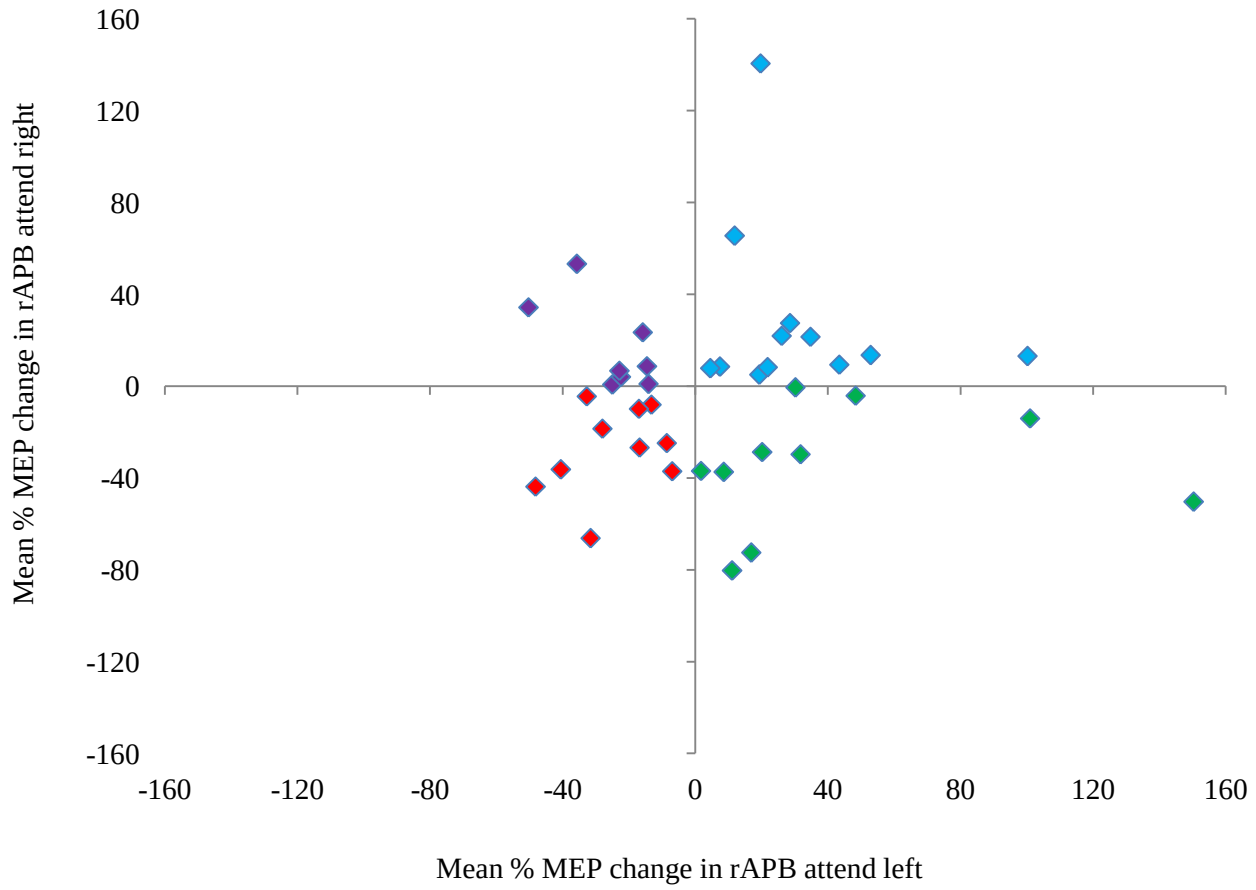

Figure D-1 Consistency of PAS effects across sessions. Participants could be classified into four different responder groups based on mean % MEP change in the rAPB in the attend right and attend left conditions. Group 1 (Blue) demonstrated LTP-like effects in both sessions. Group 2 (Red) demonstrated consistent LTD-like effects in both sessions. Group 3 (Green) demonstrated LTP-like effects in the attend left condition but LTD-like effects in the attend right condition. Group 4 (Purple) demonstrated LTP-like effects in the attend right condition but LTD-like effects in the attend left condition The criterion used to classify LTP-like and LTD-like reponses was >/< 0 % MEP change.

D.1 Analyses

This analysis was performed on the data from all individuals as there was not enough individuals to use the 20% responder analysis. An exploratory 4 x 2 x 2 x 2 x 2 mixed ANOVA testing the repeated measures of time (5, 15, 25, 35 mins post), hemisphere (targeted, non-targeted), and session (1 attend right, 2 attend left) and the between subjects factors age (young and older) and responder group (1 LTP-like effects in both attention conditions (n = 12), 2 LTD-like effects in both conditions (n = 10), 3 LTP-like effects in the attend left condition and LTD-like effects in the attend right condition (n = 10), and 4 LTP-like effects in the attend right condition and LTD-like effects in the attend left condition (n = 8)) on the mean percentage MEP change relative to each individual's baseline.

D.2 Results

Overall MEP change induced by PAS varied across the post time points as indicated by a significant main effect of time; $F(3,96) = 4.09$, $p = .011$, $\eta_p^2 = .113$. Follow up comparisons revealed that MEP change was significantly greater at 25 mins post than at 5 mins post ($t(96) = 3.10$, $p = .024$) but did not differ between any other time points (ps > .05). As expected, MEP change also differed as a function of response group as indicated by a significant main effect of response group $F(3,32) = 5.12$, $p = .005$, $\eta_p^2 = .324$. Figure D-2 depicts MEP change in the targeted and non-targeted pathways for each of the responder groups in the attend right and attend left conditions. Although a response group by session interaction was expected because the different groups were classified based on MEP change in rAPB in the two sessions, interestingly the manifestation of PAS-induced MEP change across bilateral corticospinal pathways was found to vary as a function of response group and attention, as indicated by a significant Response group x Session x Hemisphere interaction; $F(3,32) = 5.27$, $p = .005$, $\eta_p^2 = .331$. Follow-up comparisons focusing on the differences in MEPs across hemispheres revealed that while MEP change did not differ across the targeted and non-targeted pathways in either the attend right or attend left sessions in Group 1 ($t(11) = 0.32$, $p = .755$, $t(11) = 1.34$, $p = .208$ respectively) or Group 2 ($t(9) = 1.67$, $p = .130$, $t(9) = 0.35$, $p = .734$ respectively), plasticity effects did differ between the targeted and non-targeted pathways in the attend right and attend left sessions in Group 3 ($t(9) = 3.14$, $p = .012$, $t(9) = 2.77$, $p = .022$ respectively) and marginally in group 4 ($t(7) = 2.34$, $p = .052$, $t(7) = 2.14$, $p = .070$ respectively).

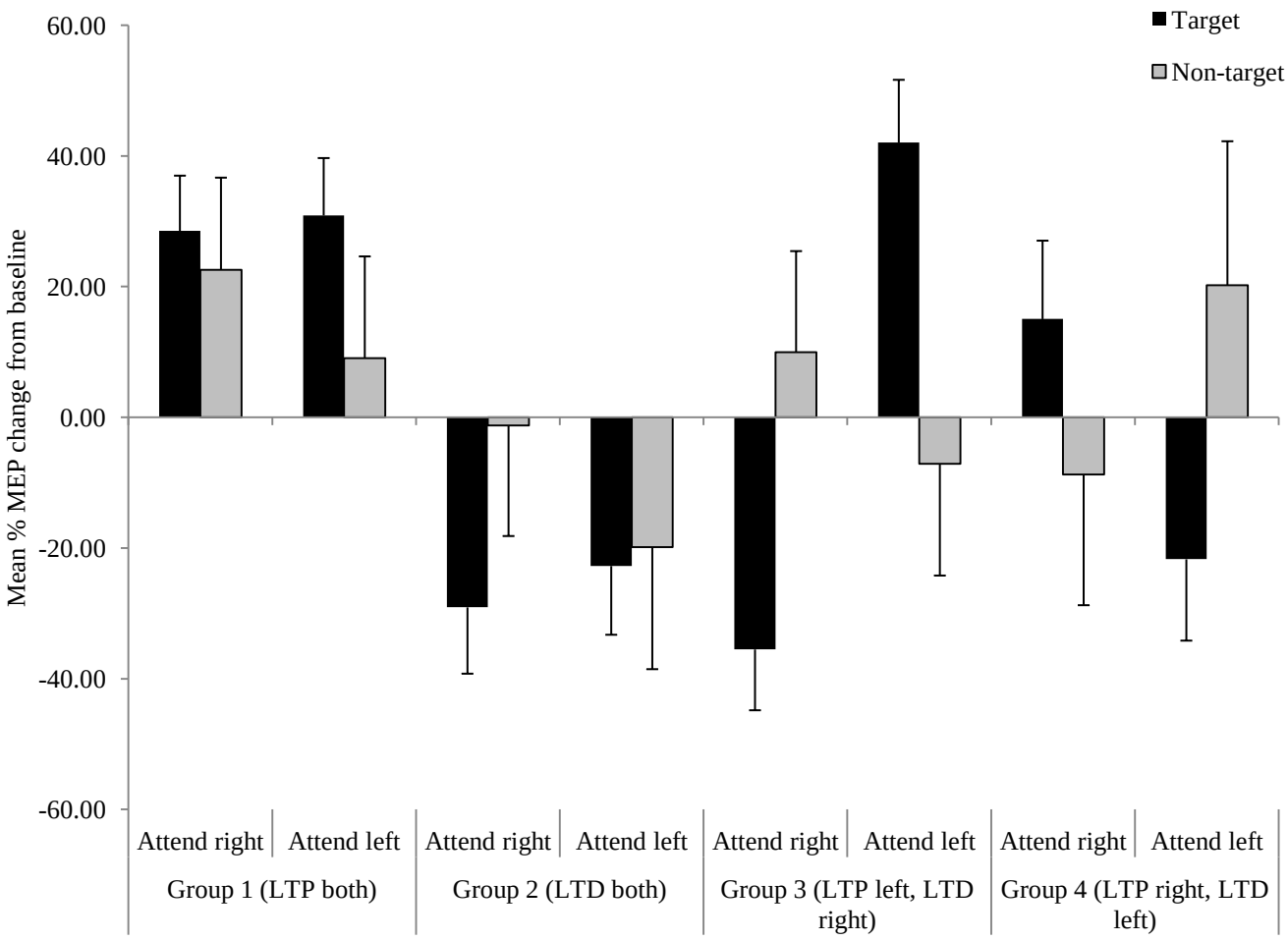

Figure D-2 Manifestation of PAS-induced plasticity across bilateral corticospinal pathways. Plasticity effects were similar across bilateral corticospinal pathways in individuals who responded with consistent LTP-like or LTD-like effects in the right APB across sessions (>/< 0% change). In participants who did not respond consistently in the right APB across sessions, plasticity effects manifested in opposite directions in the targeted and non-targeted pathway. Error bars denote SEM.

D.3 Interpretation

This analysis shows that the corticospinal plasticity induced in individuals who show consistent LTP-like or LTD-like effects in the target hemisphere across the separate sessions manifests similarly in the non-targeted hemisphere. However, in individuals who respond inconsistently in the target hemisphere across the sessions, plasticity manifests in opposite directions in the target and non-target hemispheres. Similar effects across bilateral pathways supports the hypothesis of Experiment 3 and demonstrates that plasticity can be induced bilaterally in young and older adults. The differential manifestation of corticospinal plasticity across bilateral motor pathways in individuals responding inconsistently across sessions is also an interesting observation. Although there was only a small number of participants in each group of this analysis, the results seem to

suggest that there may be an interaction between intra-subject reliability and cross-hemisphere plasticity. However, the fact that attention was manipulated across the sessions complicates this conclusion. The interaction between attention, intra-individual reliability and cross-hemisphere plasticity warrants further investigation. It is for future research to determine the individual factors that might best predict the magnitude and manifestation of plasticity across bilateral corticospinal pathways. This will aid in tailoring rehabilitation strategies to best facilitate plasticity and recovery from brain injury in different groups of individuals.