Neural correlates of upper limb unimanual motor practice

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By

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Preface and Candidate's Role

Sections of this thesis have been published in peer reviewed journals or submitted to preprint servers as multi-authored manuscripts. This section aims to define the role of the candidate and that of the co-authors for each study.

Study one contribution: Justin Andrushko co-designed the study with Layla Gould, Ron Borowsky and Jon Farthing. All authors contributed to participant recruitment. Justin Andrushko, Layla Gould, Doug Renshaw and Jon Farthing were involved in data acquisition. Justin Andrushko handled data analysis, and manuscript preparation. All authors provided feedback and final approvals on the manuscript and data interpretation prior to publication. This manuscript has already been published and Justin Andrushko is the first author.

Study one citation: This chapter has been previously published in *Neuroscience* and formatted to fit in the thesis.

> Andrushko, J. W., Gould, L., Renshaw, D. W., Ekstrand, C., Hortobágyi, T., Borowsky, R., & Farthing, J. P. (2021). High force unimanual handgrip contractions increase ipsilateral sensorimotor activation and functional connectivity. *Neuroscience*, *452*(C), 111-125. https://doi.org/10.1016/j.neuroscience.2020.10.031.

The results in this study were presented at international conferences:

1. *International meeting:* Canadian Society for Exercise Physiology – October, 2019
2. *International meeting:* Organization for Human Brain Mapping – June, 2019

Study two contribution: This study was designed as part of a larger clinical trial. The study design was primarily led by Jon Farthing and Layla Gould, however, all authors provided valuable input prior to study commencement. Justin Andrushko, Layla Gould, Doug Renshaw and Shannon Forrester were involved in data collection. Data analysis and manuscript preparation was carried out by Justin Andrushko, and all co-authors were involved in providing feedback and approval of data interpretation and the manuscript draft.

Study two citation: This study chapter has been submitted to the bioRxiv preprint server and is formatted to fit in the thesis.

> Andrushko, J. W., Gould. L., Renshaw, D. W., Forrester, S., Kelly, M. E., Linassi, G., Mickleborough, M., Oates, A., Hunter, G., Borowsky, R., & Farthing, J. P. (2021). Ipsilesional motor cortex activation with high force unimanual handgrip contractions with the less-affected limb in participants with stroke. *bioRxiv*. https://doi.org/10.1101/2021.06.25.449456.

Study three contribution: This study was carried out at the University of Oxford in the United Kingdom while Justin Andrushko was serving as a visiting research in the Nuffield Department of Clinical Neurosciences. Justin Andrushko, Jon Farthing, Charlotte Stagg, Jacob Levenstein and Catharina Zich were all involved in study design. Justin Andrushko and Jacob Levenstein co-recruited participants and collected data for this study. Evan Edmond analyzed data omitted from this thesis. Will Clarke and Emir Uzay developed the MRI sequences to carry out this study. Aspects of this study are also included in Jacob Levenstein's thesis at the University of Oxford. Justin Andrushko analyzed all data included in this study chapter and wrote the manuscript. All authors are involved in providing feedback and approval prior to manuscript submission. This manuscript is not currently submitted for published.

Study three citation: This chapter is currently in preparation and will be submitted to a pre-print server prior to submission for peer-review. The manuscript is formatted to fit in the thesis.

> Andrushko, J. W., Levenstein, J. M., Zich, C., Edmond, E., Clarke, W. T., Uzay, E., Farthing, J. P., Stagg, C. J. Repeated submaximal unimanual handgrip contractions alter cortical functional connectivity and response times with the contralateral hand. *In preparation.*

The results in this study were presented at local conferences:

1. *Local meeting:* University of Saskatchewan Life and health Sceinces Research Expo – May, 2021
2. *Local meeting:* University of Saskatchewan, Neuroscience Research Symposium – March, 2020

Abstract

The primary control centres for unimanual motor control reside in the contralateral hemisphere. Ipsilateral sensorimotor brain activity has been observed during unimanual motor tasks, and the functional properties of this ipsilateral activity are debated. Cross-education is the interlimb transfer of a practice motor behaviour (skill or strength) to the homologous contralateral limb. A leading theory for cross-education proposes the interlimb transfer manifests from ipsilateral cortical activity during unimanual motor tasks, resulting in motor-related neuroplasticity giving rise to contralateral limb improvements. Cross-education has been effectively utilized in clinical settings for motor recovery in individuals with a stroke that present with a unilateral impairment. Yet, based on stroke-related neuroplastic changes with interhemispheric inhibition, ipsilateral/ipsilesional hemispheric activity would likely be inhibited when the less-affected limb is active. Therefore, investigating ipsilateral brain activity with unimanual tasks is pertinent in neurologically intact and stroke-impaired participants. **The purpose** of this thesis was to i) investigate the neural correlates of the sensorimotor network with parametrically increasing unimanual handgrip contractions in healthy and stroke-impaired individuals, and ii) determine the effect of handgrip motor fatigue on resting-state cortical activity in the sensorimotor network and motor performance and learning in the contralateral hand. **Study One:** Two experiments were carried out; experiment one used magnetic resonance imaging (MRI) to investigate the cortical activation and functional connectivity patterns during three different submaximal handgrip contractions (25%, 50%, 75% maximum voluntary contraction [MVC]) with the right hand. In experiment two, the tasks were replicated outside of the MRI using electromyography to measure the muscle activation patterns in the wrist flexors in both limbs during the right-hand motor task. In experiment one, brain activation and functional connectivity within the ipsilateral sensorimotor areas were found to increase parametrically with the increases in handgrip force, and data from experiment two suggest that the increased cortical patterns are not likely driving involuntary muscle contractions in the opposite limb. **Study Two:** One experiment was carried out to investigate how unimanual parametrically increasing handgrip contractions with the less-affected limb modulates cortical activity in participants with stroke. Higher force contractions increased brain activity in the ipsilesional hemisphere like what was observed in the first experiment in study one with neurologically intact participants. Yet, patterns of functional connectivity differed between groups, with the participants with stroke showing lower

connectivity across the three contraction forces with no differences between conditions. **Study Three:** Study three also involved two separate experiments. In experiment one, resting-state functional connectivity and button press response times with both hands were assessed before and after participants performed nine-minutes of repeated handgrip contractions at either 5% or 50% MVC with their right hand. The handgrip contractions at 50% MVC resulted in motor fatigue and improved response times in the left hand, a phenomenon not observed with the 5% MVC condition. For the 50% MVC condition, increases in functional connectivity were found between the right ipsilateral primary motor cortex (iM1) and the right orbitofrontal cortex and between the supplementary motor areas (SMA) in each hemisphere. M1-M1 connectivity also changed with a subtle decrease after the motor task. In experiment two, the impact of repeated right handgrip contracts on contralateral motor learning and cross-education of a serial reaction time task was investigated. Motor performance and learning with the left hand improved similarly between conditions, however, greater cross-education was observed at post-learning, with the 50% MVC condition exhibiting faster response times without a decrement to performance accuracy. **Conclusion:** This thesis identifies the involvement and modulation of ipsilateral cortical activation and functional connectivity during and after right handgrip contractions at high-force and/or with fatiguing efforts in healthy and neurologically impaired individuals. These cortical modulations may be utilized to enhance acute motor performance which could have clinical applications for unilateral motor rehabilitation in stroke survivors.

Acknowledgements

I would like to thank my supervisor Dr. Jonathan Farthing for his guidance, mentorship, and friendship over the past six years, spanning back to when I started my Master of Science degree under his supervision. Throughout these years you have helped instill in me qualities that will guide me throughout my professional and personal future. For all of my successes to date, none of them would have been made possible without your support. I value our relationship and look forward to strengthening it through future collaborations and joint applications. I hope this isn't the end, rather the start of something incredible, where I hope to work with my friend and mentor for many years to come. It is insufficient to put on paper how much I value and appreciate having you as my supervisor throughout my graduate studies, but I am forever grateful and will always be in his debt.

To my doctoral committee, Dr. Saija Kontulainen, Dr. Joel Lanovaz and cognate member Dr. Ron Borowsky, thank you for your valuable insight and guidance throughout this process. Each of you have served as outstanding examples of professional mentors and academics for which I hope to model myself after as I pursue a career in academia.

To Dr. Charlotte (Charlie) Stagg, where to begin? There are simply so many things to be thankful for; inviting me to speak about my work at the University of Oxford back in 2018 which was the beginning of a professional relationship that I will cherish throughout my life. Thank you for hosting me as a visiting student and researcher within your lab for six months in 2019. Without this opportunity literally one third of the completed doctoral work in this thesis would not have been possible. Finally, thank you for all your guidance and mentorship, the skills I have learned from you and your students and trainees – Jacob Levenstein, in particular but also Catharina Zich, and Evan Edmond – have provided me with a unique skill set that I am now able to apply to my future research in the areas of kinesiology, neuroscience, physical therapy and exercise.

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Dedication

I would like to dedicate this thesis to my girlfriend Victoria Baxter and my family. Victoria and I met shortly after my arrival in Saskatoon in 2015, and throughout my master's and doctoral degrees Victoria has been incredibly supportive and loving. Her presence over the past six years has made my time as a graduate student by far the most special time of my life. Further, I am a first-generation university student and graduate. My family has taken great pride in this fact, and I am proud to have done this for my family. My family has always been supportive of whatever my goals might be and have always wished me success. For that, I would also like to dedicate this thesis to my mother Lucia Andrushko, my father Walter Andrushko, my brother Trevor Andrushko, and last but certainly not least, my three cats Jesse, Jane, and Jay.

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List of Abbreviations

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
AUC	Area under the curve
BET	Brain extraction tool
BDNF	Brain-derived neurotropic factor
BOLD	Blood oxygen level dependent
CE	Cross-education
cM1	Contralateral primary motor cortex
DMN	Default mode network
EEG	Electroencephalography
EMG	Electromyography
EPI	Echo planar imaging
FDI	First dorsal interosseous
FEAT	FMRI Expert Analysis Tool
FILM	FMRIB's Improved Linear Model
FLIRT	FMRIB’s linear Image Registration Tool
FLAME	FMRIB's Local Analysis of Mixed Effects
fMRI	Functional magnetic resonance imaging
FMRIB	Functional magnetic resonance imaging of the brain
FNIRT	FMRIB’s Nonlinear Image Registration Tool
FSL	FMRIB Software Library
FWHM	Full width half maximum
GABA	Gamma-aminobutyric acid
GLM	General linear model
ICA	Independent component analysis
ICA-AROMA	Independent Component Analysis Automatic Removal of Motion Artifacts
ICA-FIX	FMRIB's ICA-based Xnoiseifier

IHI	Interhemispheric inhibition
iM1	Ipsilateral primary motor cortex
M1	Primary motor cortex
M1-M1	Functional connectivity between the primary motor cortices in each hemisphere
M1a	Anterior primary motor cortex
M1p	Posterior primary motor cortex
MEG	Magnetoencephalography
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MNI	Montreal neurological institute
MP-RAGE	Magnetization-prepared rapid acquisition with gradient echo
MVC	Maximum voluntary contraction
NIBS	Non-invasive brain stimulation
PET	Positron emission tomography
PM	Premotor cortex
PMd	Dorsal premotor cortex
PMv	Ventral premotor cortex
RM-ANOVA	Repeated measures analysis of variance
RMS	Root-mean-square
ROI	Region of interest
RT	Response time
S1	Primary somatosensory cortex
SI	Surround inhibition
SICI	Short interval intracortical inhibition
SMA	Supplementary motor area
SMA-SMA	Functional connectivity between the supplementary motor areas in each hemisphere
SRTT	Serial reaction time task
T1	Structural brain scan

tDCS	Transcranial direct current stimulation
TE	Echo time
TMS	Transcranial magnetic stimulation
TR	Repetition time

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Chapter 1

1.0 Introduction

Unimanual sensorimotor control is an essential ability often taken for granted in able-bodied individuals. The ability to grasp and manipulate objects, and the ability to scale pinch force to an optimal level that allows for effective object control are essential sensorimotor skills throughout activities of daily living. However, in the event of an injury resulting in unilateral impairment, whether it be orthopedic or some insult to the nervous system (e.g., stroke), the importance of unimanual sensorimotor function is clear. To fully understand how to optimize unimanual recovery and control it is first important to study the neural correlates of unimanual control in healthy, neurologically intact individuals and then translate that work into clinical populations.

In humans, control of voluntary movement is governed by the cerebral sensorimotor network, which involves regions of the frontal and parietal lobes (Chenji et al., 2016). The frontal lobe regions include the primary motor cortex (M1); the supplementary motor area (SMA) - which can be divided into SMA-proper and pre-SMA (Goldberg, 1985; Tanji, 1994); the pre-motor cortex (PM) - which can be divided into the dorsal-PM (PMd) and ventral-PM (PMv) (Gallese et al., 1996; Rizzolatti et al., 1996); and the anterior cingulate cortex (ACC) (Asemi et al., 2015). In the anterior or rostral segment of the parietal lobe, the primary somatosensory cortex (S1) runs parallel with the M1 in a dorsoventral direction. Separated by the central sulcus, M1 and S1 are closely linked, each containing a human somatotopic homunculus that is nearly identical in each region in terms of location and size of body segments.

The primary control centres for unimanual motor control reside in the contralateral sensorimotor network. However, strict contralateral control over unimanual motor control is not always the case. There is evidence that the ipsilateral sensorimotor network becomes active, or more excitable, if the task is complex (Verstynen et al., 2005; Van Den Berg et al., 2011; Buetefisch et al., 2014), involves an element of fatigue (Takahashi et al., 2009), or if the unimanual force level is high (Perez and Cohen, 2008; Tazoe and Perez, 2014). The precise role of the ipsilateral activation and excitability remains unknown.

A neuromuscular phenomenon of unimanual motor practice is interlimb transfer (Lee and Carroll, 2007; Ruddy and Carson, 2013; Manca et al., 2018). Interlimb transfer is termed *cross-education* (CE), and refers to the increased motor output (i.e., force generation, skill) of the

opposite, untrained limb following a period of unilateral exercise training (Manca et al., 2021). The neural underpinnings of CE have remained elusive; however, the conditional activation and increased excitability of the ipsilateral sensorimotor network previously mentioned remains an intriguing avenue of investigation. The clinical applicability of CE as a feasible intervention strategy to offset the deleterious effects of unilateral orthopedic or neurological insult is intriguing given the challenges associated with rehabilitation from unilateral impairment (Andrushko et al., 2018a).

The purpose of this thesis was to: i) investigate how unimanual motor practice (strength and skill), and fatigue modulate ipsilateral cortical activity in healthy individuals and stroke survivors; and, ii) to determine how unimanual motor training impacts motor learning and cross-education with a contralateral limb. The literature review in chapter two surveys the existing literature on the neural correlates and adaptations associated with unimanual motor learning, neuromuscular fatigue, cross-education, and motor recovery in stroke survivors, while primarily focusing on the cerebral sensorimotor network.

Chapter 2

2.0 Review of Literature

2.1 Mechanisms of neuroplasticity and motor learning

Motor learning can be loosely defined as the processes aimed at learning and refining new voluntary movement skills through practice (Nieuwboer et al., 2009). To understand how specific motor training interventions can 'prime' the sensorimotor system for enhancing motor training adaptations, it is important to first conceptualize the neurophysiological mechanisms that facilitate the 'rewiring of the brain' (i.e., neuroplasticity) with motor training in general.

At a neuronal level there are two primary sites where motor training related neuroplasticity are thought to occur (Chen et al., 2015). There is the presynaptic axon terminal/bouton, and the postsynaptic dendritic spines (Chen et al., 2015). In the early phases of motor training, there is a reduction in the density and number of inhibitory neuronal axon boutons, which results in an acute reduction of inhibition on the postsynaptic excitatory (glutamatergic) dendritic spines (Chen et al., 2015). The reduction in inhibition thereby promotes the formation of new dendritic spines after motor training (Kida and Mitsushima, 2018). Excitatory dendritic spine formation promotes preferential neuroplasticity via increased excitability to the motor trained muscles and the formation of new neuronal connections related to the newly acquired motor skill (Xu et al., 2009). The acute reduction in inhibition is later followed by a recovery of inhibition through the reformation of inhibitory neuronal axon bouton synapses (Kida and Mitsushima, 2018). The recovery of inhibition then aids in the competitive selection of dendritic spines through 'pruning' of non-essential synapses (Hayama et al., 2013). Through this process use-dependent or 'Hebbian' plasticity occurs (Hebb, 1949; Shatz, 1992; Zenke et al., 2017), and forms the theoretical basis for which repetitive motor practice aids in the formation and mastery of motor performance (Xu et al., 2009). Therefore, any form of intervention that can reduce GABAergic inhibition within the brain prior to, or during motor training may in fact be utilized to enhance motor training outcomes. In support of this, previous research has demonstrated a mechanistic link between reductions in GABA concentrations within the primary motor cortex (M1) and enhanced motor learning (Floyer-Lea et al., 2006; Stagg et al., 2011a), and a recent study found that motor learning was impaired when cortical

inhibition was increased through a 10 mg dose of Baclofen, a GABA agonist drug (Johnstone et al., 2021).

2.2 Neural correlates of motor learning

The process of learning and retaining a skill motor movement is thought to involve a process of acquiring and storing a 'motor memory' within the brain much like cognitive forms of memory formation (e.g., working memory). Memory storage is thought to reside through chemical and/or physical changes to neurons in specific cell bundles of the brain (Ruddy and Carson, 2013; Tonegawa et al., 2015; Josselyn and Tonegawa, 2020). The neurons populating the region(s) where these adaptations occur are termed 'engrams' (Schacter et al., 1978). Memory engrams are relevant to motor behaviour in that the process of successful motor learning theoretically depends on the strengthening of engrams related to motor behaviour (i.e., motor engrams). The theory of engrams dates back to 1904 when Richard Semon first introduced the term 'engram' as a neural mechanism of memory, (translated to English in 1921 (Semon, 1921)). Engrams were originally believed to be localized to a specific region of the brain termed an 'engram ensemble' which are organized in a somatotopic manner (Monfils et al., 2005; Josselyn and Tonegawa, 2020). However, this belief led to difficulty in successfully locating the physical engrams of memory (Josselyn and Tonegawa, 2020). Prior recent research proposed that a motor engram is not stored in one specific location as a complete motor memory, rather, through the chemical and/or physical adaptations of neurons in multiple areas of the brain (Berlot et al., 2018). Multiple regions of engram ensembles create a functionally connected network termed an 'engram complex' (Josselyn and Tonegawa, 2020). Through multiple engram ensembles of potentially generalizable motor commands, the successful storage and retrieval of a motor memory is achieved through the sequential activation of an engram complex, essentially summating to the final, desired, motor behaviour (Berlot et al., 2018; Yokoi and Diedrichsen, 2019).

Through a combination of animal models and human research, several correlates of motor learning and memory have emerged. Neuronal excitability is a known determinant of a neuron's successful assignment to an engram. Neurons in a given brain region compete for inclusion in an engram ensemble for a given memory, with the more excitable neurons becoming part of the engram (Kim et al., 2016; Josselyn and Tonegawa, 2020). Additional adaptions that

correlate with motor memory and learning include: increases in dendritic spinal density and synaptic strength (Xu et al., 2009; Yang et al., 2009; Lisman et al., 2018); task dependent functional connectivity between multiple brain regions (Ranganath et al., 2005; Sami et al., 2014); increased dopamine (Rioult-Pedotti et al., 2015); increased brain-derived neurotropic factor (BDNF) (Klintsova et al., 2004); and decreases in GABA concentrations (Stagg et al., 2011a).

2.2.1 A Bayesian approach to motor learning

The successful execution of sensorimotor behaviour such as catching a ball involves the appropriate management of variability. In this example, there is variability in the task (e.g., velocity and trajectory of the ball being thrown, etc.) and in the intrinsic sensorimotor system to successfully catch the ball (e.g., placement of the hand, appropriate absorption of force, etc.). The ability to learn the task relies on one's ability to accurately predict and reduce the amount of variability. Bayesian inference (Cox, 1946) is the process where the probability for the hypothesis is updated as new knowledge is acquired and combined with prior information (Körding and Wolpert, 2004). Bayesian inference can explain how people improve and learn proficient execution of sensorimotor tasks with repeated practice. With practice of a sensorimotor task, more information is acquired through the management of errors and successes. The information is applied to update the hypothesis for better predicting the probability of a certain outcome (e.g., catching a ball).

A Bayesian approach to conceptualize motor learning and adaptations to sensorimotor control is relevant to not only the direct practice for unimanual tasks but can also help explain, at least in part, the CE phenomenon in the opposite untrained limb. With cumulative practice of a unilateral sensorimotor task, greater knowledge is obtained, which then is used to update the hypotheses for executing a given task. This knowledge can be generalized to the opposite untrained limb to improve the task execution in the absence of directly training that limb (Hewitson et al., 2018).

2.3 Cross-education of motor training

CE is a neuromuscular phenomenon that refers to the increased motor output (i.e., force generation, skill) of the opposite, untrained limb following a period of unilateral exercise training (Lee and Carroll, 2007; Ruddy and Carson, 2013; Manca et al., 2018, 2021). The magnitude of

the effect after strength training has been previously described as an ~8% increase in strength in the untrained limb, or a ~50% increase in strength relative to the enhancement in the trained limb (Carroll et al., 2006). A more recent systematic review and meta-analysis reported CE to enhance the absolute strength of an untrained limb in the magnitude of 18% in young healthy individuals, 17% in healthy older adults, and even higher in neuromuscular impaired individuals with a mean effect of 29% (Green and Gabriel, 2018). The meta-analysis by Manca et al. (2017) quantified the absolute improvement in an untrained limb as 9.4% for upper limbs, 16.4% for lower limbs (11.8% pooled across limbs) and reported differences in the effect dependent on the type of contraction used in strength training. Eccentric muscle actions were shown to improve untrained limb strength by 17.7%, whereas isotonic dynamic training (concentric + eccentric) and concentric training improved untrained limb strength by 15.9% and 11.3%, respectively. Isometric strength training was the least effective contraction type for enhancing strength in the untrained limb with a mean effect of 8.2% (Manca et al., 2017). The CE effect was first described in the late 19th century (Scripture et al., 1894), although it wasn't until the 21st century that researchers began to investigate its clinical utility for aiding in motor recovery of an impaired limb (Andrushko et al., 2018a). Current evidence points towards a neural origin for CE (Farthing et al., 2007; Lee et al., 2010; Ruddy and Carson, 2013; Ruddy et al., 2017b; Manca et al., 2018), but the exact underpinnings of the effect remain unresolved.

2.3.1 Neural mechanisms of cross-education

There are currently two dominant hypotheses that broadly theorize the neural mechanisms of CE; these two hypotheses are the *Bilateral Access* hypothesis, and the *Cross-Activation* hypothesis (Lee et al., 2010; Ruddy and Carson, 2013). The *Bilateral Access* hypothesis (figure 2.1) suggests that motor training leads to neuroplastic changes in the trained hemisphere, and this 'learned' motor behaviour is shared, or transferred through commissural fibres projecting to the contralateral hemisphere, and then used when executing motor tasks with homologous muscles of the contralateral limb. The *Cross-Activation* hypothesis (figure 2.2) suggests that unimanual motor training leads to increased excitability and/or activation in the motor network bilaterally; and subsequent adaptations resulting from the increased excitability and/or activation in the ipsilateral motor areas are thought to form a duplicate engram complex giving rise to the performance enhancement observed in the untrained limb (Parlow and Kinsbourne, 1989). Early

research proposed an additional model termed the *Callosal Access* model, which was developed on the premise that the left hemisphere was dominant for motor behaviour in right-handed individuals, whereby regardless of which limb is trained, the motor engram is only stored in the left hemisphere (Taylor and Heilman, 1980). The *Callosal Access* model suggested that after right limb training, the left limb would only have indirect access to the stored motor engram in the left ipsilateral hemisphere through transcallosal connections via the corpus callosum. In contrast, the preferential direction of left to right limb was thought to be a result of the ipsilateral motor engram storage after left limb training, giving direct access of the motor engram for the right untrained limb via decussated pathways. Lateralized cortical motor dominance was suggested to cause poorer transfer in the right to left limb direction when compared to the left to right direction of transfer (Hicks, 1974; Taylor and Heilman, 1980). The *Callosal Access* model has since fallen out of favour due to several studies failing to find support for the theory, with contralateral cortical activity more typically observed during unimanual tasks regardless of the active limb (Borowsky et al., 2002). Both the *Bilateral Access* hypothesis and the *Cross-Activation* hypothesis have merit and may not be mutually exclusive from one another (Colomer-Poveda et al., 2019).

A systematic review by Colomer-Poveda and colleagues concluded that the excitability of the ipsilateral M1 (iM1) via transcranial magnetic stimulation (TMS) stimulation protocols was evident in chronic CE based strength training studies. In contrast, for acute (single session) CE strength training studies, ipsilateral excitability was not consistently observed (Colomer-Poveda et al., 2019). Given the increased ipsilateral excitability in chronic studies, *Cross-Activation* mechanisms may explain late-phase CE adaptations, whereas, the lack of change to ipsilateral excitability in acute resistance training studies lends support for the *Bilateral Access* mechanisms for early-phase CE effects. However, it is important to note that these findings do not completely invalidate the *Cross-Activation* hypothesis for acute phases of CE, nor the *Bilateral Access* hypothesis for late-phase CE. Ipsilateral cortical and/or subcortical areas of the brain may still be contributing to the acute CE effects that are not observable with non-invasive brain stimulation protocols to the M1. Similarly, contralateral and ipsilateral areas of the brain, outside of M1, may be contributing to late-phase CE effects. An examination of the time course of brain activation data during unilateral strength training is critically needed to attempt to answer these questions. Both theories aim to explain the location of motor engram storage that is

accessible to the untrained limb. Based on the evidence presented in section 2.1 it is likely that motor memory is stored in an engram complex, made up of several distinct engram ensembles distributed throughout the cortical and subcortical areas of the brain.

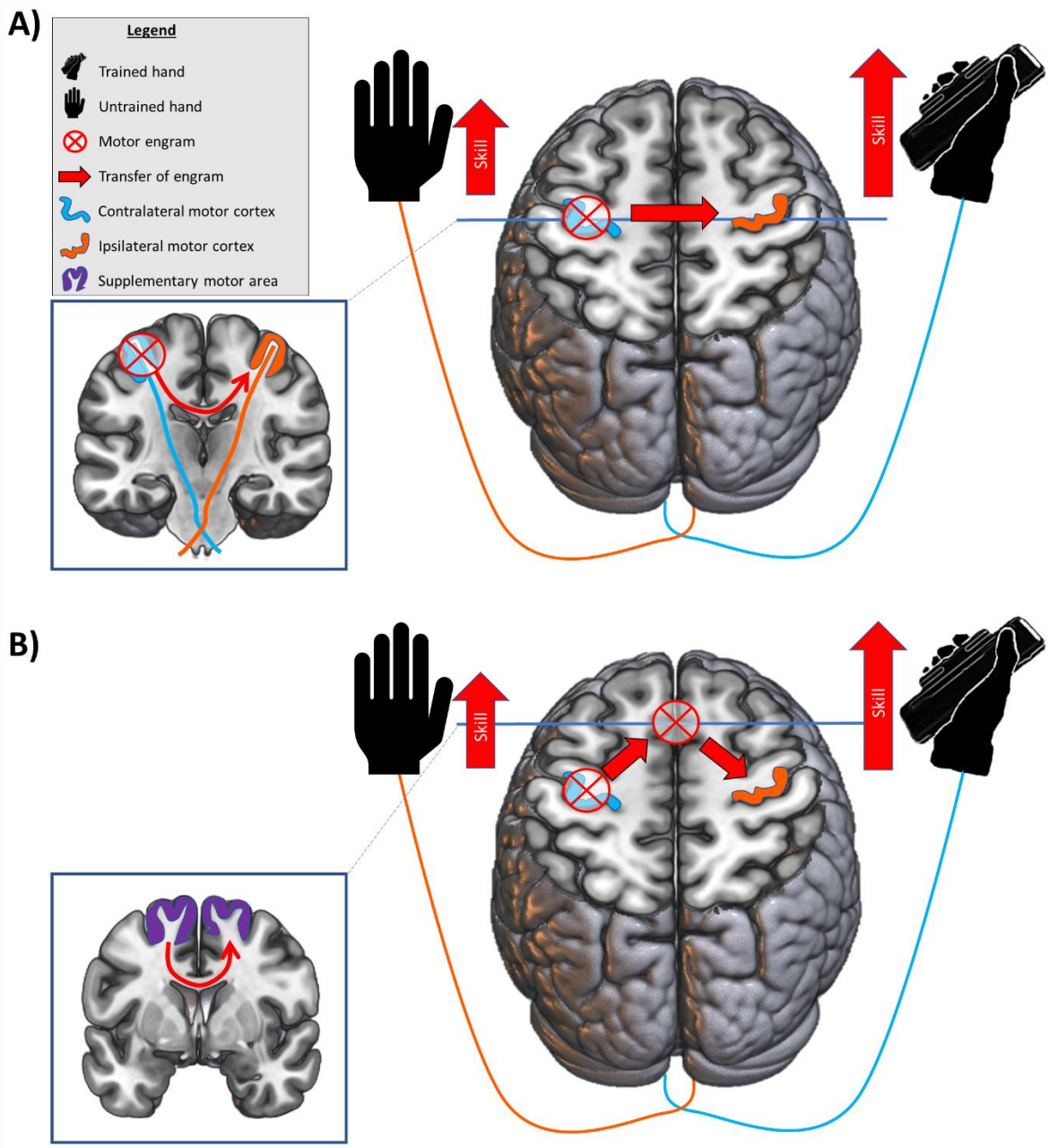

Figure 2.1 Bilateral-Access hypothesis. This theory has two proposed mechanisms; **A)** The motor engram is stored in the contralateral motor cortex and the information is shared with the ipsilateral motor cortex through homologous connections in the corpus callosum. **B)** The motor engram is stored in the contralateral motor cortex but the information is transferred 'up-stream' through the transcallosal homologous connections between supplementary motor area in each hemisphere or some other pre-motor region in the brain.

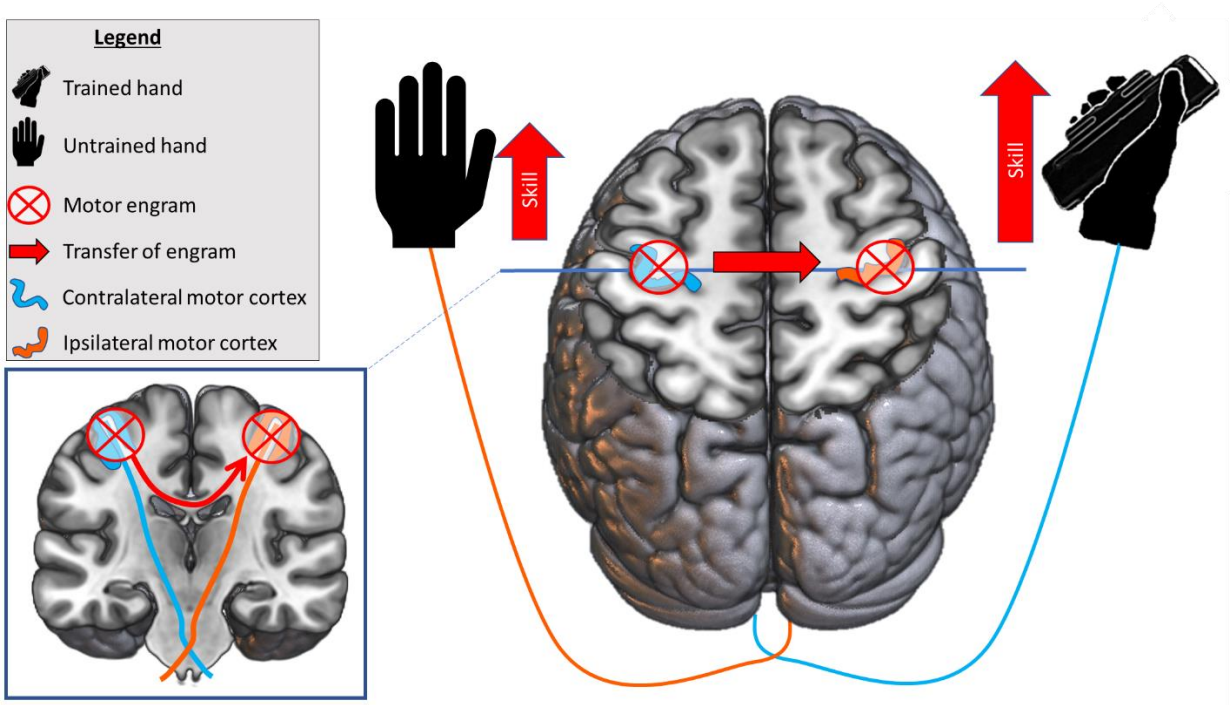

Figure 2.2 Cross-Activation hypothesis. This theory proposes that unilateral voluntary motor training results in bilateral motor cortex activation, which then produces a motor engram in each hemisphere.

2.3.1.1 Interhemispheric inhibition

The corpus callosum is a transcallosal structure of myelinated axons that connects homologous and heterologous regions between the two hemispheres of the brain. The corpus callosum can regulate cross-talk through the inhibition or disinhibition of neuronal signals travelling through these pathways (Daskalakis et al., 2002). Many of the sensorimotor areas have transcallosal projections that connect to various regions within the sensorimotor network in the contralateral hemisphere. The most abundant commissural connections through the corpus callosum seem to project to homologous regions (Ruddy et al., 2017a). In the order of fibre density for homologous commissural connections, the connections between SMAproper in each hemisphere contains the greatest fibre density, followed by PMd, preSMA, CMA, anterior M1 (M1a), PMv, S1 and lastly, posterior M1 (M1p) contains the least dense transcallosal connections between homologous pairs (Ruddy et al., 2017a). Measuring interhemispheric inhibition (IHI) is typically accomplished using paired-pulse TMS protocols, with a conditioning stimulus applied to the M1 in one hemisphere an instant before a test stimulus is delivered to the opposite M1. The

conditioning stimulus inhibits the size of the motor evoked potential (MEP) produced by the test stimulus in the contralateral M1 (cM1) (Ferbert et al., 1992; Hanajima et al., 2001).

Four studies have observed a decrease in IHI with unimanual strength exercise paradigms (Perez and Cohen, 2008; Camus et al., 2009; Hortobágyi et al., 2011; Howatson et al., 2011). In the study by Howatson et al., the authors observed a decrease in IHI with both concentric and eccentric contractions (Howatson et al., 2011). However, with the high-intensity eccentric contractions (90% maximum voluntary contraction [MVC]) the IHI from the exercised contralateral hemisphere to the non-exercised ipsilateral hemisphere was nearly abolished. Perez and Cohen (2008) found that with parametric increases of forceful wrist flexion contractions (10, 30 and 70% MVC) the IHI from the cM1 to the iM1 parametrically decreased. This negative correlation suggests that effortful contractions may give rise to improvements in the CE of strength given the observed decrease in IHI between homologous motor cortices. Concerning CE after chronic strength training, there are three studies that have directly investigated the impact of IHI on the interlimb transfer of strength (Camus et al., 2009; Hortobágyi et al., 2011; Manca et al., 2016). Camus et al. (2009) witnessed CE effects in a single session that were accompanied with a decrease in IHI after motor training with a unimanual pinch force task. In the study by Hortobágyi et al. (2011), participants engaged in 20 training sessions of isolated first dorsal interosseus (FDI) abduction exercise. The authors found that IHI and muscular strength improvement in the untrained first dorsal interosseus (FDI) muscle over the 20 training sessions were negatively correlated, where strength increase was correlated with decreased IHI. In contrast Zult et al.(2016) investigated CE using a mirror paradigm (i.e., using a mirror to theoretically activate the mirror neuron system in the 'resting' limb during motor practice) and observed an increase in IHI in those that experienced greater CE. Further, Manca et al. (2016) had participants perform unilateral high-intensity FDI focused pinch grip training in a CE experiment, but failed to observe any differences in IHI between experimental and control groups (Manca et al., 2016). Perez et al. (2007b) investigated CE with a serial reaction time task (SRTT) to assess procedural knowledge transfer between limbs. Perez et al. (2007b) found that both sequence-specific blocks and non-specific random blocks (i.e., randomly presentation of button presses with no sequence to learn) effectively transferred to the non-trained limb. IHI however was only correlated with the transfer of general motor performance improvements

(faster response times on random blocks with no repeating sequence) but did not correlate with the transfer of the sequence-specific task blocks that represented the procedural knowledge.

2.3.1.2 Intracortical modulation

Intracortical inhibition (ICI) and facilitation (ICF) are processes whereby the cortical excitability within a given M1 is modulated (Wagle-Shukla et al., 2009). Modulation of intracortical excitability is thought to be regulated through gamma-aminobutyric acid (GABA)ergic tone within the motor cortex, with decreases in GABA supporting ICF and/or a decrease in ICI, while an increase in GABA would increase inhibition and thereby preventing ICF (Avoli et al., 1997; McDonnell et al., 2006). Based on the *Cross-Activation* hypothesis (Section 2.3.1, figure 2.2) changes to intracortical excitability within the untrained M1 would be a sensible adaptation to unimanual training that would enhance motor performance of the untrained limb. However, in Ruddy and Carson's review (Ruddy and Carson, 2013), the authors conclude that changes to intracortical excitability within the untrained M1 were not probable mechanisms contributing to CE effects due to a lack of correlations between the changes to the brain and behaviour. A recent 2018 meta-analysis came to a similar conclusion, where short-interval intracortical inhibition (SICI) of the untrained M1 was observed after unimanual motor training but these changes to SICI were not correlated with the motor performance of the untrained limb (Manca et al., 2018).

Although ICI is reported as an unlikely mechanism of CE, changes in the ipsilateral untrained M1 are often observed (Manca et al., 2018). Changes in the iM1 are represented differently (i.e., increases vs. decreases in ICI) depending on the type of muscle contraction used in the motor training. Concentric/shortening muscle contractions do not systematically modulate SICI or ICF in acute or chronic strength training studies (McCombe Waller et al., 2008; Hortobágyi et al., 2011), whereas studies involving strength training with eccentric muscle actions report decreases in ICI and increases in ICF in the ipsilateral, untrained M1 (Howatson et al., 2011; Kidgell et al., 2015). The conclusions made by Ruddy and Carson, that ICI was incidental and not causal of CE, was based on concentric focused strength training literature (Ruddy and Carson, 2013). An investigation of CE with the use of a mirror to activate the mirror neuron system (i.e., mirror therapy model) utilized isometric contractions and witnessed strong CE effects without changes to SICI (Zult et al., 2016). The fact that CE occurs regardless of the contraction type being used and in the absence of iM1 changes to intracortical excitability or

inhibition lends support for the conclusion that it is not a dependent factor for CE to occur (Ruddy and Carson, 2013; Manca et al., 2018). However, these changes to the iM1 intracortical excitability and inhibition cannot readily be ruled out as a contributing factor to the effect and explain, at least in part, why eccentric muscle actions tend to yield greater interlimb transfer of strength (Hortobágyi et al., 1997; Seger et al., 1998; Farthing and Chilibeck, 2003).

2.3.4 Surround inhibition

Surround inhibition (SI) is a neural process whereby an excited neuron inhibits the neural activity of its neighbouring or 'surrounding' neurons (Beck and Hallett, 2011). SI is regulated through GABAergic transmission which neurons use to focus neural activity to perform a desired motor behaviour, where a reduction in proximal neural regions (antagonistic muscles for example) need to be quiescent for the task to be performed efficiently (Mink, 1996; Ziemann et al., 1996). Akkad et al. (2016) used TMS to deliver MEPs to a resting abductor digiti minimi while the participants performed 10% MVC contractions with their first dorsal interosseous muscle and demonstrated that as proficiency in motor training improved, SI decreased in the motor cortex. More recently Bächinger et al. (2019) reported that less proficient movements, and declining motor performance (decreased coordination of agonist-antagonist pairs) after fatigue were associated with a breakdown of SI. SI may be required in acute phases of motor learning to focus and train the neural pattern of activation, but once this pathway and activation pattern is well established, SI is no longer required and diminishes. Regarding CE, SI is thought to play a role, at least in the early phases of learning, in focusing transcallosal neural activity to the homologous region in the contralateral motor network (Ruddy and Carson, 2013), which may contribute to the observed specificity of CE to the contralateral trained homologous muscle group and task.

2.3.5 Summary of candidate cortical mechanisms

The *Bilateral-Access, Cross-Activation* hypotheses, SI, interhemispheric, and intracortical modulation have been surveyed in the previous sections that highlight the primary candidate brain mechanisms likely involved in CE effects. Intracortical modulation is known to change with CE but a link between CE and these changes remains poor (Ruddy and Carson, 2013; Manca et al., 2018). The specificity of CE effects to the contralateral homologous muscle makes

SI a logical candidate for the effect, yet, there is no direct evidence that connects changes in SI with CE. Finally, there is limited evidence that identifies IHI as a leading candidate mechanism for CE (Manca et al., 2018). In summary, the neural contributions to CE of motor training remains poorly understood. Gaining a deeper understanding of the neural contributions to CE is needed for the advancement and translation of this work into clinical settings where it has the greatest potential as an adjunct mode of therapy for unilateral injury.

2.4 Stroke and motor behaviour

A stroke occurs when cortical or subcortical brain tissue is deprived of oxygen from a cessation of blood supply through either a blockage of blood vessels (ischemic stroke) or a broken blood vessel causing the brain to bleed (hemorrhagic stroke) (Yu et al., 2016). The result of such an occurrence is cell death and the formation of what is known as a lesion (i.e., dead brain tissue). Approximately 15 million people have a stroke annually, of which five million suffer from permanent disability (Mittmann et al., 2012). Due to advancements in modern medicine, the survivability of individuals experiencing a stroke has greatly increased (Adamson et al., 2004; Donkor, 2018). However, due to the loss or decline in motor function from the lesioned brain tissue, stroke is one of the leading causes of severe disability (Adamson et al., 2004; Donkor, 2018). Therefore, understanding brain behaviour in stroke survivors and identifying rehabilitation methods that promote neuroplasticity that subserves functional recovery is an important and persistent research objective.

2.4.1 Interhemispheric competition model

The interhemispheric competition model at its roots proposes that lateralized brain activation during unilateral motor tasks serves to suppress or inhibit the brain activity in the opposite hemisphere through transcallosal connections (Kinsbourne, 1974; Murase et al., 2004; Bütefisch et al., 2008; Grefkes et al., 2008; Nowak et al., 2009; Hordacre and Goldsworthy, 2018). Further, when this model is applied to lesioned brains, there is evidence to suggest that a bi-directional alteration in the interhemispheric communication presents. Whereby, a release in the IHI occurs from the lesioned (ipsilesional) to the non-lesioned ‘healthy’ (contralesional) hemisphere. However, the opposite response is observed in the other direction. Where the contralesional hemisphere displays a strong inhibitory response onto the ipsilesional hemisphere (Kinsbourne, 1974; Murase et al., 2004; Bütefisch et al., 2008; Grefkes et al., 2008; Nowak et al., 2009;

Hordacre and Goldsworthy, 2018). In stroke survivors, contralesional brain activation and structural plasticity is often observed during movements of the paretic limb (Schaechter and Perdue, 2008; Buetefisch, 2015; Alawieh et al., 2017; Dodd et al., 2017). The interhemispheric competition model posits that this contralesional brain activity is a maladaptive compensatory response, given that it exhibits a strong inhibitory response on the ipsilesional hemisphere, thereby 'competing' for neural resources and reducing the opportunity for ipsilesional plasticity and recovery (Kinsbourne, 1974; Murase et al., 2004; Bütefisch et al., 2008; Grefkes et al., 2008; Nowak et al., 2009; Hordacre and Goldsworthy, 2018). Based on the interhemispheric competition model, many therapies have been developed to promote ipsilesional plasticity. These therapies include cortical stimulation methods to either inhibit the contralesional hemisphere, or excite the ipsilesional hemisphere (Kinsbourne, 1974; Murase et al., 2004; Bütefisch et al., 2008; Grefkes et al., 2008; Nowak et al., 2009; Hordacre and Goldsworthy, 2018).

Additionally, constraint-induced-movement-therapy is a conventional therapeutic approach utilized in stroke recovery interventions (Grotta et al., 2004). Constrained-induced-movement-therapy is a rehabilitation method in which the less-affected limb is constrained, and the stroke survivor is forced to try and use their affected limb to perform motor tasks. The constraint-induced-movement-therapy approach to motor recovery is also rooted in the notion that contralesional brain activation is maladaptive and that movements with the less-affected limb would promote contralesional hemispheric activation and therefore reduce the opportunity for ipsilesional plasticity to occur. Although the interhemispheric competition model has been widely accepted and used as the foundation for which therapies are developed, the model is somewhat controversial, as good functional motor recovery has been observed in individuals with sustained contralesional brain activation during movements with the impaired limb (Dodd et al., 2017). The interhemispheric competition model has also been called an overly simplistic perspective on post-stroke neuroplasticity and motor recovery (Waters et al., 2017; Hordacre and Goldsworthy, 2018).

2.4.2 Cross-education for motor recovery with stroke survivors

Based on the interhemispheric competition model of stroke recovery the implementation of CE would be deemed contraindicated. Given that CE focuses on motor training with the less-affected limb in stroke survivors, this form of therapy would therefore promote contralesional brain

activation and plasticity. There is limited evidence that has demonstrated successful motor recovery of the affected limb in stroke survivors with the implementation of CE (Dragert and Zehr, 2013; Urbin et al., 2015; Sun et al., 2018; Dehno et al., 2021). Further, the effectiveness of the relative CE *'transfer'* (i.e., gain in affected limb relative to the training improvement in the less-affected limb) has been reported to be as high as 91.2% (Dragert and Zehr, 2013) and 83.3% (Sun et al., 2018), suggesting that CE is not only effective, but may actually be magnified in stroke survivors compared to healthy individuals which typically see relative *'transfer'* to the untrained limb in the range of ~50% (Carroll et al., 2006).

As previously stated, the neural mechanisms of CE are poorly understood, but to expand on this further, the neural mechanisms underpinning CE in stroke survivors with lesioned tissue, remains unclear. Therefore, a logical research objective is to determine the brain behaviour during unimanual motor tasks with the less-affected limb of stroke survivors in order to gain insight in which cortical or subcortical regions are active and how this brain activity may impact the CE effect.

2.5 Neuromuscular fatigue and cortical excitability

Exercise to task failure is known to increase cortical excitability and descending neural drive to the exhausted muscle (Benwell et al., 2006). These acute neural changes are thought to act as compensatory mechanisms to overcome fatigue and maintain task performance (Benwell et al., 2006). An acute reduction to GABA has been observed (Maruyama et al., 2006; Takahashi et al., 2009), lasting up to 5-10 minutes after the cessation of fatiguing exercise. A brief reduction in GABA and an increase in cortical excitability may present a 'window of opportunity' where the nervous system is '*primed'* for ensuing motor practice, thereby augmenting the effectiveness of the motor practice adaptation.

Neuromuscular fatigue, which can be defined as an acute reduction in motor performance (Enoka and Stuart, 1992), commonly involves two components: peripheral fatigue, and central fatigue. Peripheral fatigue refers to a disturbance in the neuromuscular system at sites that are distal to the neuromuscular junction, with a disturbance in calcium handling, reduced adenosine triphosphate production, and an accumulation of phosphate occurs (Boyas and Guével, 2011). Whereas, central fatigue refers to disturbances in the central nervous system, encompassing the brain and/or spinal cord (Boyas and Guével, 2011). A recent study observed that fatigue caused a

decrease in M1 inhibition (SICI) and an increase in M1 excitability (MEPs) as measured with TMS (Bächinger et al., 2019). However, the decrease in inhibition was a result of a breakdown in SI, which Bächinger et al. (2019) purport was the likely cause of the observed decrease in neuromuscular coordination via increased coactivation of agonist and antagonist pairs. These findings explain at least in part, the decrements in motor performance commonly associated with fatigue.

The study by Bächinger et al. (2019) highlights the impact of fatigue on reducing acute motor output, which can easily be used to confirm that fatigue is detrimental to motor performance. However, the negative connotation commonly associated with fatigue for training and learning may not be completely warranted. Fatiguing contractions have been shown to increase cortical excitability (Löscher and Nordlund, 2002; Aboodarda et al., 2016; Bächinger et al., 2019) and decrease cortical inhibition in both the cM1 (Benwell et al., 2006; Bächinger et al., 2019) *and* in the iM1 (Maruyama et al., 2006, 2012; Takahashi et al., 2009). A reduction in cortical inhibition may be beneficial for motor training adaptations given the findings that an acute decrease in GABA is linked to improved motor learning (Floyer-Lea et al., 2006; Stagg et al., 2011a; Bachtiar and Stagg, 2014). Reasonably, decreased inhibition in the iM1 may be beneficial for the effectiveness of subsequent unilateral motor training of the opposite non-fatigued limb since that limb would not experience declines in the peripheral neuromuscular system resulting from direct motor activity.

A prior TMS study, designed to examine the effect of fatigue on corticospinal excitability, found that two sustained unilateral contractions with the biceps brachii of 100 seconds each, increased MEP size from both the contralateral hemisphere to the fatigued limb and from the ipsilateral hemisphere to the non-fatigued limb (Aboodarda et al., 2016). The authors concluded that the supraspinal output was increased as a means of compensating for the peripheral declines in muscle performance (Aboodarda et al., 2016). In contrast, a recent study investigated the effect of fatigue on motor learning over four consecutive days (Branscheidt et al., 2019). The authors observed that the fatigue condition blunted motor learning, but by the end of day three and four, the fatigue condition matched, and nearly exceeded motor performance of the non-fatigued condition (although not statistically different). The authors concluded that fatigue is detrimental to motor learning, but the experiment may have been underpowered to detect a chronic effect at day four. There is apparent controversy relating to fatigue as a

detriment to motor learning and chronic training adaptations, particularly pertaining to the ipsilateral motor pathway, and this warrants further investigation.

2.6 Statement of the problem

The optimization of unimanual motor training of strength and skill is important, especially in individuals recovering from a unilateral injury (e.g., orthopedic or stroke). Conducting well controlled mechanistic studies in patient populations are difficult because patient populations can be heterogenous in how their brain and behaviour present. Therefore, investigating the neural correlates of unimanual motor behaviour in healthy individuals provides important information about nervous system behaviour in various settings such as executing forceful contractions and sequenced motor skills. Next it is imperative to take a translational approach to the problem and investigate how brain function differs in neurologically impaired individuals (e.g., stroke survivors) and determine how to modulate cortical activity in these populations to optimize neural plasticity for functional recovery. Finally, identifying methods to enhance or prime neural activity associated with desired motor outcomes in healthy individuals is also important. The effective use of fatiguing contractions is one potential method to augment subsequent motor practice and investigating methods to enhance neural adaptations associated with desirable motor behaviour may be beneficial to inform future clinical research on best practices to facilitate motor recovery in impaired populations.

2.7 Objectives

The objective of this thesis was to investigate i) the neural correlates of the sensorimotor network with parametrically increasing right-hand unimanual grip force during a visuomotor task to determine the cortical dynamics that govern unimanual motor behaviour in healthy (chapter 3) and stroke survivors (chapter 4), and ii) the effect of handgrip motor fatigue on resting-state cortical activity in the sensorimotor network and how these variables impact the motor performance and CE of a unimanual response time and sequence learning task (chapter 5).

Thesis Transition – Study One

As indicated in the literature review, one of the main objectives of this thesis was gain an understanding of the neural correlates of unimanual motor behaviour, with particular interest in the modulation of the ipsilateral hemisphere and identifying methods to enhance the cross-education effect. The first experiment in study one identified that brain activation and functional connectivity of the iM1 parametrically modulates with increased submaximal handgrip force. Further, the second experiment in study one provides a level of confidence that the ipsilateral cortical modulation is not simply related to mirror activity of the opposite resting limb. Study one set the stage for follow up investigations that explore alternative methods to modulate cortical activity in order to gain a fulsome understanding of how different exercise methods specifically target ipsilateral brain activity and functional connectivity.

Chapter 3

Study One - High-force unimanual handgrip contractions increase ipsilateral sensorimotor activation and functional connectivity

3.0 Introduction

Imaging and brain stimulation studies provide evidence that our classical understanding of primarily lateralized contralateral motor control offers an incomplete view of unimanual voluntary force generation by identifying widespread sensorimotor brain network activity. Indeed, when healthy humans generate unimanual force, the primary motor cortex (M1) becomes activated in each hemisphere (Hortobágyi et al., 2003; Kobayashi et al., 2003; Zijdewind et al., 2006; Sun et al., 2007; Perez and Cohen, 2008; Hendy et al., 2017). However, the scaling and hemispheric-specificity of network activation with unimanual voluntary force generation remain unclear (Kim et al., 1993; Thickbroom et al., 1998; Kobayashi et al., 2003; Perez and Cohen, 2008; Buetefisch et al., 2014). The key centres that control each upper extremity reside primarily in the opposite cerebral hemisphere (Cincotta and Ziemann, 2008). The contralateral hemisphere projects approximately 90% of descending corticospinal pyramidal tracts across the body through the decussation in the medulla forming the lateral corticospinal tract, with the remaining ~10% of the tracts descending ipsilaterally forming the anterior corticospinal tract (Amaral, 2000). Previous literature proposed that ipsilateral activation could afford additional neural drive for the generation of unimanual force (Kobayashi et al., 2003; Jankowska and Edgley, 2006). However, scientific inquiry into the functional role has been inconsistent, with findings that suggest iM1 plays an inhibitory (Kobayashi et al., 2003) and facilitatory (Perez and Cohen, 2008) role in unimanual motor behaviour.

Most prior studies that examined ipsilateral brain activity with unilateral motor tasks have used single and paired-pulse TMS measures (Hess et al., 1986; Stedman et al., 1998; Tinazzi and Zanette, 1998; Muellbacher et al., 2000; Hortobágyi et al., 2003; Perez and Cohen, 2008; Vercauteren et al., 2008; Hendy et al., 2017). A potential limitation to utilizing non-invasive brain stimulation to measure the neuromuscular responses is that even with low stimulation intensities, the spatial extent (Doty and Negrão, 1973) and repetitive discharge frequencies (Maier et al., 2013; Lemon and Kraskov, 2019) of the stimulated cortical tissue are much greater than would be under natural conditions (see review by Carson (2020)). Previously, studies have

utilized fMRI to examine lateralization of brain 'activation' with increased unimanual force generation (Dettmers et al., 1995; Thickbroom et al., 1998; Dai et al., 2001; Van Duinen et al., 2008). With fMRI, brain 'activation' can be determined by examining the blood-oxygen level dependent (BOLD) signal, providing an indication of oxygen uptake by active neurons, which is highly correlated with brain activation (Golkowski et al., 2017a). Brain imaging can increase our understanding of the magnitude, hemispheric specificity, and the relationship between activation intensity and temporal correlates (i.e., functional connectivity) of motor centres involved in unimanual voluntary force generation. This is achieved by determining the BOLD signal 'activation' and connectivity between motor centres within and between hemispheres during unimanual motor tasks (Fling et al., 2012; Rosen et al., 2013; Stagg et al., 2014). Hebbian theory suggests neurons that fire together, wire together (Hebb, 1949; Shatz, 1992; Zenke et al., 2017), which is to suggest that if regions of the brain are temporally correlated or '*functionally connected*' they are likely to be functionally involved in the desired behaviour (Bi and Poo, 2001). To link the TMS evidence with that of fMRI and MR spectroscopy, there is some evidence to suggest that IHI and functional connectivity are negatively correlated (Fling et al., 2012; Rosen et al., 2013) with higher levels of functional connectivity associated with lower levels of IHI. There is also evidence that a decrease in GABA concentration within the cM1 correlates with greater functional connectivity across the sensorimotor network bilaterally (Stagg et al., 2014), suggesting that interhemispheric temporal connectivity is enhanced when inhibition is decreased. Therefore, using fMRI to measure functional connectivity and the BOLD signal has the potential to offer insights into interhemispheric and intracortical balance in a non-perturbed state. To our knowledge, no previous fMRI studies have investigated the functional connectivity of the sensorimotor network during forceful unimanual contractions.

3.1 Objectives

The objective of this study was to determine the effects of parametrically increasing right-hand grip force on activation and connectivity of the sensorimotor network within and between hemispheres.

3.2 Hypotheses

The hypotheses were that i) BOLD signal in contralateral and ipsilateral sensorimotor areas would increase with greater handgrip forces, and ii) functional connectivity of the sensorimotor

network would increase bilaterally during the higher force handgrip contractions, suggesting that neural activity in ipsilateral sensorimotor regions scales with force.

3.3 Methods

3.3.1 Ethical approval

This study conformed to the standards set by the Declaration of Helsinki and was approved by the University of Saskatchewan Biomedical Research Ethics Boards: Bio# 01-125. Researchers were not blinded during data collection or analyses.

3.3.2 Experiment one

3.3.2.1 Participants

Thirteen healthy adults (Data are presented as mean ± standard deviation; 11 right-handed, 2 left-handed, age: 28 ± 6 yrs, height: 170.9 ± 9.8 cm, mass: 75.1 ± 16.7 kg) participated in the study. All participants were screened using an MRI patient safety questionnaire, and handedness was self-reported. Participants were instructed to refrain from exercise for 24 hours prior to the MRI session. Written informed consent was obtained from each participant prior to study commencement, and participants were blinded from the study's hypotheses.

3.3.2.2 Experimental design and fMRI parameters

Participants attended two fMRI sessions where they completed three experimental conditions during each session, involving submaximal unimanual isometric handgrip contractions (25%, 50%, 75% of MVC) with the right hand. Data from two sessions were averaged to reduce variance for each participant in analyses. An MRI-compatible hand clench dynamometer (Biopac Systems Inc. Aero Camino Goleta, CA) was used for this study. All scans were done in a Siemens 3T MAGNETOM Skyra MRI scanner (Siemens Healthcare, Erlangen, Germany). Scanning sessions were separated by a minimum of 48 hours. Each session began with whole-brain anatomical scans acquired using a high-resolution magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence consisting of 192 T1-weighted echo-planar imaging (EPI) slices (1 mm slice thickness with no gap), with an in-plane resolution of 1 × 1 mm (field of view = 256 × 256; repetition time [TR] = 1900 ms; echo time [TE] = 2.08 ms). Following the whole brain anatomical scans, participants performed three right-handed MVCs with 60 seconds of rest between trials. No brain scans were taken during each MVC. After the maximum handgrip force

was determined, the three submaximal handgrip conditions (25, 50, 75%) were completed in random order during fMRI brain scans. For each of the functional tasks, T2*-weighted single-shot gradient-echo EPI scans were acquired using an interleaved ascending sequence, consisting of 105 volumes (TR = 1650 ms; TE = 30 ms) of 25 axial slices of 4-mm thickness (1-mm gap) with an in-plane resolution of 2.7 mm × 2.7 mm (field of view = 250) using a flip angle of 90°. The top 2 coil sets (16 channels) of a 20-channel Siemens head-coil (Siemens Healthcare) were used. Scans consisted of a 10-volume alternating block design beginning with five volumes for stabilization (task, rest; 105 volumes total). During scans the participants wore MRI compatible goggles and viewed a projection of a computer screen running a custom-built LabView (version 8.6) interface. Participants saw clear target lines and go/no-go flashing lights and were cued when to contract or relax. The LabView interface was triggered by the MRI to ensure the task was synchronized with each TR.

3.3.2.3 Behavioural motor task

Participants performed 5 sets × 5 repetitions of grip contractions at each prescribed contraction force during separate scanning runs. In a block design, task blocks composed of 1650 ms (i.e., corresponding to the TR for the T2* imaging) contractions alternating with 1650 ms of rest (16.5 seconds total task block), separated by rest blocks of complete rest (16.5 seconds total rest block). Target lines were presented relative to the individual's peak MVC and force feedback was presented as a vertical force bar that was responsive to each participant's grip contraction (i.e., harder contraction resulted in the bar rising vertically). Two virtual 'lights' were present on the motor task interface to cue participants. A green/black light turned green to instruct the participant to contract to the target line and turned black to indicate when to stop contracting. A second red/black light remained black during task blocks and turned red during rest blocks to indicate a sustained rest. The red light switched to black moments before the next task block as an indicator that the next task series of contractions was about to begin. During each contraction force condition, participants were instructed to relax their non-active left arm and hand to prevent mirror activity. Previous research has demonstrated by consciously attempting to relax the non-active limb mirror activity can be negligible or abolished (Hortobágyi et al., 2011).

3.3.2.4 fMRI preprocessing

Functional MRI data processing was carried out using FMRI Expert Analysis Tool (FEAT) Version 6.00, as part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Jenkinson et al., 2012). For each participant, the T1 structural images from each session were merged into a single mean T1 template. The session two T1 image was first aligned with the T1 image from session one using FMRIB's Linear Image Registration Tool (FLIRT: (Jenkinson and Smith, 2001; Jenkinson et al., 2002)). Next, 'fslmaths' was used to create a mean participant specific T1 structural template. The mean T1 template image was used for registering session one and two functional data in order to avoid asymmetry-induced bias between sessions (Reuter and Fischl, 2011; Reuter et al., 2012). Boundary based registration was used to register the functional image to the high-resolution mean T1 structural template image, followed by registration to standard space images. Registration of the functional images to mean T1 structural template images was carried out using FLIRT: (Jenkinson and Smith, 2001; Jenkinson et al., 2002), and the registration to the standard space images was carried out using FMRIB's Nonlinear Image Registration Tool (FNIRT; (Andersson et al., 2007a, 2007b)).

The following pre-statistic processing was applied: motion correction using Motion Correction FMRIB's Linear Image Registration Tool (MCFLIRT; (Jenkinson et al., 2002)); non-brain removal using Brain Extraction Tool (BET; (Smith, 2002)); spatial smoothing using a Gaussian kernel of FWHM 6mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor (Pruim et al., 2015).

Next, Independent Component Analysis Automatic Removal of Motion Artifacts (ICA-AROMA) was used to identify and remove motion-related noise from the functional data (Pruim et al., 2015). Following the ICA-AROMA data clean up, data were high pass temporal filtered with a 0.01 Hz cut off frequency. Time-series statistical analyses were carried out using FMRIB's Improved Linear Model (FILM) with local autocorrelation correction (Woolrich et al., 2001). Z (Gaussianised T/F) statistic images were constructed non-parametrically using Gaussian Random Field theory-based maximum height thresholding with a corrected significance threshold of $p = 0.05$ (Worsley, 2001).

3.3.3 Statistical analysis

3.3.3.1 Handgrip force

For each of the five task blocks (five contractions in each task block) the mean force was calculated. Next, a mean of the two sessions was determined for each task block and was normalized to the mean MVC force (Mean of session one and two) and expressed as a relative value (% MVC). A condition (25, 50, 75% MVC) × time (blocks 1-5) repeated measures analysis of variance (RM-ANOVA) was used to determine motor performance.

3.3.3.2 Task-based functional activation

To assess the activation of brain activity in the three conditions, a multi-session and multi-subject repeated measures analysis was carried out. This analysis method involved three levels. First-level analysis involved analyzing individual scans with a binary block design (1's for when activation should occur and 0's for when the participant should be at rest) was used in the general linear model (GLM) design. Second-level analysis involved creating across session subject means for each condition using a Fixed Effects voxelwise analysis with a corrected *p*-value threshold of ≤ 0.05. The third level analysis involved analyzing group-level statistics using FMRIB's Local Analysis of Mixed Effects (FLAME) 1 with voxelwise statistical thresholding (corrected *p*-value threshold ≤ 0.05). For the third level analysis a '*triple-t*' test was run, which generated three contrast maps (75 > 50% MVC; 75 > 25% MVC; 50 > 25% MVC) to investigate the significant differences between conditions.

3.3.3.3 Motor cortex region of interest signal change

Regions of interests (ROI) for the M1 in each hemisphere were based on the Brainnetome atlas (cM1: A4ul_l; iM1: A4ul_r) (Fan et al., 2016). A condition (25, 50, 75% MVC) × hemisphere (cM1, iM1) RM-ANOVA was used to test for an interaction between conditions and hemisphere for percent signal change. To assess significant main effects and/or the higher order interaction, Bonferroni post-hoc testing was used. Additional data analyses involve separate Spearman's correlations with relative handgrip force (% MVC) and the percent signal change in the cM1 and iM1. Relative handgrip force was entered as a continuous variable using all data from each condition (each participant contributed three data points to the correlation model).

Statistical analyses were carried out in Jamovi version 1.2.16 (The Jamovi Project, 2020) using R version 3.6 (R Core Team, 2019) with packages afex (Singmann, 2018) and emmeans (Lenth, 2018).

3.3.3.4 Functional connectivity

To assess the functional connectivity of the motor network during the three different contraction force conditions, the 50 volumes corresponding with the task blocks were extracted and merged across time. Rest volumes were removed to avoid the potential impact that rest-related activity may have on the functional connectivity analysis (Steel et al., 2016; Cole et al., 2018). First, a non-constrained dimensionality independent component analysis was carried out using temporal concatenation implemented in Multivariate Exploratory Linear Decomposition into Independent Components (MELODIC) Version 3.15, part of FSL. The non-constrained analysis resulted in 28 independent components. After evaluating the components for fit, it was determined that the 28 components over split the data and reducing the number to 10 components prevented over splitting of known spatial networks. Spatial networks were cross-correlated with known spatial distributions (Smith et al., 2009) to confirm that constraining the ICA to 10 components appropriately identified known networks.

Data were masked to remove non-brain voxels; voxel-wise de-meaned; normalized for the voxel-wise variance; whitened (remove the temporal autocorrelation resulting from intrinsic smoothness in voxel time-series data) and projected into a dimensional subspace using probabilistic Principal Component Analysis. Laplace approximation was used to estimate the number of dimensions in the data (Minka, 2000; Beckmann and Smith, 2004). The whitened observations were temporally decomposed into sets of time-series vectors; the participant domain, and across the spatial domain (maps). This was achieved by optimizing for non-Gaussian spatial source distributions using a fixed-point iteration technique (Hyvärinen, 1999). Estimated Component maps were divided by the standard deviation of the residual noise, and a threshold was determined by fitting a mixture model to the histogram of intensity values (Beckmann and Smith, 2004).

All three conditions across both sessions (78 total scans) were included in the group level map at the MELODIC stage. Between condition contrasts were carried out using dual regression with exchangeability blocks used to pair within-subject runs for the permutation testing to determine the null distribution (i.e., three conditions across both sessions were included in one exchangeability block for each participant) (Nickerson et al., 2017). Dual regression involves three stages. First, the concatenated fMRI dataset from the MELODIC stage was decomposed into the 10 predefined spatial maps (determined from the group level ICA analysis) resulting in a

4D space-time dataset with 10 independent timeseries for each participant. Second, the 10 independent time-series were regressed as temporal regressors in a multiple regression, into the same 4D dataset resulting in 10 spatial maps for each participant (one spatial map for each group-level component). The network of interest was then split into individual runs using '*fslsplit*', then for each condition the runs were averaged across sessions for each individual using '*fslmaths*'. For each participant and condition, the mean of the two sessions for the network of interest were then merged back together using '*fslmerge*' resulting in 39 imaging files (13 participants with three conditions each) rather than the original 78 files (13 participants, three conditions with two sessions). Finally, an across condition *F*-test, condition means, and differences between conditions (75 > 50% MVC; 75 > 25% MVC; 50 > 25% MVC) were tested using permutation-testing with FSL's tool *randomise* using threshold-free cluster enhancement (TFCE) statistics (Smith and Nichols, 2009). The sensorimotor network and the default mode network were assessed with this approach. The default mode network was assessed as a control measure to ensure data stability between the three conditions in a non-task related network. A manual Bonferroni correction was used to adjust the alpha level for statistical significance in order to correct for the comparison of multiple components ($\alpha = 0.025$; [0.05/2]).

An ROI approach was used to determine the network strength (i.e., parameter estimates) of the cM1 and iM1 for each individual and condition. The same Brainnetome atlas ROI masks used on the functional activation data were used for the functional connectivity analyses (cM1: A4ul_l; iM1: A4ul_r) (Fan et al., 2016). The network strength provides an indication of each individual's 'contribution' to the overall group level sensorimotor network, whereby a larger parameter estimate indicates that a given individual or condition has stronger functional connectivity to the rest of the network. A condition (25, 50 75% MVC) × hemisphere (cM1, iM1) RM-ANOVA was used to test for interactions between conditions and hemisphere for network strength. To assess significant main effects and/or the higher order interaction Bonferroni post-hoc tests were used. Further data analyses involved using separate Spearman's correlations to determine if relative handgrip force correlated with the overall network strength, and cM1 and iM1 network strength.

3.3.4 Experiment two

3.3.4.1 Participants

A separate cohort of 11 right-handed participants (age: 30.6 ± 6.1 yrs, height: 176.3 ± 31.7 cm, mass: 81.2 ± 34.2 kg) were recruited to participate in a single session control experiment where the same submaximal tasks (25%, 50% and 75% MVC) were performed with EMG recordings of the wrist flexor muscles in each hand. The setup was similar to the MRI environment, with participants laying supine while watching a computer screen during task performance. Participants were instructed to limit body movement during the tasks. The purpose of the control experiment was to quantify muscle activity in the active and non-active arms during the handgrip task.

3.3.4.2 Muscle activity acquisition

EMG electrodes (VERMED NeuroPlus; 2.5 cm^2, Ag/Ag) were placed over the flexor carpi radialis (FCR) muscles in each arm. Electrodes were placed one-third of the distance from the medial epicondyle to the radial styloid following the recommendations from Buschbacher and Prahlow (2000) and Zehr (2002). EMG data was recorded using Biopac amplifier MP150 (Biopac Systems Inc. Aero Camino Goleta, CA) with a sampling rate of 2000.

3.3.4.3 EMG processing

The EMG signal from each MVC and the submaximal conditions were demeaned, then filtered with a fourth order Butterworth digital filter with a high-pass cut-off of 10 Hz, and a low-pass cut-off of 500 Hz. The root-mean-square (RMS) of the filtered EMG signal was then calculated with a moving RMS (window length of 250 ms). The onset and offset of each of the 25 contractions over the course of the five task blocks were determined. The mean RMS EMG for each contraction was then normalized to the mean RMS of the EMG from the peak MVC for each arm respectively (left arm EMG normalized to left arm MVC, right arm EMG normalized to right arm MVC). There were two missing data points across all participants, due to two separate participants missing a repetition when visually cued, this resulted in 24 total contractions for a given condition. The missing data points were replaced with the median value for the given repetition within the respective condition. Next, a within subject normality assessment was conducted using a Kolmogorov-Smirnov test. Where violated, outlier data points

were assessed, removed and replaced with median values. After outlier removal, for each participant the mean normalized EMG activity for each block (five contractions) was calculated for each condition. The mean values were then carried forward for analysis using a condition (25%, 50%, 75% MVC) × time (five blocks) RM-ANOVA. Where there were violations to sphericity, a Greenhouse-Geisser correction was used.

3.4 Results

3.4.1 Experiment one

3.4.1.1 Behavioural task

Mean handgrip MVC force across the two sessions was 43.6 ± 15.6 kg-Force. Participants were accurate with the motor task performance at 25% MVC (Relative: 26.1 ± 1.9% MVC; Absolute: 11.4 ± 4.3 kg-Force) and 50% MVC (Relative: 51.3 ± 4.1% MVC; Absolute: 22.3 ± 7.8 kg-Force). However, the 75% MVC condition was a mean 5% under their target value (Relative: 69.9 ± 8.3% MVC; Absolute: 30.7 ± 12.0 kg-Force).

A significant condition × time interaction was observed, Greenhouse-Geisser corrected $F(1.6,19.7)= 4.819$, $p = 0.025$, $\eta_p^2 = 0.287$, in addition to main effects of condition (Greenhouse-Geisser corrected, $F(1.0,12.5)= 168.454$, $p < 0.001$, $\eta_p^2 = 0.934$), and time (Greenhouse-Geisser corrected, $F(1.5,17.5)= 2.773$, $p = 0.102$, $\eta_p^2 = 0.188$).

3.4.1.2 Task-based functional activation

3.4.1.2.1 Contrasts

Three contrasts were analyzed (75% > 50% MVC; 75% > 25% MVC; 50% > 25% MVC). Contrast maps between 75 > 50% MVC and 50 > 25% MVC failed to detect significant differences between conditions. The 75 > 25% MVC contrast map revealed several significant clusters of activation (figure 3.1; table 3.1). Regarding the motor related areas of the cerebrum, a notable cluster of activation was observed over the iM1 hand knob region. Suggesting that the 75% MVC condition resulted in a stronger BOLD signal in the iM1 compared to the 25% MVC condition (table 3.1). For non-threshold magnitude difference images (contrast of parameter estimates) between the three conditions see figure 3.2 (Chen et al., 2017).

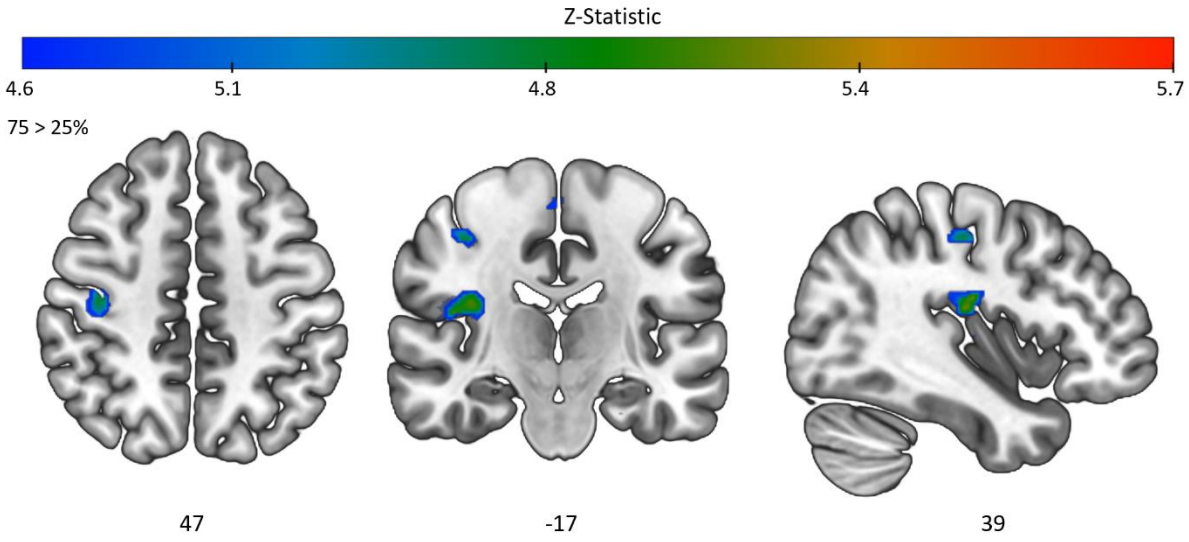

Figure 3.1 75 > 25% MVC contrast map for the 105-volume block design activation analysis. Threshold z = 4.6 ($p < 0.05$). Figure is in radiological view (left side of brain on the right; right side of brain on the left).

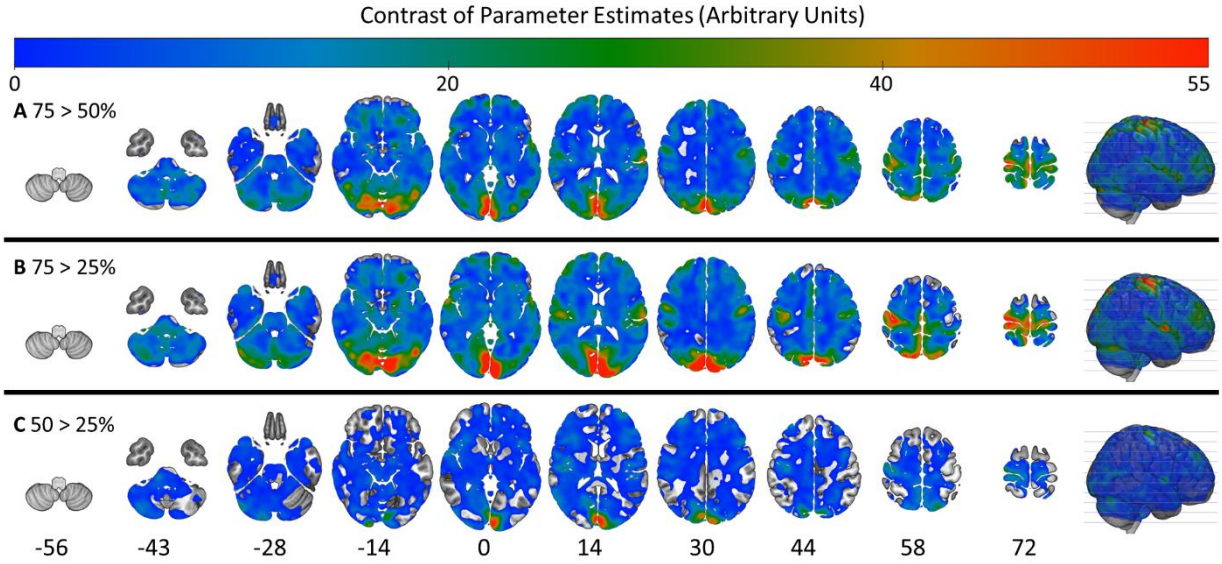

Figure 3.2 Non-threshold brain activation magnitude difference maps. Colour bar represents the contrast of the parameter estimates for A) 75 > 50% MVC, B) 75 > 25% MVC, and C) 50 > 25% MVC conditions. Figure is in radiological view (left side of brain on the right; right side of brain on the left).

Table 3.1 Condition activation contrast maps. Peak Z-statistic voxel for significant clusters

75 > 50% MVC					
Voxels	Z-MAX	Z-MAX MNI Coordinates (mm)			AAL Label
		X	Y	Z	
2	5.02	-2	68	2	Frontal Superior Medial Left
1	4.82	8	-42	20	Cingulum Post Right
75 > 25% MVC					
Voxels	Z-MAX	Z-MAX MNI Coordinates (mm)			AAL Label
		X	Y	Z	
293	5.61	10	-88	34	Cuneus Right
267	5.55	36	-16	18	Insula Right
112	5.29	38	-16	48	Precentral Right
67	5.13	0	-20	64	Supplementary Motor Area Right
47	5.35	62	4	2	Temporal Pole Superior Right
36	5.21	8	-34	-40	Cerebellum 9 Right
32	5.87	18	-4	-2	Pallidum Right
25	4.78	-6	-92	8	Calcarine Left
20	5	36	-22	68	Precentral Right
19	4.81	-56	-6	28	Postcentral Left
17	5.23	-20	2	-14	Amygdala Left
16	4.89	-12	-40	-20	Cerebellum 4 5 Left
10	4.82	-40	-6	-18	Fusiform Left
7	5.04	-70	-36	-4	Temporal Middle Left
5	5.1	-10	60	-6	Frontal Medial Orbital Left
5	4.75	6	-24	-24	Cerebellum 3 Right
4	4.69	4	-92	16	Cuneus Left
3	4.94	-42	-8	-12	Temporal Superior Left
3	4.68	8	-20	48	Cingulum Middle Right
3	4.86	42	54	30	Frontal Middle Right
3	4.84	54	46	10	Frontal Inferior Triangular Right
2	4.66	52	-12	56	Precentral Right
2	4.8	-26	-18	-26	ParaHippocampal Left
2	4.86	-2	68	22	Frontal Superior Medial Left
2	4.72	-54	-12	20	Postcentral Left
2	4.75	-50	-16	16	Rolandic Operculum Left
2	4.78	40	-14	6	Insula Right
2	4.81	10	-26	-16	Cerebellum 3 Right
2	5.03	-2	68	2	Frontal Superior Medial Left
2	4.72	4	-20	-8	Thalamus Right
2	4.73	-28	-24	-6	Hippocampus Left
2	4.66	-10	-28	72	Paracentral Lobule Left
1	4.71	62	6	12	Rolandic Operculum Right
1	4.69	-10	64	36	Frontal Superior Left
1	4.68	2	-82	-6	Calcarine Left
50 > 25% MVC					
Voxels	Z-MAX	Z-MAX MNI Coordinates (mm)			AAL Label
		X	Y	Z	
None					

3.4.1.3 Region of interest analysis - Activation

For the percent BOLD signal change within each ROI, a significant main effect of hemisphere, $F(2,24)=139.73$, $p < 0.001$, $\eta_p^2 = 0.921$ and a condition × hemisphere interaction was observed, $F(2,24)= 6.60$, $p = 0.005$, $\eta_p^2 = 0.355$. The main effect of condition was not significant, $F(2,24)=3.18$, $p = 0.059$, $\eta_p^2 = 0.209$. BOLD signal change was significantly larger in the cM1 compared to the iM1, with a mean signal change difference of 0.52%. To understand the interaction, data for each hemisphere were split, and conditions compared. A RM-ANOVA for the cM1, failed to observe a significant condition main effect, $F(2,24)= 0.682$, $p = 0.515$, $\eta_p^2 = 0.054$ indicating that the percent signal chance in the cM1 was not different between conditions. However, a RM-ANOVA for the iM1 revealed a significant main effect of condition, $F(2,24)= 9.393$, $p < 0.001$, $\eta_p^2 = 0.439$. Bonferroni post-hoc tests demonstrated that iM1 signal change in the 75% condition was greater than 25% ($p < 0.001$) and 50% ($p = 0.017$), but 50 and 25% conditions were not significantly different ($p = 0.784$).

3.4.1.3.1 iM1 signal change (Brainnetome atlas; A4ul_r)

Relative handgrip force (% MVC) was significantly correlated with the iM1 signal change, $\rho(38)= 0.553$, $p = 0.001$, 95% confidence interval (CI): 0.279 – 0.744 (figure 3.3A).

3.4.1.3.2 cM1 signal change (Brainnetome atlas: A4ul_l)

Relative handgrip force (% MVC) was not correlated with cM1 signal change, ρ(38)= 0.210, *p* = 0.199, 95% CI: -0.122 – 0.500 (figure 3.3B)

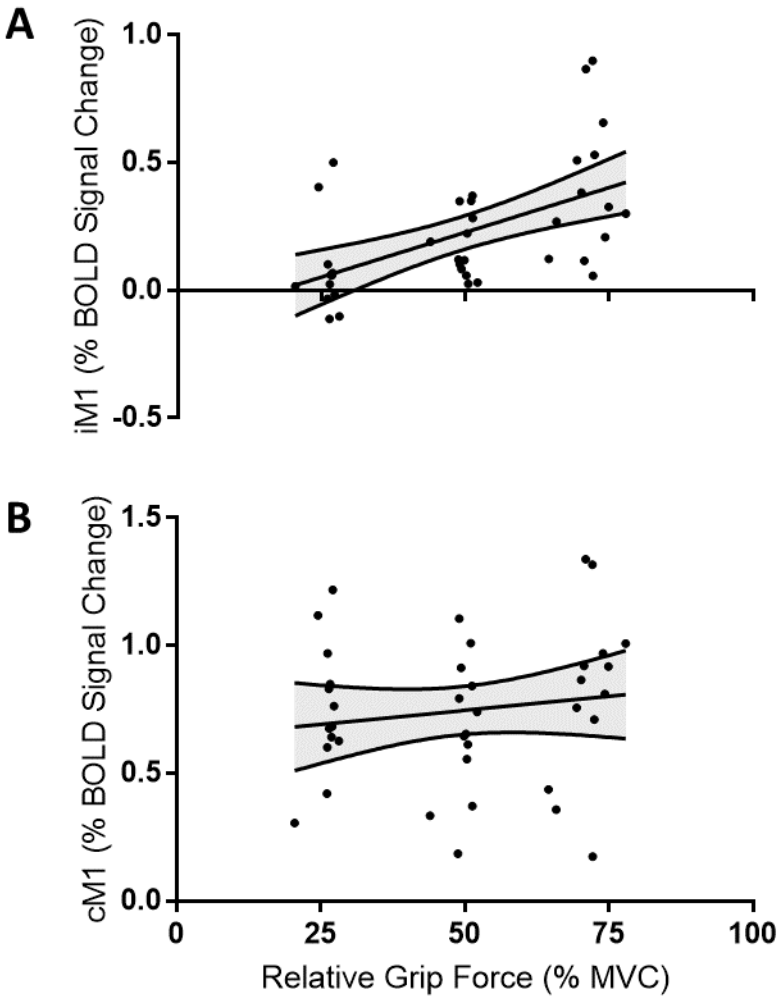

Figure 3.3 Region of interest analyses for the percent BOLD signal change of the contralateral (cM1; Brainnetome atlas: A4ul_l) and ipsilateral hand/arm region of the motor cortex (iM1: Brainnetome atlas: A4ul_r). Spearman's correlations were run with relative handgrip force (% MVC) and: A) ipsilateral motor cortex, ρ(38)= 0.553, *p* = 0.001, and B) contralateral motor cortex, ρ(38)= 0.210, *p* = 0.199. Scatter plots display 95% confidence intervals around the slope (grey band).

3.4.1.4 On-task functional connectivity

3.4.1.4.1 Sensorimotor network contrasts

The 1 minus family wise error rate (1-FWE) corrected *p*-value statistical maps generated from *randomise* with TFCE statistical processing reveal a significant difference in the left, contralateral hemisphere over the pre- and postcentral gyrus (table 3.2). This significant cluster of stronger network connectivity is lost after the Bonferroni correction. A total of six significant clusters were present in the 75 > 25% MVC connectivity contrast within the sensorimotor network. Of interest to the hypothesis, the strongest cluster resides over the ipsilateral precentral gyrus, indicating that higher force handgrip contractions, specifically 75% compared to 25% MVC, increases the sensorimotor network functional connectivity between the ipsilateral and contralateral hemispheres. There were no significant differences observed between 50% and 25% conditions (figure 3.4).

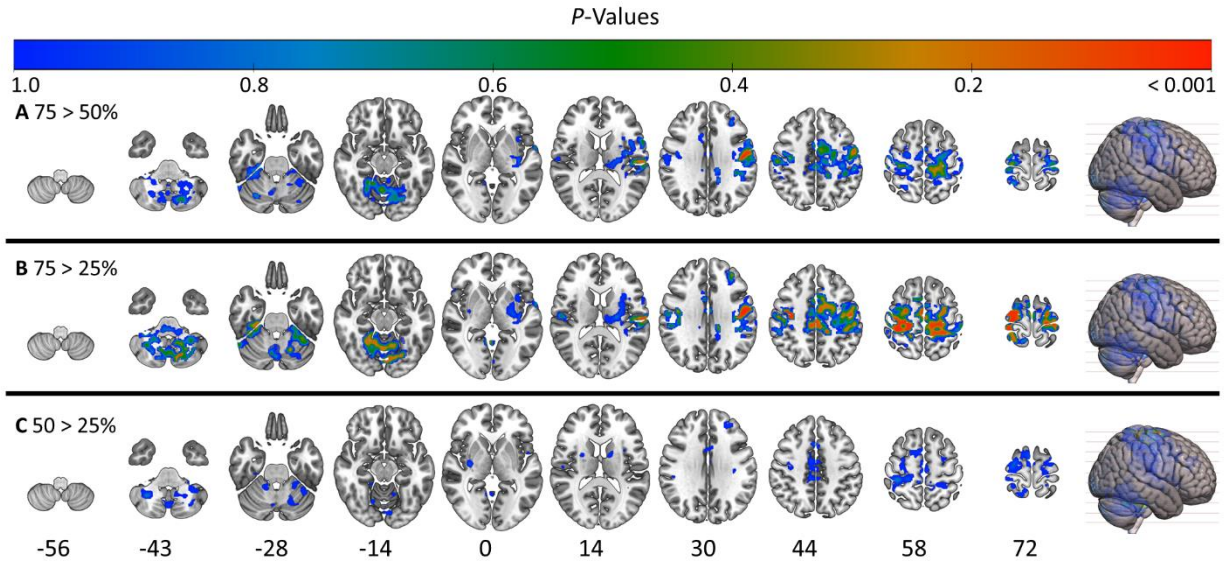

Figure 3.4 Sensorimotor network functional connectivity contrast maps for **A)** 75 > 50% MVC, **B)** 75 > 25% MVC, and **C)** 50 > 25% MVC conditions. There were significant differences between **B)** 75 > 25% MVC conditions after Bonferroni corrections. Figure is in radiological view (left side of brain on the right; right side of brain on the left).

Table 3.2 Sensorimotor network connectivity contrast maps. Peak *P*-statistic voxel for significant clusters

75 > 50% MVC					
Voxels	*P*-MAX	*P*-MAX MNI Coordinates (mm)			AAL Label
		X	Y	Z	
25	0.034	-50	-12	34	Postcentral left

75 > 25% MVC					
Voxels	*P*-MAX	*P*-MAX MNI Coordinates (mm)			AAL Label
		X	Y	Z	
538	0.007*	26	-24	74	Precentral Right
75	0.018*	-50	-12	28	Postcentral Left
18	0.04	-14	-30	62	Paracentral Lobule Left
4	0.038	-20	-76	-42	Cerebellum Crus 2 Left
2	0.047	-22	-24	76	Postcentral Left
1	0.05	-22	-28	78	Postcentral Left

50 > 25% MVC					
Voxels	*P*-MAX	*P*-MAX MNI Coordinates (mm)			AAL Label
		X	Y	Z	
None					

* Significant at Bonferroni corrected $\alpha = 0.025$

3.4.1.5 Region of interest analysis – Sensorimotor network connectivity

For network strengths within each ROI, the condition × hemisphere interaction did not reach significance, $F(2,24)= 0.11$, $p = 0.89$, $\eta_p^2 = 0.009$. However, main effects of condition ($F(2,24)=6.84$, $p = 0.004$, $\eta_p^2 = 0.363$) and hemisphere ($F(2,24)=79.73$, $p < 0.001$, $\eta_p^2 = 0.869$) were observed. Significant differences were found between 75% and 50% conditions (mean difference = 11.52, $t(24)= 2.63$, $p = 0.037$) and 75% and 25% conditions (mean difference = 15.61, $t(24)= 3.57$, $p = 0.004$), but no difference was observed between 50% and 25% conditions, (mean difference = 4.07, $t(24)=0.93$, $p = 0.626$). As expected for the main effect of hemisphere, the cM1 network strength was stronger in magnitude than the iM1 (mean difference = 30.89 arbitrary units).

3.4.1.5.1 Sensorimotor network strength

Relative handgrip force (% MVC) was significantly correlated with the sensorimotor network strength, $\rho(38)= 0.393$, $p = 0.013$, 95% CI: 0.079 – 0.6362 (figure 3.5A).

3.4.1.5.2 iM1 network strength (Brainnetome atlas; A4ul_r)

Relative handgrip force (% MVC) was significantly correlated with the iM1 network strength, $\rho(38)= 0.528$, $p < 0.001$, 95% CI: 0.246 – 0.728 (figure 3.5B).

3.4.1.5.3 cM1 network strength (Brainnetome atlas: A4ul_l)

Relative handgrip force (% MVC) was significantly correlated with the cM1 network strength, $\rho(38)= 0.379$, $p = 0.017$, 95% CI: 0.062 – 0.626 (figure 3.5C).

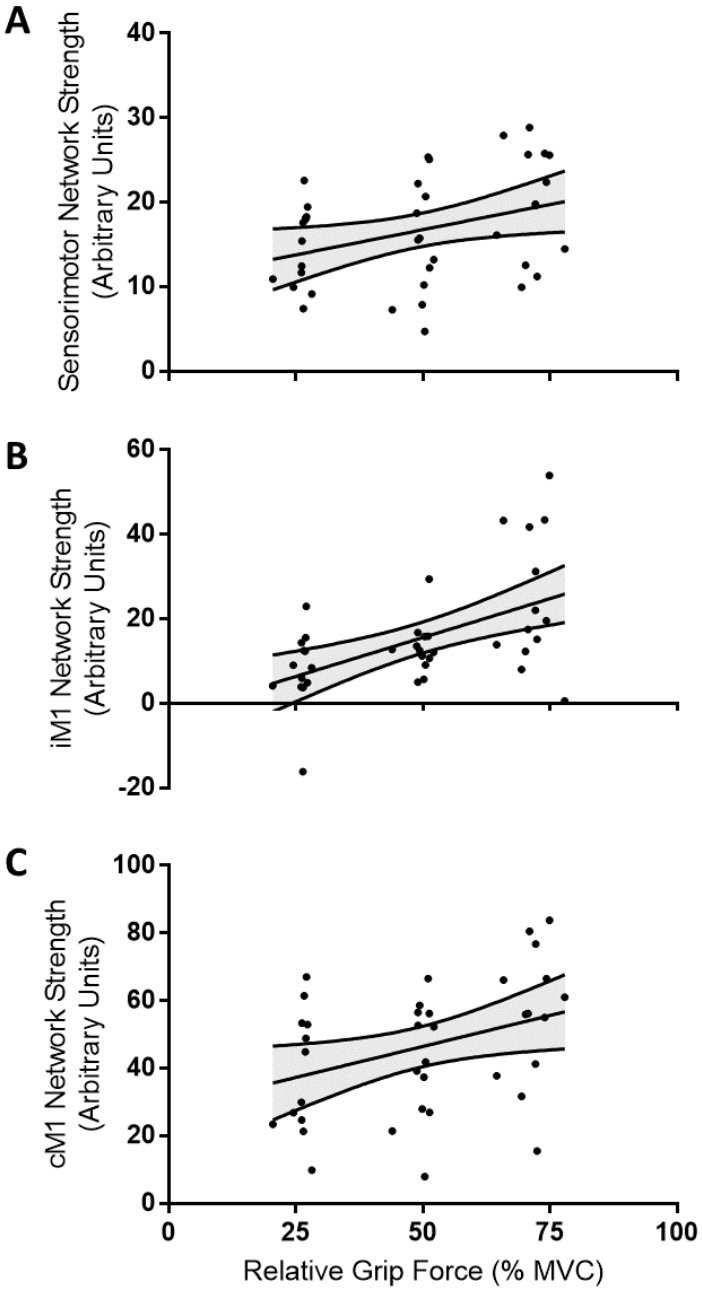

Figure 3.5 Region of interest Spearman's correlations for relative handgrip force (% MVC) and the functional connectivity network strengths of **A)** the entire sensorimotor network, $\rho(38)= 0.393$, $p = 0.013$, **B)** ipsilateral motor cortex (iM1; Brainnetome atlas, A4ul_r), $\rho(38)= 0.528$, $p < 0.001$, and **C)** contralateral motor cortex (cM1: Brainnetome atlas, A4ul_l), $\rho(38)= 0.379$, $p = 0.017$. Scatter plots display 95% confidence intervals around the slope (grey band).

3.4.1.5.4 Default mode network contrasts

The default mode network was analyzed as a control measure for data stability in a non-motor related network. The contrast analyses from *randomise* with TFCE statistical processing failed to reveal any significant differences between the three conditions for the on-task functional connectivity of the default mode network, indicating stability of the network with parametric increase in handgrip force (figure 3.6).

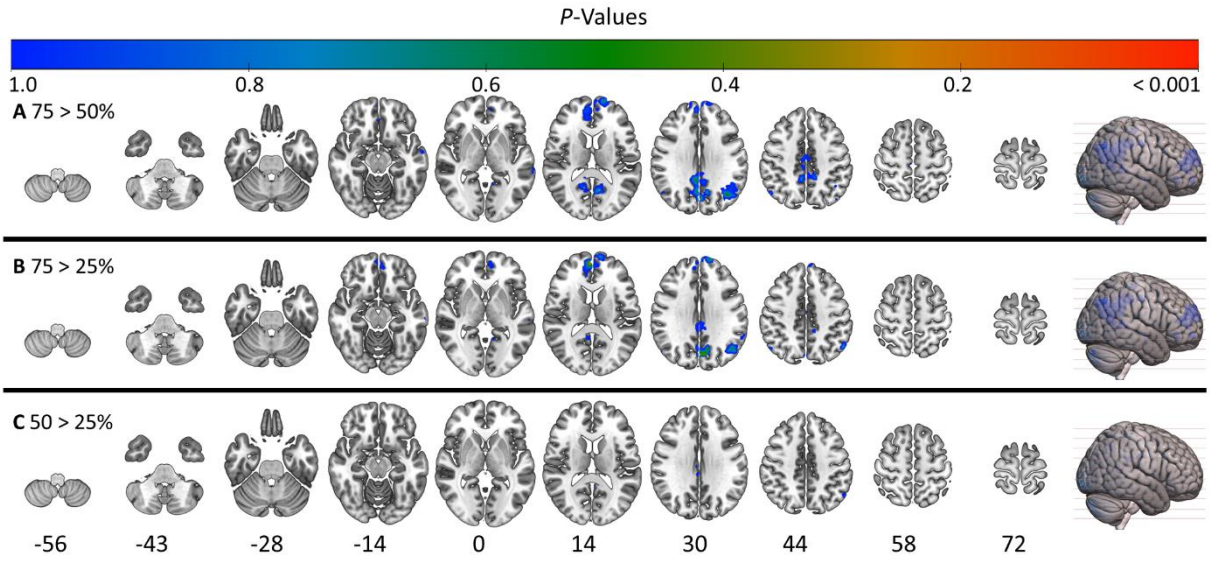

Figure 3.6 Default mode network functional connectivity contrast maps for **A)** 75 > 50% MVC, **B)** 75 > 25% MVC, and **C)** 50 > 25% MVC conditions. Figure is in radiological view (left side of brain on the right; right side of brain on the left). There were no significant contrasts.

3.4.2 Experiment two

3.4.2.1 Right active arm force

There was a significant condition × time interaction, $F(8,40)=3.192$, $p = 0.003$, $\eta_p^2 = 0.242$ and a significant Greenhouse-Geisser corrected condition main effect, $F(1.0,10.1)=166.534$, $p < 0.001$, $\eta_p^2 = 0.943$. The main effect of time did not reach statistical significance, Greenhouse-Geisser corrected $F(1.8,18.3)=1.285$, $p = 0.298$, $\eta_p^2 = 0.114$. To break down the significant interaction, post-hoc tests were used to determine that the 75% condition had a significant decrease in handgrip force in the fifth block (block 1 vs. 5, $p = 0.006$). There were no other significant differences over time for any of the conditions. Post-hoc testing for the main effect of condition

was used to determine that 25% (Marginal mean = 10.58 ± 2.84 kg-Force), 50% (Marginal mean = 20.28 ± 5.41 kg-Force) and 75% (Marginal mean = 29.28 ± 7.55 kg-Force) MVC conditions were all significantly different from each other (all $p < 0.001$).

3.4.2.2 Right active arm EMG

There was a significant main effect of condition, Greenhouse-Geisser corrected $F(1.1,11.4)= 30.928$, $p < 0.001$, $\eta_p^2 = 0.756$. However, the condition × time interaction (Greenhouse-Geisser corrected $F(1.5,15.0)= 0.663$, $p = 0.488$, $\eta_p^2 = 0.062$) and main effect of time ($F(1.5,15.2)= 1.455$, $p = 0.259$, $\eta_p^2 = 0.127$) failed to reach significance. Post-hoc testing for the main effect of condition was used to determine that 25% MVC (Marginal mean = 0.291 ± 0.234 MVC) was significantly different than 50% (Marginal mean = 0.535 ± 0.097 MVC, $p = 0.001$), and 75% (Marginal mean = 0.742 ± 0.045 MVC, $p < 0.001$) MVC conditions. Additionally, 50% was also significantly different than the 75% MVC condition ($p = 0.005$) (see figure 3.7A).

3.4.2.3 Left inactive arm EMG – Mirror activity

The condition × time interaction failed to reach significance, Greenhouse-Geisser corrected $F(1.9,19.2)= 0.771$, $p = 0.471$, $\eta_p^2 = 0.072$. Additionally, main effects of condition ($F(2,20)= 1.323$, $p = 0.289$, $\eta_p^2 = 0.117$) and time (Greenhouse-Geisser corrected $F(1.6,16.2)= 2.023$, $p = 0.170$, $\eta_p^2 = 0.168$) also failed to reach significance, indicating that the mean normalized RMS of the EMG signal for the 25% (Marginal mean = 0.025 ± 0.027 MVC), 50% (Marginal mean = 0.020 ± 0.013 MVC) and 75% (Marginal mean = 0.035 ± 0.035 MVC) were not different (see figure 3.7B).

An additional analysis was carried out with the left arm EMG data without median replacements of missing data points, to ensure the findings are robust and not influenced by the data replacement method. For this analysis the condition × time interaction failed to reach significance, Greenhouse-Geisser corrected $F(1.6,15.5)= 0.525$, $p = 0.557$, $\eta_p^2 = 0.050$. The main effects of condition ($F(2,20)= 1.740$, $p = 0.201$, $\eta_p^2 = 0.148$) and time ($F(1.3,12.9)= 2.050$, $p = 0.176$, $\eta_p^2 = 0.170$) also failed to reach significance.

3.4.2.4 Left inactive arm baseline EMG

For the baseline EMG signal recorded from the left arm, the mean normalized baseline noise in the EMG signal for the 25% MVC was 0.014 ± 0.005, for 50% MVC was 0.016 ± 0.006, and for the 75% MVC condition was 0.016 ± 0.009.

To assess whether the EMG signal during contractions differed from the baseline noise a 3 × 6 condition (25%, 50%, 75% MVC) × time (Baseline noise, blocks 1-5) RM-ANOVA was run for the left arm normalized EMG signal. There was a significant main effect of time, $F(5,50) = 3.195$, $p = 0.014$, $\eta_p^2 = 0.242$. However the condition × time interaction ($F(10,50) = 0.944$, $p = 0.496$, $\eta_p^2 = 0.086$) and the main effect of condition ($F(2,20) = 1.345$, $p = 0.283$, $\eta_p^2 = 0.119$) failed to reach significance. Bonferroni post-hoc testing for the main effect of time failed to detect any significant differences (All $p > 0.05$). These data suggest that the mirror activity across the three conditions was not significantly different than the baseline noise recorded prior to starting the motor task.

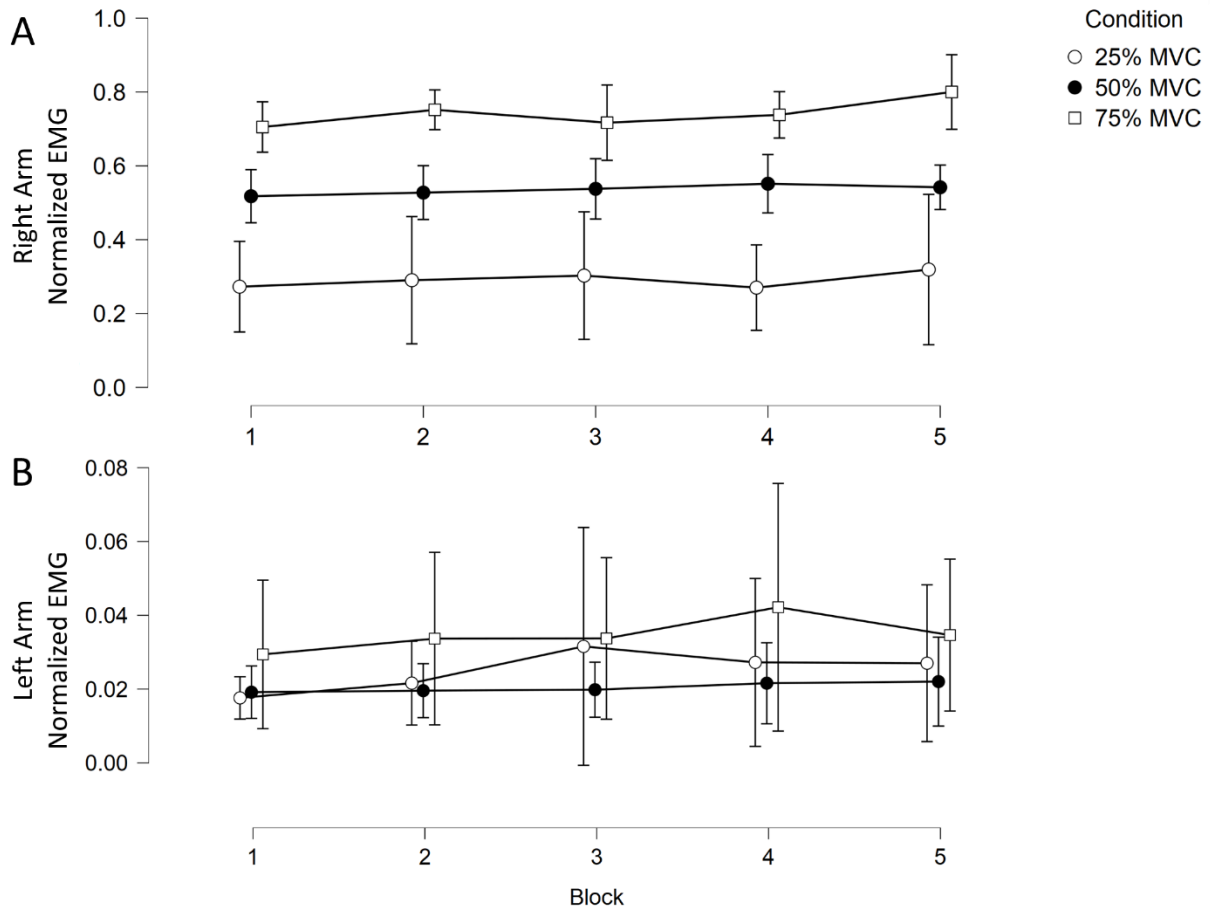

Figure 3.7 Control experiment. EMG normalized to MVC for **A)** the right, active arm, and **B)** the left, non-active arm for each of the three conditions (25%, 50%, 75% MVC) across the five task blocks. Error bars = 95% confidence intervals.

3.5 Discussion

To our knowledge, this is the first study to examine the effects of parametrically increasing unimanual handgrip force on activation and 'on-task' functional connectivity (i.e., functional connectivity during task blocks only) within the sensorimotor network and specifically within the primary motor cortices bilaterally (cM1, iM1).

Concurrent bilateral sensorimotor activity, specifically in the iM1 during unimanual motor tasks, has been investigated in several prior studies utilizing TMS or neuroimaging techniques (Hortobágyi et al., 2003; Zijdewind et al., 2006; Perez and Cohen, 2008; Hendy et al., 2017) and has been inconsistently observed in neuroimaging studies. The inconsistent observation is likely due to the differences in the type of task being performed (Buetefisch et al., 2014). There is evidence to suggest that the iM1 activity depends on task complexity (Verstynen

et al., 2005; Buetefisch et al., 2014). Verstynen et al. (2005) observed greater iM1 brain activation with complex movements such as sequenced movements with multiple fingers compared to a single finger tapping task. Further, greater iM1 activation was also observed with a more difficult sequence compared to an easier one, suggesting that the complexity of the movement and cognitive demand both recruited iM1 greater than a simpler finger tapping task. The handgrip task employed in the present experiments differed in that the task itself did not change, rather only the requisite force output to achieve the targets changed between conditions. An increase in both ipsilateral and contralateral sensorimotor areas BOLD signal may support the notion that the ipsilateral hemisphere aids the contralateral sensorimotor network to enhance force output under high-force conditions (Jiang et al., 2012), but this remains an untested hypothesis, and the purpose of ipsilateral cortical activity remains controversial (Kobayashi et al., 2003). The present study sought to investigate the neural correlates within the sensorimotor network, and more specifically, the activation and network connectivity strength of the cM1 and iM1 with parametrically increasing handgrip contraction forces. The novel data from the current study suggest that both the magnitude of 'activation' and connectivity strength within the iM1 scales with increasing force of unimanual handgrip contractions (figures 3.3 and 3.5).

For the 75 > 25% MVC activation map there were several clusters of significantly greater activation. Within the cerebral sensorimotor network there were significant clusters covering the iM1 hand knob area, the ipsilateral premotor area and one covering the SMA bilaterally. Another notable cluster was observable in the occipital lobe. It is possible that the 75% MVC condition preferentially activated the visual cortex in the occipital lobe to a greater extent than the 25% MVC condition. For each condition, the force feedback bar displayed the full range from 0-100% MVC, with only the target line changing. With a target line at 75% MVC compared to 25% MVC, the visual force feedback bar that represents the contraction is larger for the higher force condition and provided a greater visual stimulus. Another perhaps more plausible interpretation for the cluster of activation in the occipital lobe is that the cluster is in the vicinity of the superior sagittal sinus and the Torcular Herophili (confluence of sinuses) which is the intersection for the superior sagittal sinus, straight sinus, transverse sinuses and the occipital sinus. False activations are common in this area and around other veins (Salimi-Khorshidi et al., 2014; Eklund et al., 2019), and therefore any interpretation of the activation contrasts in the occipital lobe should be made with caution. Outside of the 75 > 25% contrast, there were two significant clusters in the

75 > 50% MVC contrast, one in the frontal superior medial gyrus and another in the posterior cingulum. Aside from those two small clusters (2 and 1 voxel respectively), no other differences were observed in the 75 > 50% or 50 > 25% MVC activation contrasts.

Importantly, prior work has reported robust iM1 activation with motor tasks requiring substantially less force than the 25% MVC condition in the present study but with greater task complexity (Buetefisch et al., 2014; Uehara and Funase, 2014). In relation to the ipsilateral activation with task complexity, an increase in contraction force, although not necessarily more complex in terms of the motor task itself compared to previous work investigating brain activation with task complexity (Verstynen et al., 2005), resulted in greater ipsilateral activation (figure 3.1). Anecdotally, participants reported higher task difficulty with the 50% and 75% MVC gripping task, and therefore an increase in task demand may require greater neural activity to suppress unwanted motor behaviours (e.g. reciprocal inhibition) or reflect activation of synergistic muscles (Perez and Cohen, 2008), which could be a driving factor for the ipsilateral activation. Unfortunately, the lack of peripheral measures of muscle activity with EMG in the primary experiment prevents us from directly linking the ipsilateral brain data to resting limb muscle activity, but there is a convincing relationship between the recorded increase in right handgrip force and signal change in the ipsilateral, right, M1 (figure 3.3).

The data from experiment two suggest that the mirror activity in the left, non-active arm did not scale with the right, active arm. Significant parametric increases in right arm EMG activity were observed, similar to the target force output for each condition (25% condition = 29.1% normalized EMG; 50% condition = 53.5% normalized EMG; 75% condition = 74.2% normalized EMG). Yet there were no significant differences between conditions in the left, non-active arm. Mirror activity was low, ranging from 2 – 3.5% of MVC across the three conditions. Although these data are not definitive, the mirror activity in experiment two was quantifiably low and therefore we suggest that the ipsilateral brain activation observed in the primary experiment is unlikely to be driving motoneuron activation of the non-active arm.

An additional consideration is the involvement of muscle fatigue in the higher force conditions. Previous literature has demonstrated that in the presence of muscular fatigue, cortical excitability (Aboodarda et al., 2016), functional connectivity (Jiang et al., 2012) and EMG amplitude (Enoka and Duchateau, 2008) increase, whereas intracortical inhibition via TMS (Maruyama et al., 2006; Takahashi et al., 2009) decreases in the iM1. The increase in the ‘on-

task' functional connectivity observed in the iM1 with greater handgrip force, paired with the small decrease in force output across time for the 75% MVC condition in experiment two may be an indication that the higher force contractions resulted in some level of muscular fatigue. However, in experiment two the EMG data did not change over time, within a condition, suggesting muscle fatigue did not alter muscle activation. Investigating the contribution of fatigue within a similar paradigm remains an empirical question for future work.

We show that when healthy humans perform high-force handgrip contractions, ipsilateral and contralateral sensorimotor areas activate, coupled with an increase in the functional connectivity within the sensorimotor network. Further, the iM1 activation and network strength scales with grip force in a manner different from the cM1. In the primary experiment there was a notable lack of scaling within the cM1 with parametric increases in handgrip force. It is plausible that in the specific handgrip task, the cM1 contribution was near maximized with the 25% MVC condition, and greater force output within the 50% and 75% conditions was a result of iM1 or other cortical centres contributing to the increased force output. The increase in 'on-task' functional connectivity across the sensorimotor network including cM1 and iM1 (figure 3.5) may lend support for this notion, as an increase in handgrip force resulted in greater synchronicity between sensorimotor areas in each hemisphere, which may have aided in force output. It should be noted however that this is a hypothesis that these data are unable to directly address and warrants further investigation.

3.6 Limitations and future directions

Future research may benefit from utilizing MR spectroscopy, electro-encephalography, or magnetoencephalography to gain better insight into the functional premise of ipsilateral brain activity and functional connectivity during unimanual motor behaviour. We recorded contraction force and EMG during the movements in a second experiment with a different cohort of participants outside of the MRI. This allowed us to gain insight into the potential involvement of mirror activity in the non-active arm. Although, an extension of this work requires the careful examination of EMG muscle activity and the force profile of the homologous muscles within the active and non-active limbs for both right and left-handed contractions during MRI scans. Additionally, experiment one included two left-handed participants, and there are reports that activation (Begliomini et al., 2008; Grabowska et al., 2012) and connectivity (Pool et al., 2014, 2015) differ between individuals of different hand dominance. To address this potential

confound, we reanalyzed these data with the two left-handed participants removed and observed the same effects in each hemisphere, and therefore opted to include the two left-handed participants in the analyses. Future research should consider investigating differences between left and right-handed participants with a similar paradigm. Finally, the lack of cM1 scaling with parametric increases in handgrip force was an unexpected finding that warrants replication in future studies.

3.7 Conclusions

This study examined the effects of parametrically increasing handgrip force on brain activation and functional connectivity within the sensorimotor network. While an increase in ipsilateral sensorimotor activation and excitability have been observed in previous literature (Hortobágyi et al., 2003; Kobayashi et al., 2003; Zijdewind et al., 2006; Sun et al., 2007; Perez and Cohen, 2008; Hendy et al., 2017), our data suggest that the cM1 signal change, although greater in magnitude (i.e. % signal change), does not appear to scale with handgrip force to the same extent as the iM1. We present evidence that high-force handgrip contractions result in an observable increase in iM1 BOLD signal change that scales with handgrip force. Further, our novel analyses examining the on-task functional connectivity of the sensorimotor network during unimanual handgrip contractions suggest that the sensorimotor network strength as a whole, and within iM1 and cM1 ROI, correlates with relative handgrip force.

Thesis Transition – Study Two

Study two builds off the work from study one, by applying the same experimental paradigm to 11 stroke survivors with hemiparesis. Study one demonstrated that ipsilateral cortical activity and functional connectivity are increased with higher force unimanual handgrip contractions. These data provided a sound basis for which CE may be governed based on the cross-activation hypothesis. A remaining research question was how varying submaximal unimanual handgrip contractions with the less-affected limb in stroke survivors would impact ipsilesional brain activity. Given that the interhemispheric competition model proposes that contralesional brain activity inhibits brain activity in the ipsilesional hemisphere, it remained to be determined if the increases in cortical activation observed in study one would be present in stroke survivors.

Chapter 4

Study Two - Ipsilesional motor cortex activation with high-force unimanual handgrip contractions of the less-affected limb in participants with stroke

4.0 Introduction

Ischemic stroke, characterized by neuronal cell death due to cerebrovascular disruptions (Yu et al., 2016), is the second leading cause of death globally and one of the leading causes of severe disability (Katan and Luft, 2018). Approximately 15 million people experience stroke annually, of which five million suffer from permanent disability (Mittmann et al., 2012). When the occluded vessel serves motor-relevant cortical or subcortical areas, sensorimotor impairments often manifest. Most often the impairment presents as an asymmetry in motor output that is lateralized to the limb(s) contralateral to the cerebral hemisphere that suffered the lesion. Identifying rehabilitation methods that have the potential to improve motor functional outcomes is of clear importance and a persistent research question.

In neurologically intact humans, voluntary movements are governed typically by contralateral hemispheric control (Borowsky et al., 2002; Cincotta and Ziemann, 2008), which is thought to have an active inhibitory effect on the ipsilateral hemisphere through transcallosal projections intended to suppress unwanted movements (interhemispheric inhibition [IHI]; (Hübers et al., 2008; Sehm et al., 2016)). However, in stroke survivors this transcallosal inhibitory control is altered and the contralesional hemisphere (opposite side to the lesion) exhibits an increased level of activation during movements with the affected limb. There is debate regarding brain stimulation and motor training paradigms that modulate either ipsilesional (same side as the lesion) or contralesional hemispheric brain activity (Buetefisch, 2015). The *interhemispheric competition model* (Kinsbourne, 1974; Murase et al., 2004; Bütefisch et al., 2008; Grefkes et al., 2008; Nowak et al., 2009) suggests bi-directional changes to IHI post-stroke, whereby the ipsilesional hemisphere exhibits a reduced capacity to inhibit brain activity in the non-affected contralesional hemisphere. In contrast, when the contralesional hemisphere is active it appears to have an increased capacity to inhibit the damaged hemisphere (Kinsbourne, 1974; Murase et al., 2004; Bütefisch et al., 2008; Grefkes et al., 2008; Nowak et al., 2009). Due to the changes in inhibitory control, this model suggests contralesional brain activity is maladaptive and ‘competes’ with the ipsilesional hemisphere for cortical control over movement.

Therefore, the interhemispheric competition model suggests that contralesional brain activity reduces the opportunity for ipsilesional neuroplasticity that would promote contralateral motor control as is seen in neurologically intact individuals. Based on this model, conventional treatments for improving motor impairment focus on finding ways to inhibit contralesional and promote ipsilesional brain activity that would then subserve preferential neuroplasticity and recovery of the ipsilesional hemisphere. However, the premise based on the interhemispheric competition model that all therapies should focus on inhibiting contralesional brain activity is somewhat controversial, as good functional motor recovery can occur in individuals that display sustained contralesional brain activation during affected limb movements (see review by (Dodd et al., 2017)).

There is evidence supporting the implementation of contralesional, less affected limb approaches in chronic stroke survivors to improve motor recovery (Dragert and Zehr, 2013; Urbin et al., 2015; Sun et al., 2018; Dehno et al., 2021). These studies are based on the concept of cross-education - a neuromuscular phenomenon referring to the increased motor output (i.e., force generation or skill-based movements) of the opposite, untrained limb following a period of unilateral motor training (Manca et al., 2021). The first empirical evidence of cross-education dates back to a case study in 1894 (Scripture et al., 1894), yet the neural mechanisms driving the effect are not completely understood. The implementation of cross-education into the rehabilitation process for promoting recovery of the more-affected limb after stroke paradoxically conflicts with the interhemispheric competition model. Given that voluntary exercise with the less-affected limb promotes brain activity in the contralesional hemisphere, it presumably would inhibit ipsilesional brain plasticity. Yet, the exemplar studies cited above suggest motor recovery is enhanced.

A leading theory describing the neural underpinnings of cross-education is the cross-activation hypothesis (Lee and Carroll, 2007; Ruddy and Carson, 2013). The cross-activation hypothesis suggests unilateral voluntary movements facilitate bilateral brain activation, which results in motor training-related neuroplasticity in both hemispheres (Lee et al., 2010; Ruddy and Carson, 2013; Manca et al., 2018). In neurologically intact humans, previous research has observed ipsilateral brain activation with high-force (Study 1, experiment 1), fatiguing (Benwell et al., 2006; Jiang et al., 2012), or complex (Verstynen et al., 2005) unilateral voluntary movements. Further, greater cross-education is known to occur when the exercise is performed at

high intensities (Urbin et al., 2015), high velocities (Farthing and Chilibeck, 2003), and when eccentric muscle actions are incorporated into the unilateral training regimen (Farthing and Chilibeck, 2003; Manca et al., 2017). Therefore, if unilateral motor tasks with the less-affected limb result in ipsilesional brain activation in individuals with stroke, targeted exercise with the less-affected limb provides a potential avenue for enhancing neuroplasticity subserving motor recovery, while shedding light on the efficacy of cross-education as an adjunct therapy for individuals with stroke (i.e., in addition to constraint-induced-movement-therapy).

4.1 Objectives

The objective of this study was to determine if high-force unilateral handgrip contractions performed with the less-affected limb in stroke survivors result in increased cortical activation and functional connectivity between the primary motor cortex (M1) and the supplementary motor area (SMA) within the ipsilesional hemisphere. Further, utilizing participants from study one experiment one, a secondary purpose is to determine if unilateral handgrip contractions performed with the less-affected limb result in differences in ipsilateral/ipsilesional brain activation and functional connectivity between participants with and without a history of stroke.

4.2 Hypotheses

The primary hypotheses are that high-force unilateral handgrip contractions will result in greater brain activation and functional connectivity within the ipsilesional hemisphere in both groups.

4.3 Methods

4.3.1 Ethical approval

This study conforms to the standards set out by the Declaration of Helsinki and was approved by the University of Saskatchewan Biomedical Research Ethics Board (Ethics # Bio 16-157).

4.3.2 Participants

Using a partial eta squared (η_p^2) effect size of $\eta_p^2 = 0.439$ based on a main effect of condition for the modulation of ipsilateral M1 (iM1) cortical activation between 25%, 50%, and 75% maximum voluntary contraction (MVC) from the first experiment in study one, it was determined that an estimated total sample size of eight was needed (G*Power 3.1.9.2; $1-\beta = 0.95$, $\alpha = 0.05$). Eleven stroke survivors volunteered to participate in a larger intervention (clinicaltrials.gov: NCT02948725), of which their baseline data was used in the present study

(Data are presented as mean ± standard deviation; 3 female, 8 male, 10 right-handed, 1 left-handed, age: 60 ± 11 yrs, height: 173.3 ± 6.4 cm, mass: 81.7 ± 18.3 kg). Additionally, 13 neurologically intact controls (11 right-handed, 2 left-handed, age: 28 ± 6 yrs, height: 170.9 ± 9.8 cm, mass: 75.1 ± 16.7 kg) from the first experiment in study one were used to conduct between group comparisons.

Participants with stroke were eligible to participate if they met the following inclusion criteria: 18 years or older, within 18 months of stroke recovery, medically stable, and have moderate to severe upper limb hemiparesis as diagnosed using the Chedoke McMaster Stroke Assessment. Participants with stroke were excluded if they had significant cognitive impairment or aphasia affecting understanding as assessed by a clinician, severe upper limb spasticity preventing any movement of the proximal arm and shoulder, had a diagnosis of hemorrhagic or bilateral stroke, had a history of other severe upper limb musculoskeletal injury or other neurological diseases, had intracranial metal clips or cardiac pacemaker, or anything that would preclude an MRI, and finally if participants had any condition that would preclude the participant's ability to attend follow-up visits in the opinion of the investigator.

Written informed consent was obtained from all participants prior to participating. This study conforms to the standards set out by the Declaration of Helsinki and was approved by the University of Saskatchewan Biomedical Research Ethics Board (Ethics # Bio 16-157).

4.3.3 Functional assessment

The participants with stroke completed the Fugl Meyer upper limb assessment (Sullivan et al., 2011) and the Chedoke-McMaster impairment inventory: stage of recovery for arm and hand (Gowland et al., 1993) to determine motor function in the more-affected limb. Additionally, the Waterloo handedness questionnaire was used to acquire an estimate of their hand dominance pre-stroke (Bryden, 1977).

4.3.4 Experimental outline

Participants attended one fMRI session where they completed three experimental conditions in a randomized order, each condition involved repeated submaximal unimanual isometric handgrip contractions at either 25%, 50%, or 75% of MVC with their less-affected hand (stroke participants) or their right hand for the neurologically intact control participants. The session began with a structural MRI brain scan, followed by three MVCs with the less-affected limb

(stroke) or the right hand of the control participants. One minute of rest was given between each MVC attempt to avoid fatigue. The MVCs were used to set the target force for each of the three submaximal conditions. Next, participants performed the three experimental conditions in a randomized order with a functional brain scan occurring during the performance of each condition.

4.3.5 Behavioural motor task

Participants performed 5 sets × 5 repetitions of grip contractions at each prescribed submaximal force during separate scanning runs with an MRI-compatible hand clench dynamometer (Biopac Systems Inc. Aero Camino Goleta, CA). In a block design, task blocks composed of 1650 ms (i.e., corresponding to the repetition time [TR] of the T2* fMRI scan) contractions alternating with 1650 ms of rest (16500 ms total task block), separated by rest blocks of complete rest (16500 ms total rest block). During scans the participants wore MRI compatible goggles and viewed a projection of a computer screen running a custom-built LabView (version 8.6) interface. Participants saw clear target lines and go/no-go flashing lights and were cued when to contract or relax. The LabView interface was triggered by the MRI to ensure the task was synchronized with each TR. Target lines were presented relative to the individual's peak MVC and force feedback was presented as a vertical force bar that was responsive to each participant's grip contraction (i.e., harder contraction resulted in the bar rising vertically). Two virtual 'lights' were present on the motor task interface to cue participants. A light turned green to instruct the participant to contract to the target line and turned black to indicate when to stop contracting. A second light remained black during task blocks and turned red during rest blocks to indicate a sustained rest. The red light switched to black moments before the next task block as an indicator that the next task series of contractions was about to begin. During each contraction force condition, participants were instructed to relax their non-active more-affected arm and hand to prevent mirror activity. Using this same experimental paradigm in the second experiment of study one, the muscle activity of non-active arm was not significantly active compared to baseline noise in an electromyography control experiment with young neurologically intact adults.

4.3.5.1 Handgrip motor task processing

The handgrip force data was processed in Matlab using custom scripts (The Mathworks Inc, 2018). Prior to analysis, data were processed with a fourth-order 100 Hz low pass Butterworth filter and full-wave rectified. Next the onset and offset of each contraction was determined and the mean of each contraction was calculated. Data were normalized to the mean Kg-force of the highest MVC and expressed as a percentage of MVC. The mean of each task block (five contractions) was calculated and used in subsequent analyses.

4.3.6 fMRI Parameters

All scans were done in a Siemens 3T MAGNETOM Skyra MRI scanner (Siemens Healthcare, Erlangen, Germany). At the start of the session the whole-brain anatomical scan was acquired using a high-resolution magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence consisting of 192 T1-weighted echo-planar imaging slices (1 mm slice thickness with no gap), with an in-plane resolution of 1 × 1 mm (field of view = 256 × 256; TR = 1900 ms; echo time [TE] = 2.08 ms). For each of the experimental conditions T2*-weighted single-shot gradient-echo planar imaging scans were acquired using an interleaved ascending sequence, consisting of 105 volumes (TR = 1650 ms; TE = 30 ms) of 25 axial slices of 4-mm thickness (1-mm gap) with an in-plane resolution of 2.7 mm × 2.7 mm (field of view = 250) using a flip angle of 90°. The top 2 coil sets (16 channels) of a 20-channel Siemens head-coil (Siemens Healthcare) were used. Scans consisted of a 10-volume alternating block design beginning with five volumes for stabilization (task, rest; 105 volumes total).

4.3.6.1 fMRI pre-processing

Functional MRI data processing was carried out using FMRI Expert Analysis Tool (FEAT) Version 6.00, as part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Boundary based registration was used to register the functional image to the high-resolution T1-weighted structural image. Registration of the functional images to the T1-weighted structural image was carried out using FLIRT: (Jenkinson and Smith, 2001; Jenkinson et al., 2002), and the registration to the standard space images was carried out using FMRIB’s Nonlinear Image Registration Tool (FNIRT; (Andersson et al., 2007a, 2007b)).

The following pre-processing was applied: motion correction using Motion Correction FMRIB's Linear Image Registration Tool (MCFLIRT; (Jenkinson et al., 2002)); non-brain removal using Brain Extraction Tool (BET; (Smith, 2002)); spatial smoothing using a Gaussian kernel of FWHM 6mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor (Pruim et al., 2015).

Next, Independent Component Analysis Automatic Removal of Motion Artifacts (ICA-AROMA) was used to identify and remove motion-related noise from the functional data (Pruim et al., 2015). Following the ICA-AROMA data clean up, data were high pass temporal filtered with a 0.01 Hz cut off frequency. Time-series statistical analyses were carried out using FMRIB's Improved *Linear Model* (FILM) with local autocorrelation correction (Woolrich et al., 2001). Z (Gaussianised T/F) statistic images were constructed non-parametrically using Gaussian Random Field theory-based maximum height thresholding with a corrected significance threshold of $p = 0.05$ (Worsley, 2001).

4.3.6.1.1 On-task functional connectivity

To assess the on-task functional connectivity during the three different conditions, the 50 volumes corresponding with the task blocks were extracted and merged across time. Rest volumes were removed to avoid the potential impact that rest-related activity may have on the functional connectivity analysis (Steel et al., 2016; Cole et al., 2018) and the same pre-processing steps previously indicated were used on the 50 volume on-task scans. Following data processing, the mean timeseries of the ipsilesional (stroke)/ipsilateral (controls) and contralesional (stroke)/contralateral (controls) M1 and SMA (iM1, cM1, iSMA, cSMA respectively) were extracted from native-space using reverse transformed region of interest (ROI) masks from the Brainnetome atlas (left M1: A4ul_l; right M1: A4ul_r; left SMA: A6m_l; right SMA: A6m_r) in MNI standard space (Fan et al., 2016). Correlation analyses were then carried out using custom Matlab scripts (The Mathworks Inc, 2018). First a correlation matrix was calculated that represented the edge strength between each network node. Next, a Fishers r-to-z transformation was carried out on the Pearson's *r* values. The Z-scores for the edge strengths were then used for analyses.

4.3.7 Statistical analyses

Analyses were carried out in R (R Core Team, 2019), using linear mixed effects (LME) analyses, with participants treated as random effects to account for repeated measures. The following R packages were used; lmerTest package (Kuznetsova et al., 2017), tidystats (Sleegers, 2020), ggplot2 (Wickham, 2016).

4.3.7.1 Handgrip force

To assess the relative handgrip force between groups and conditions, fixed effects of group (stroke, healthy), condition (25%, 50%, 75% MVC), and block (5 blocks of contractions during each condition), in addition to interactions of group × condition, condition × block, group × block, and a group × condition × block were included in the model.

4.3.7.2 Brain activation

To assess the brain activation in each hemisphere during the three different conditions for each group, two separate LME analyses were carried out for each hemisphere for the percent signal change of the M1 and SMA, with fixed effects of group, condition, and a group × condition interaction.

4.3.7.3 On-task functional connectivity

To assess the functional connectivity from the 50-volume 'on-task' data separate analyses were carried out to assess interhemispheric homologous functional connectivity of M1's bilaterally (cM1-iM1) and SMA's bilaterally (cSMA-iSMA). Further, intrahemispheric connectivity was assessed between the M1 and SMA (M1-SMA) within each hemisphere, respectively. For each of these dependent variables, LME analyses were carried out, with fixed effects of group, condition, and a group × condition interaction included in each model.

4.4 Results

Table 4.1 Injury-specific characteristics and functional scores for participants with stroke

Participant*	Days Since Stroke	Lesion volume mm^3	Lesioned-Hemisphere	Waterloo Handedness	FM Score	Chedoke-McMaster	
						Hand	Arm
1	56	67635	Right	-20	10	1	2
2	79	223988	Right	19	12	1	1
3	161	64132	Left	19	55	6	5
4	328	99	Right	20	44	4	4
5	97	1628	Left	20	32	5	1
6	103	1302	Right	3	54	5	5
7	211	3165	Left	20	16	2	2
8	228	17309	Right	20	36	3	3
9	51	Not visible	Right	20	26	3	2
10	170	2206	Left	20	14	2	2
11	139	250316	Left	13	10	2	1
Mean:	148	63178	N/A	14.0	28.1	3.1	2.5
SD:	84	95406	N/A	12.4	17.3	1.7	1.5

* Participant numbers do not reflect study assigned participant identification

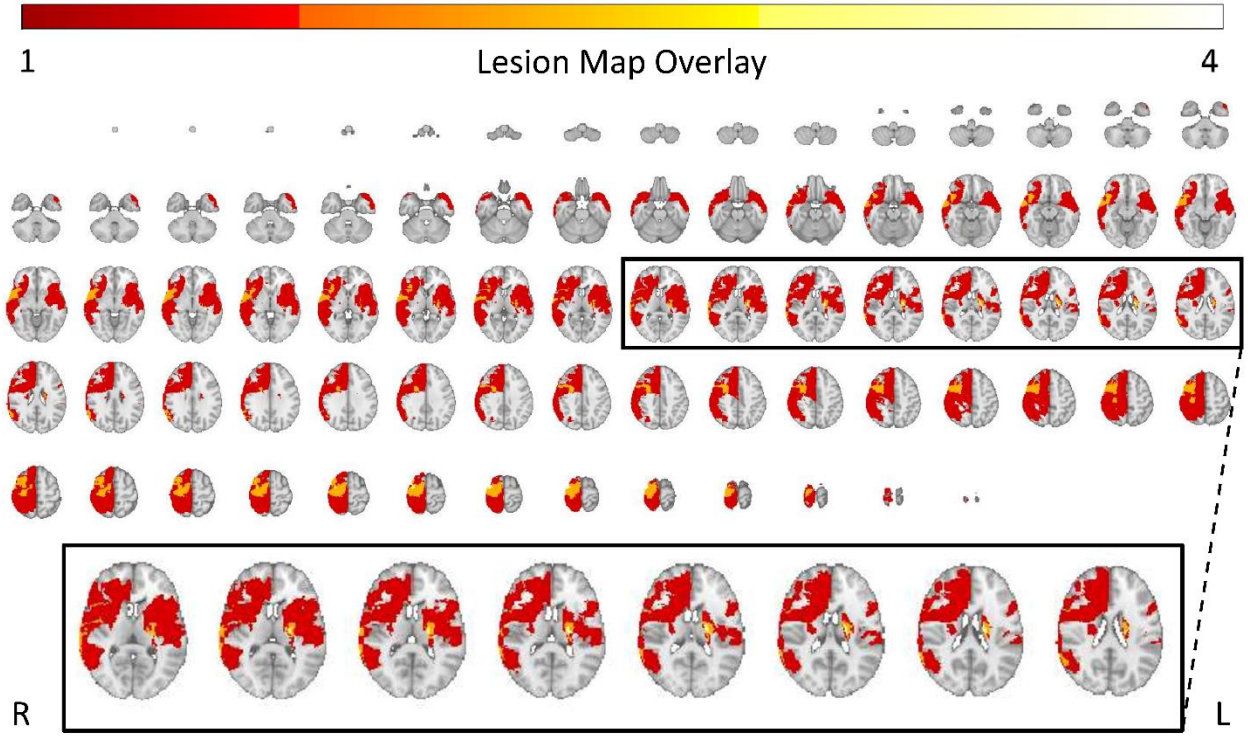

Figure 4.1 Stroke lesion map overlay. Image is in radiological view (left hemisphere displayed on the right; right hemisphere displayed on the left). Lesion masks are in MNI152 2mm standard space. Colour bar represents the number of participants that share a lesion location.

4.4.1 Motion and data removal

Data from three participants with stroke during the 75% MVC condition was deemed unusable due to high levels of motion artifact that was task correlated (table 2). An additional scan in the

25% condition was missing due to a technical error during data collection. Furthermore, of the usable scans, several data points for each ROI were not included in analyses due to lesion intrusion within the ROI masks in stroke survivors. This resulted in a total data loss of three ROIs for the iM1 (two left, one right), and two ROIs for the iSMA (two right). After data removal for the 25% MVC condition, a total of seven ROIs for the iM1, 10 for the unaffected M1, eight ROIs for the affected SMA, and 10 for the unaffected SMA were deemed usable. For the 50% MVC condition, a total of eight ROIs for the affected M1, 11 for the unaffected M1, nine for the affected SMA and 11 for the unaffected SMA were included in analyses. Finally, for the 75% MVC condition there were five usable ROIs for the affected M1, eight ROIs for the unaffected M1, six ROIs for the affected SMA, and nine ROIs for the unaffected SMA.

Table 4.2 Motion metrics and data removal

		25% MVC		**50% MVC**		**75% MVC**	
		Motion (mm)		**Motion (mm)**		**Motion (mm)**	
Participant**	**Group**	**Relative**	**Absolute**	**Relative**	**Absolute**	**Relative**	**Absolute**
1	Stroke	0.11	0.19	0.14	0.25	0.13	0.30
2	Stroke	0.16	0.15	0.16	0.14	0.21	0.44
3	Stroke	No Scan	No Scan	0.52	0.18	8.95*	1.34*
4	Stroke	0.08	0.12	0.10	0.45	0.13	0.29
5	Stroke	0.09	0.23	0.26	1.01	0.46*	1.12*
6	Stroke	0.05	0.08	0.11	0.23	0.29	0.64
7	Stroke	0.13	0.14	0.15	0.16	0.18	0.56
8	Stroke	0.19	0.35	0.19	0.27	0.20	0.22
9	Stroke	0.17	0.25	0.15	0.26	0.78*	2.11*
10	Stroke	0.16	0.13	0.15	0.17	0.19	0.24
11	Stroke	0.20	0.30	0.23	0.31	0.27	0.46
12	Control	0.05	0.08	0.07	0.22	0.10	0.35
13	Control	0.06	0.12	0.08	0.25	0.13	0.58
14	Control	0.10	0.28	0.11	0.36	0.10	0.25
15	Control	0.11	0.27	0.05	0.10	0.06	0.07
16	Control	0.03	0.06	0.04	0.13	0.06	0.20
17	Control	0.07	0.11	0.06	0.10	0.07	0.13
18	Control	0.06	0.13	0.11	0.31	0.11	0.22
19	Control	0.10	0.26	0.12	0.21	0.19	0.56
20	Control	0.05	0.11	0.06	0.27	0.11	0.29
21	Control	0.04	0.13	0.06	0.17	0.17	0.59
22	Control	0.04	0.11	0.06	0.21	0.15	0.73
23	Control	0.04	0.06	0.07	0.15	0.15	0.36
24	Control	0.04	0.07	0.05	0.09	0.06	0.17

* Scan removed due to high motion or task correlated motion

** Participant numbers do not reflect study assigned participant identification

Table 4.3 Group and condition means and standard deviations for the dependent variables of interest

	Stroke			Healthy		
Variables of interest	**25% MVC**	**50% MVC**	**75% MVC**	**25% MVC**	**50% MVC**	**75% MVC**
Force (% MVC)	25.3 ± 6.3	50.3 ± 3.7	71.9 ± 6.1	26.8 ± 1.7	50.3 ± 2.6	72.4 ± 4.8
iM1 (% Δ)	0.07 ± 0.21	0.18 ± 0.22	0.26 ± 0.23	0.09 ± 0.25	0.22 ± 0.2	0.48 ± 0.28
cM1 (% Δ)	0.52 ± 0.36	0.64 ± 0.53	0.77 ± 0.47	0.69 ± 0.36	0.8 ± 0.24	0.89 ± 0.24
iSMA (% Δ)	0.35 ± 0.41	0.34 ± 0.38	0.41 ± 0.3	0.59 ± 0.29	0.69 ± 0.31	0.77 ± 0.28
cSMA (% Δ)	0.43 ± 0.42	0.48 ± 0.41	0.55 ± 0.36	0.64 ± 0.31	0.71 ± 0.24	0.77 ± 0.26
cM1-iM1 (Z-Score)	0.79 ± 0.51	0.85 ± 0.77	0.81 ± 0.46	0.74 ± 0.5	1 ± 0.27	1.11 ± 0.32
cSMA-iSMA (Z-Score)	1.8 ± 0.51	1.64 ± 0.69	1.54 ± 0.56	1.8 ± 0.42	2.05 ± 0.29	1.9 ± 0.44
iM1-iSMA (Z-Score)	1.12 ± 0.63	1.16 ± 0.77	0.93 ± 0.32	0.78 ± 0.51	1.2 ± 0.24	1.35 ± 0.23
cM1-cSMA (Z-Score)	1.49 ± 0.62	1.33 ± 0.65	1.38 ± 0.39	1.51 ± 0.43	1.69 ± 0.35	1.49 ± 0.49

4.4.2 Handgrip contractions

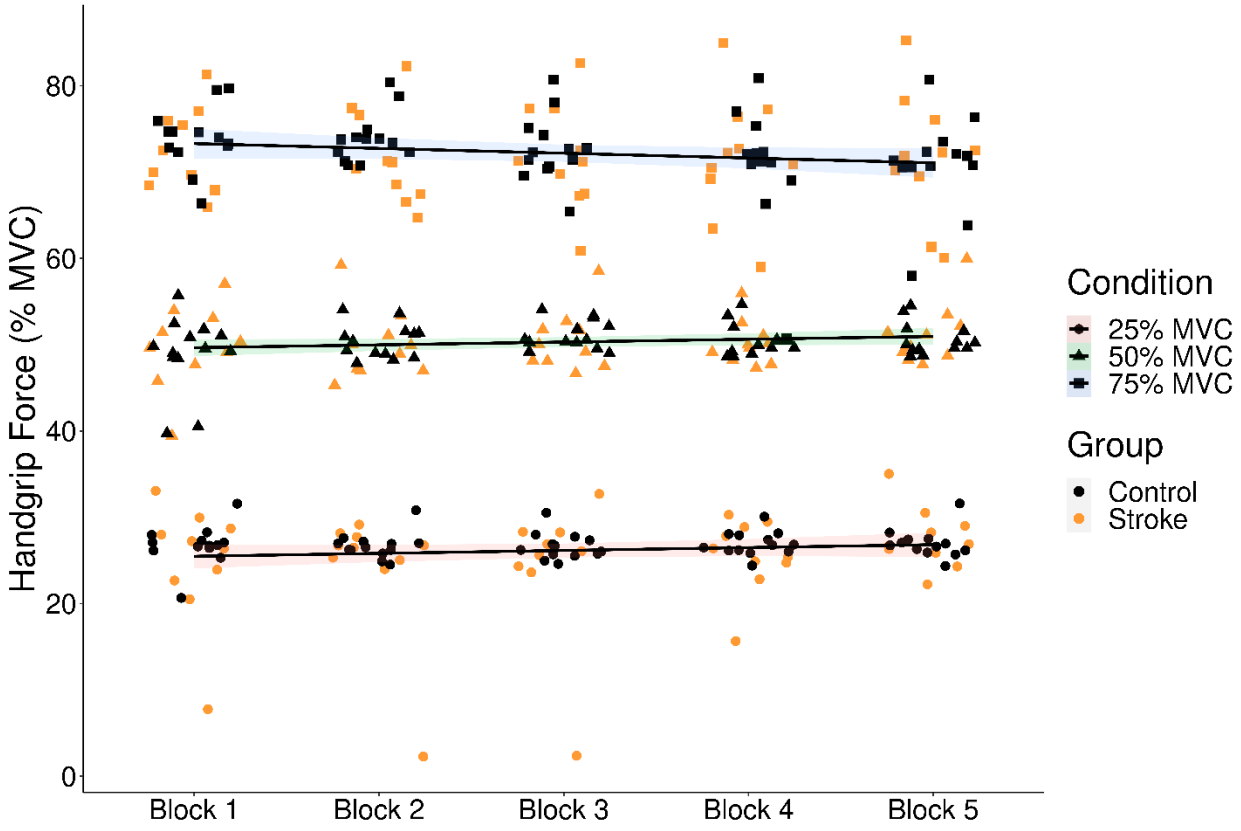

Figure 4.2 Mean relative handgrip force (y-axis: Percent MVC) for each task block (x-axis) for 25% (circles), 50% (triangles), and 75% (squares) MVC for control (black) and stroke (orange) participants. Each regression line represents each condition across blocks. Shaded area is the 95% confidence interval around the regression line.

For the relative handgrip force, a significant main effect of condition was found ($F(2, 322) = 4012.53$, $p < 0.001$). However, the main effects of group ($F(1, 23) = 0.65$, $p = 0.43$), and block

($F(4, 322) = 0.18$, $p = 0.95$) were not significant, indicating that relative handgrip force differed between conditions but not between groups or across the five blocks of contractions. Additionally, the group × condition ($F(2, 322) = 1.01$, $p = 0.36$), condition × block ($F(8, 322) = 1.13$, $p = 0.34$), group × block ($F(4, 322) = 0.95$, $p = 0.43$), and the group × condition × block ($F(8, 322) = 0.23$, $p = 0.99$) interactions all failed to reach significance. The lack of interactions indicates that motor performance within a given condition was stable and did not change as a function of time (across the five blocks) or group (figure 4.2).

4.4.3 Brain activation

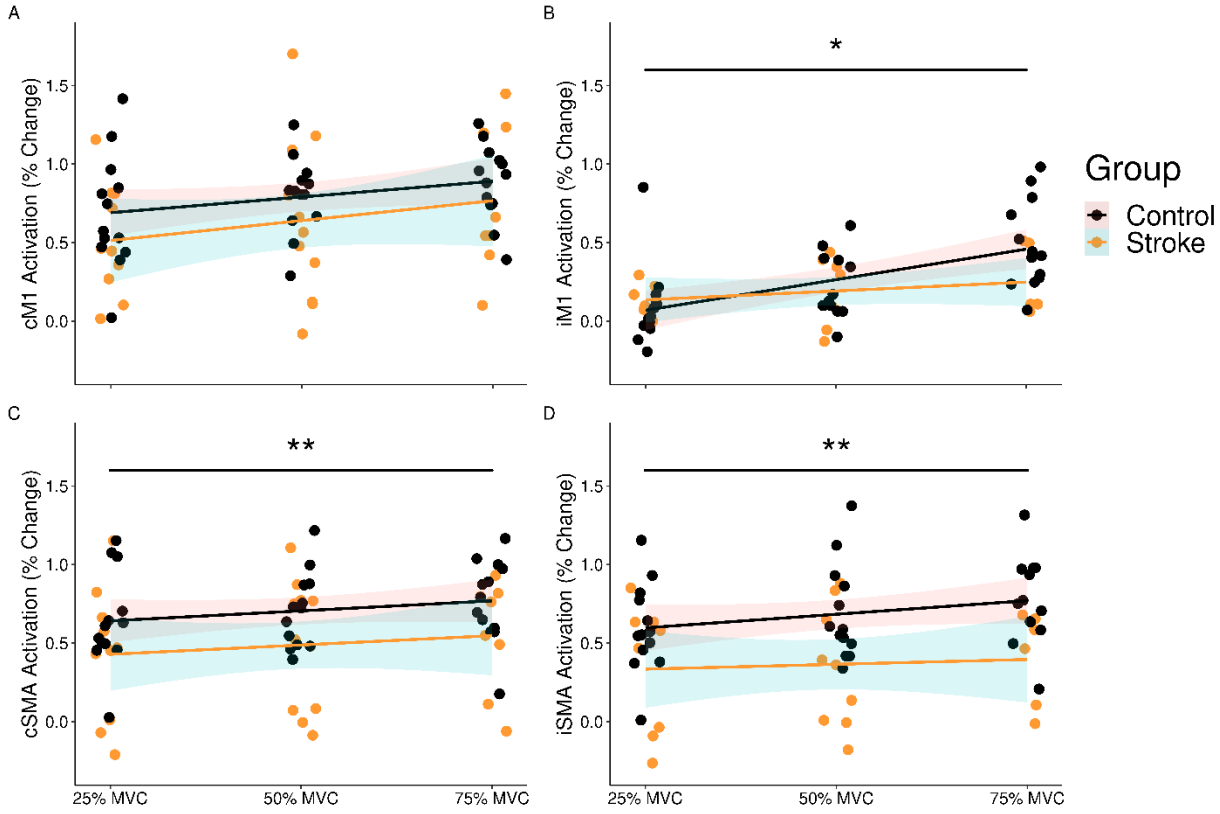

Figure 4.3 Percent change in BOLD signal for the contralateral/contralesional (c; left column), and ipsilateral/ipsilesional hemispheres (i; right column) for A) cM1, B) iM1, C) cSMA, D) iSMA for control (black) and stroke (orange) participants. * = significant main effect of condition, ($p = 0.002$). ** = significant main effect of group ($p \leq 0.05$). Scatter plots display 95% confidence intervals around the regression line.

4.4.3.1 Contralateral/Contralesional primary motor cortex (cM1)

For the cM1 brain activation, the main effects of condition ($F(2, 44.69) = 2.63$, $p = 0.083$), group ($F(1, 23.51) = 2.28$, $p = 0.14$) and the group × condition interaction ($F(2, 44.69) = 0.01$, $p =$

0.99) all failed to reach significance, indicating that there were no differences in cM1 brain activation between groups or conditions (figure 4.3A; table 4.3).

5.4.3.2 Ipsilateral/Ipsilesional primary motor cortex (iM1)

A significant main effect of condition was observed in the iM1 ($F(2, 41.56) = 7.24$, $p = 0.002$), where the brain activation in iM1 increased parametrically with higher force handgrip contractions. The main effect of group ($F(1, 22.82) = 1.82$, $p = 0.19$) and the group × condition interaction ($F(2, 41.56) = 1.14$, $p = 0.33$) were both non-significant, indicating that there were no differences between groups for any of the three conditions (figure 4.3B; table 4.3).

4.4.3.3 Contralateral/Contralesional supplementary motor area (cSMA)

For the cSMA brain activation, there was a significant main effect of group ($F(1, 23.94) = 4.28$, $p = .05$), but the main effect of condition ($F(2, 44.87) = 1.02$, $p = 0.37$), and the group × condition interaction ($F(2, 44.87) = 0.09$, $p = 0.92$) failed to reach significance. These results indicate that control participants had greater cSMA brain activation compared to participants with stroke, but there were no differences between conditions for either group (figure 4.3C; table 4.3).

4.4.3.4 Ipsilateral/Ipsilesional supplementary motor area (iSMA)

A significant main effect of group was observed in the iSMA ($F(1, 22.61) = 7.97$, $p = 0.01$), indicating that control participants had greater SMA brain activation in the ipsilateral hemisphere compared to the ipsilesional hemisphere in participants with stroke. However, the main effect of condition ($F(2, 41.41) = 0.99$, $p = 0.38$) and the group × condition interaction ($F(2, 41.41) = 0.59$, $p = 0.56$) both failed to reach significance suggesting that there were no differences between conditions for either group (figure 4.3D; table 4.3).

4.4.4 Functional connectivity

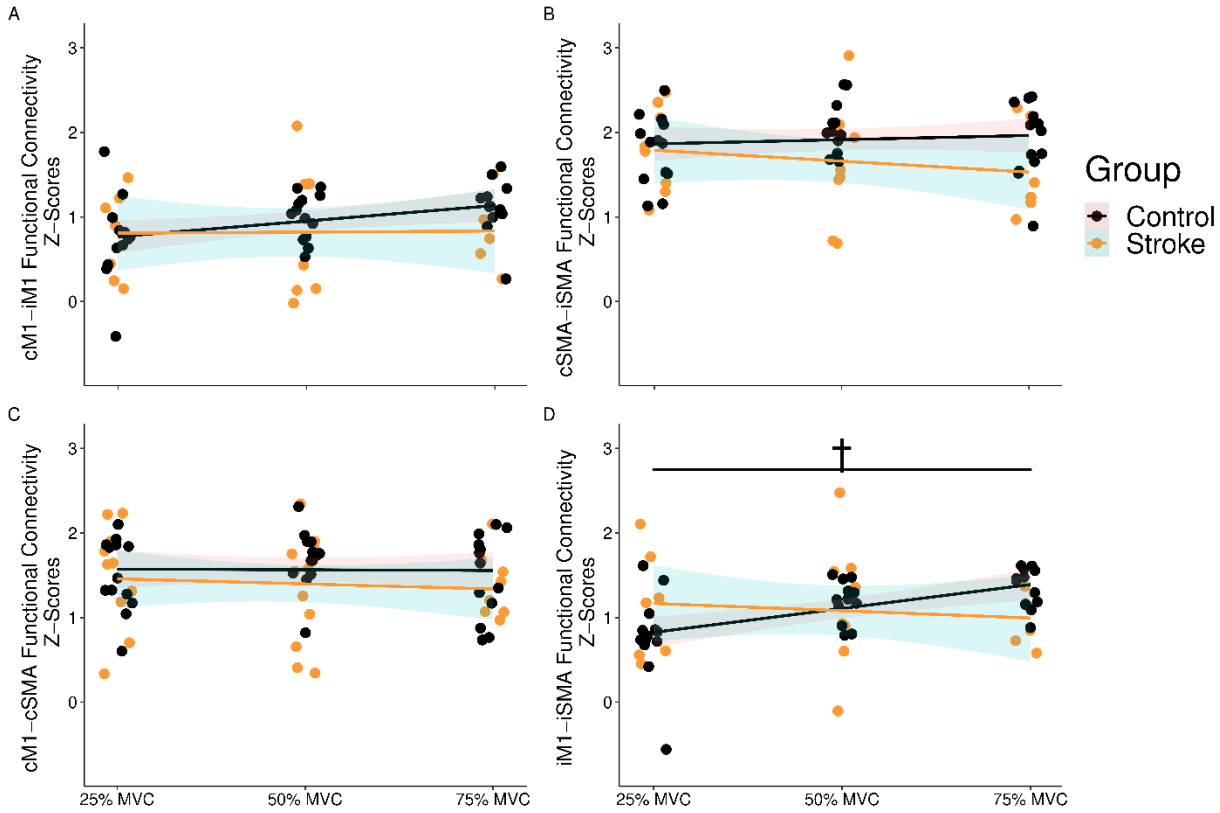

Figure 4.4 On-task functional connectivity for interhemispheric A) cM1-iM1, B) cSMA-iSMA, and intrahemispheric C) cM1-cSMA, and D) iM1-iSMA correlations. Data points are z-scores for each participant in the control (black) and stroke (orange) groups. † = significant group × condition interaction, (p =0.027). Scatter plots display 95% confidence intervals around the regression line.

4.4.4.1 cM1-iM1 Connectivity

For cM1-iM1 functional connectivity the main effects of condition (F(2, 37.97) = 1.06, p = 0.36), group (F(1, 19.51) = 1.36, p = 0.26) and the group × condition interaction (F(2, 37.97) = 1.11, p = 0.34) all failed to reach significance, indicating that there were no differences between groups or conditions for cM1-iM1 interhemispheric functional connectivity (figure 4.4A; table 4.3).

4.4.4.2 cSMA-iSMA Connectivity

For cSMA-iSMA functional connectivity the main effects of condition (F(2, 40.61) = 0.38, p = 0.69), group (F(1, 20.21) = 4.31, p = 0.051) and the group × condition interaction (F(2, 40.61) = 1.23, p = 0.30) all failed to reach significance, indicating that there were no differences in

cSMA-iSMA interhemispheric functional connectivity between groups or conditions (figure 4.4B; table 4.3).

4.4.4.3 Contralateral M1-SMA Connectivity

For the cM1-cSMA intrahemispheric functional connectivity, the main effects of condition ($F(2, 41.89) = 0.78$, $p = 0.46$), and group ($F(1, 20.73) = 1.95$, $p = 0.18$) in addition to the group × condition interaction ($F(2, 41.89) = 1.01$, $p = 0.37$) all failed to reach significance, indicating that this relationship did not differ regardless of group or condition (figure 4.4C; table 4.3).

4.4.4.4 iM1-iSMA connectivity

The analysis of the iM1-iSMA intrahemispheric functional connectivity failed to observe significant main effects of condition ($F(2, 37.88) = 1.58$, $p = 0.22$), and group ($F(1, 19.12) = 0.18$, $p = 0.68$). However, a significant group × condition interaction was observed ($F(2, 37.88) = 3.97$, $p = 0.027$), indicating that iM1-iSMA functional connectivity changed differently between conditions for each group. The control group experienced a small increase in functional connectivity with increased contraction force, whereas the opposite was observed for participants with stroke, whereby a small decrease in connectivity was observed with increasing handgrip force (figure 4.4D; table 4.3).

4.5 Discussion

This study investigated cortical brain activation and functional connectivity during unilateral handgrip contractions with 25%, 50%, and 75% MVC. In the first experiment of study one in this thesis robust parametric scaling was observed in the ipsilateral hemisphere with increases in handgrip force over two experimental sessions. The present study investigated this phenomenon in participants with stroke while performing the handgrip contractions with their less-affected hand and compared those findings to data from the first session of the neurologically intact control participants from the first experiment in study one. In participants with stroke, iM1 brain activation scaled with force similar to what was observed in the first experiment of study one. A notable difference between the control and participants with stroke was seen with the functional connectivity analyses. In the group of participants with stroke, inter- and intra-hemispheric functional connectivity did not scale with handgrip force as was previously observed (Study 1, experiment 1), and with the intrahemispheric iM1-iSMA functional connectivity analyses in this

study with neurologically intact control participants. This study presents novel findings that shed light on the impact of high-force unimanual contractions with the less-affected limb for promoting ipsilesional brain activity contrasting the underlying principle of the interhemispheric competition model.

There is evidence to suggest that the utilization of cross-education in stroke survivors can improve functional outcomes, whereby unilateral strength training of the less-affected limb aids in the motor recovery of the more-affected limb (Dragert and Zehr, 2013; Urbin et al., 2015; Sun et al., 2018; Dehno et al., 2021). Based on the cross-activation hypothesis for describing the neural mechanisms of cross-education, the phenomenon manifests through ipsilateral brain activation resulting in neuroplasticity that accounts for motor improvements in the contralateral limb (Lee et al., 2010; Ruddy and Carson, 2013). However, this form of motor training might be contraindicated given contralesional brain activation is thought to facilitate an inhibitory effect on the lesioned hemisphere (Kinsbourne, 1974; Murase et al., 2004; Bütefisch et al., 2008; Grefkes et al., 2008; Nowak et al., 2009). This study explicitly investigated this controversy by comparing brain activation and functional connectivity in the ipsilesional hemisphere during less-affected handgrip contractions in participants with stroke and compared those results to ipsilateral hemispheric brain activation and connectivity in neurologically intact control participants who completed right handgrip contractions.

4.5.1 High-force contractions modulate the motor cortex in the ipsilateral hemisphere

The main finding from this study is that an increase in iM1 brain activation was observed with increased handgrip force (figure 4.3B) in both groups. These data suggest that high-force contractions with the less-affected hand of stroke survivors did not inhibit iM1 brain activation. Previous literature supports this notion in that the BOLD signal reflect primarily excitatory rather than inhibitory neuronal activity (Logothetis et al., 2001; Sotero and Trujillo-Barreto, 2007; Logothetis, 2008). These findings contrast the assumptions of the interhemispheric competition model, where this increased contralesional brain activation driving the less-affected handgrip contractions should have an inhibitory effect on the lesioned hemisphere. Similar to previous observations, a lack of cM1 scaling with increased contraction force is not entirely surprising (Dettmers et al., 1996). Previous literature suggests that cM1 cortical activation scales for low force contractions, but the relationship is diminished once contraction force exceeds >10% MVC

(Dettmers et al., 1996). The loss of cM1 scaling with higher force contractions suggests that contraction force variance cannot be entirely accounted for with cM1 activation and corticospinal tract volleys (Cheney and Fetz, 1980; Lemon et al., 1986; Maier et al., 1993; Dettmers et al., 1996).

4.5.2 Lower SMA cortical activation in stroke survivors

The SMA is considered an important cortical region for upper limb movement planning and execution (Goldberg, 1985; Forstmann et al., 2008), and is known to have direct descending connections with upper limb alpha-motoneurons (Maier et al., 2002). The SMA is also an important region in the context of stroke due to previous observations that the non-decussated reticulospinal tract serves as a compensatory pathway for motor recovery (Baker, 2011), which has cortical origins within the SMA (Fisher et al., 2021). The SMA is an important region for cross-education given its dense interhemispheric white matter connections with its homolog in the opposite hemisphere (Ruddy et al., 2017a). Here we demonstrate that cortical activation in the cSMA and iSMA did not differ between conditions, suggesting this region does not modulate with force. However, a main effect of group for each region suggests that regardless of hemisphere or condition, SMA activation is lower in stroke survivors (figure 4.3C and D). The findings that the SMA does not modulate with force was somewhat surprising given that this region likely plays a substantial role in in stroke recovery. However, the lack of modulation does not exclude the SMA as an important cortical region for motor function. Rather, the SMA may have a more stable function in motor planning or movement generation that is not modulated by effort or force. Another possibility for the lower SMA activation and lack of modulation may be attributed to the differences in age between the stroke survivors and neurologically intact controls (stroke: mean 60 ± 11 yrs; control: 28 ± 6 yrs). Previous literature has demonstrated that with aging, atrophy occurs in the rostral segments of the corpus callosum (Hou and Pakkenberg, 2012). The SMA is located in the frontal lobe with some of the densest transcallosal fibres between homologs (Ruddy et al., 2017a). Therefore, it is possible that the lower SMA activity in the participants with stroke is related to a degradation of these regions as a result of age and not due to the stroke.

4.5.3 Functional connectivity is not modulated with force in stroke survivors

For the inter- and intrahemispheric functional connectivity analyses, the only significant observation was a group × condition interaction for the iM1-iSMA connectivity ($p = 0.027$). This interaction was influenced by an increase in connectivity strength with higher force contractions in the control participants only, whereas no change was observed in the stroke survivors (figure 4.4D). The only other finding that was nearly significant was the main effect of group for cSMA-iSMA functional connectivity ($p = 0.051$), whereby control participants displayed marginally higher levels of functional connectivity than stroke survivors across the three conditions (table 4.3). Overall, these data suggest that inter- and intrahemispheric functional connectivity are not significantly modulated with contraction force in participants with stroke. It remains to be determined if the lack of modulation paired with the lower connectivity across conditions for participants with stroke are related to a degradation in motor function, or whether the observed difference in functional connectivity is simply related to aging where differences have been observed (Tscherpel et al., 2020).

4.6 Limitations and future directions

These data provide an important foundation for future work in understanding neural correlates of unimanual motor tasks with the less-affected limb in participants with stroke, but there are several important limitations in this study. First, because the study used neurologically intact controls from the experiment one in the first study of this thesis, they were not aged-matched with the stroke group, perhaps limiting comparisons between groups. Future research should aim to replicate these findings with age-matched neurologically intact controls. Measuring brain activity during high-force contractions is difficult with fMRI, given that head motion can contaminate the data and render it unusable (Makowski et al., 2019). Further, high intensity or high-force strength-based exercises are not conventionally used in stroke rehabilitation, with most rehabilitation programs focused primarily on motor skill recovery (Langhorne et al., 2009; Belagaje, 2017). Given these two points, current literature is lacking on the fundamental understanding of how high-force, strength-based exercise may modulate neuroplasticity and promote motor recovery in stroke. With that in mind, an additional limitation was the relatively low sample size within each group. Recruiting participants with stroke to participate in research is difficult and future work may benefit from collaborative and multi-site studies to increase the

sample size to offset data loss with these high-force motor tasks in the fMRI environment. Further, an inspection of the lesion volume (table 4.1) and location (figure 4.1) clearly shows that study participants were a heterogenous sample of stroke survivors, with a maximum lesion overlap of four (i.e., number of participants with a shared lesion location). Future multi-site collaborative efforts may be able to better screen participants to obtain a more homogenous group in terms of lesion size, location, time since stroke, and functional scores, which was limited in the present study in part due to the impact of the global COVID-19 pandemic on participant recruitment. An additional limitation is that performing high-force handgrip contractions during fMRI brain scans is difficult to achieve without substantial data loss due to motion contamination. We contend that the cost-benefit trade-off to carrying out investigations into how the brain functions during these higher force contractions are of value and imperative for scientific and clinical advancement.

4.7 Conclusions

This study suggests high-force contractions with the less-affected limb may provide greater benefit to promoting iM1 activity subserving use-dependent neuroplasticity than lower force contractions. Further, the modulation of iM1 brain activation does not appear to be influenced by interhemispheric communication between these homologous regions or intrahemispheric connectivity between the SMA and M1, and is likely influenced independently or by other inter- or intrahemispheric connections not measured in the present study. The study provides a possible mechanistic basis for which cross-education may be utilized to promote iM1 brain activity in individuals with stroke. In scenarios where individuals with stroke do not have the functional capacity to engage in constraint-induced-movement-therapy, whereby the more-affected limb is directly exercised (Grotta et al., 2004), unilateral high-force contractions with the less-affected limb can provide a boost in iM1 activity that may lead to neuroplasticity of the lesioned M1 circuitry.

Thesis Transition – Study Three

Study one and two provided valuable insight into how ipsilateral cortical activity and functional connectivity are modulated with differing submaximal handgrip force levels in healthy (study 1) and stroke survivors (study 2), and observed that higher force levels increased ipsilateral cortical activation (study 1 and study 2) and functional connectivity (study 1 only). A question that remained, was how the presence of motor fatigue impacts ipsilateral cortical activity, and subsequently motor performance of each hand. The first experiment of study three identified that unimanual fatiguing handgrip contractions enhanced response time motor performance in the opposite non-fatigued limb, while also observing changes in functional connectivity between cortical regions that are known to impact motor performance within the ipsilateral hemisphere and between the two hemispheres. Further, in study three experiment two, right unimanual handgrip contractions were implemented in a left-hand motor learning paradigm to determine how fatiguing contractions impact motor learning in the opposite limb and cross-education, and observed greater cross-education in the fatiguing condition. This study contributes to the overall objective of the thesis by providing novel insights into how unimanual fatigue impacts contralateral limb motor performance and ipsilateral cortical activity.

Chapter 5

Study Three - The impact of fatigue on neural correlates of motor learning and cross-education of a serial reaction time task

5.0 Introduction

Motor skill acquisition is essential in nearly all facets of life. Able-bodied individuals may take for granted the abilities to grasp and manipulate objects, to walk or obtain the muscular strength to safely complete essential activities of daily living. However, people lose their ability to perform these tasks through a variety of injuries. Restoring function in these individuals is of clear clinical importance but how we can optimally improve behaviour is an open scientific question: both in terms of restoring motor function in orthopedic or neurologically impaired individuals (e.g., stroke patients) and in healthy populations looking to maximize motor performance (e.g., athletes).

In humans, learning of motor skills is underpinned by changes in functional connectivity across a wider network of inter-connected brain areas (Sampaio-Baptista et al., 2015; Sugata et al., 2020). A putative route to develop interventions to improve function might therefore be to modulate this connectivity. However, it is not currently clear how best to do this.

Multiple studies have shown that effective motor learning depends on the balance between neural inhibitory and excitatory processes: the induction of an excitatory/disinhibitory neural state in the primary motor cortex (M1), a key network node, significantly increases motor skill acquisition (Stagg et al., 2011a; Hendy and Kidgell, 2014; Hendy et al., 2015). The major inhibitory neurotransmitter GABA decreases during learning of a motor skill (Floyer-Lea et al., 2006; Kolasinski et al., 2019), and the magnitude of GABA decreases correlates with the extent of skill acquisition on a sequence learning task (Stagg et al., 2011a). Linking these two levels of physiological explanation, it is not yet completely clear how the local decrease in inhibition may facilitate learning, but there is evidence that local inhibition is related to network-connectivity (Stagg et al., 2014; Bachtiar et al., 2015), suggesting that perhaps an approach that reduces local inhibition may lead to increases in functional connectivity between relevant sensorimotor areas in the brain and hence greater skill acquisition.

Consistent with this, decreasing local inhibition using the non-invasive brain stimulation (NIBS) approach anodal transcranial direct current stimulation (atDCS) has been demonstrated

to lead to a decrease in GABA (Stagg et al., 2009, 2011a; Bachtiar et al., 2015; Antonenko et al., 2017), an increase in functional connectivity in the sensorimotor network (Stagg et al., 2014; Bachtiar et al., 2015) and increased motor skill acquisition, both in healthy adults (Reis et al., 2009; Stagg et al., 2011b) and in the recovery of function in stroke survivors (Kim et al., 2010; Allman et al., 2016). Though these represent promising exemplars, NIBS approaches are not without limitations: reproducibility is poor, and their neural underpinnings are poorly understood (Vallence et al., 2013). Investigating other methods to induce an optimal neural environment to improve motor learning is, therefore, an important research objective.

Unilateral fatiguing exercise has been found to increase cM1 and iM1 cortical excitability and descending neural drive, which together may aid in overcoming peripheral fatigue (Benwell et al., 2006; Aboodarda et al., 2016). Unilateral fatiguing exercise has been shown to decrease iM1 GABA-A activity, as quantified by SICI (Maruyama et al., 2006; Ni et al., 2007; Takahashi et al., 2009), and also to increase iM1 functional connectivity within the wider sensorimotor network (Jiang et al., 2012). Given the impact of cM1 inhibitory modulation on motor learning, this acute reduction in GABAergic inhibitory neural processes within the iM1 after a fatiguing bout of exercise may present a 'window of opportunity' to improve neural plasticity, making motor training with the non-fatigued limb during that disinhibited neural state a putative therapeutic tool to enhance motor learning and therefore motor rehabilitation (Maruyama et al., 2012).

Inter-limb effects after unilateral motor training approaches are relevant for motor rehabilitation. Cross-education (CE) refers to the increased motor output (i.e., force generation, skill) of the opposite, untrained limb following a period of unilateral exercise training (Manca et al., 2021). Although CE was known before the turn of the 20th century (Scripture et al., 1894) it has only recently been identified as a relevant modality of motor rehabilitation for individuals suffering from unilateral impairment (e.g., following orthopedic injury or stroke). Recent studies have identified the effectiveness of CE for preserving muscle strength and size in an immobilized limb (Farthing et al., 2009, 2011; Magnus et al., 2010; Pearce et al., 2013; Andrushko et al., 2018b; Valdes et al., 2021), and strength and range of motion of an opposite fractured arm (Magnus et al., 2013). Studies have also found CE to be effective for motor recovery in chronic stroke patients (Dragert and Zehr, 2013; Urbin et al., 2015; Sun et al., 2018). Current evidence suggests cortical mechanisms likely drive CE effects (Ruddy and Carson, 2013; Manca et al.,

2018), although the exact neural underpinnings of CE are still unknown. The iM1, ipsilateral and contralateral SMA (iSMA and cSMA respectively) are altered by fatigue (Jiang et al., 2012) and have been proposed as important sensorimotor network nodes contributing to CE (Perez et al., 2007a; Ruddy and Carson, 2013). Indeed, a compelling finding from single-arm exercise-induced fatigue is an increased functional connectivity between the iM1 and the rest of the sensorimotor network (Jiang et al., 2012), which also occurs with high-force unilateral contractions (Study 1, experiment 1), known to produce large CE effects (Urbin et al., 2015). Therefore, the increase in iM1 functional connectivity has potential relevance for CE, where the iM1 is predominantly responsible for innervating voluntary contractions of the opposite, contralateral limb.

In line with the evidence that atDCS leads to improvements in learning in the hand contralateral to the stimulated M1 (Nitsche et al., 2003; Antal et al., 2004; Boggio et al., 2006; Reis and Fritsch, 2011), atDCS applied to the iM1, either prior to (Frazer et al., 2017), or during (Hendy and Kidgell, 2014; Hendy et al., 2015) unilateral strength training enhances performance of the contralateral untrained arm. These studies give support to the hypothesis that motor training of one limb performed in a state of ipsilateral neural disinhibition may enhance inter-limb effects. As an alternative to NIBS, exercise-induced fatigue may be a suitable and universally accessible strategy for augmenting motor learning and motor rehabilitation through a decrease in GABA, and improved cortical activity and functional connectivity of resting-state sensorimotor network. Recent translational studies have supported the use of CE in clinical populations (Dragert and Zehr, 2013; Magnus et al., 2013; Urbin et al., 2015; Sun et al., 2018); however, there is currently no literature directly investigating the effects of fatigue on augmenting CE effects. Inducing a state of fatigue before a prescribed motor learning (skill or strength-based) training session may prime the motor cortices, improving the effectiveness of neural plasticity associated with CE, and enhancing the transfer effects.

5.1 Objectives

This study examined the acute effects of repeated submaximal handgrip contractions on cortical resting-state functional connectivity, motor performance and learning of a SRTT with the non-fatigued hand, and CE.

5.2 Hypotheses

The hypotheses were that a series of fatiguing right-hand submaximal handgrip contractions would augment motor performance of the non-fatigued, contralateral hand, which would be accompanied by increased interhemispheric resting-state functional connectivity between M1 and SMA, sensorimotor network nodes that are relevant for contralateral hand motor performance, learning, and CE.

5.3 Methods

5.3.1 Ethical approval

This study conforms to the standards set by the Declaration of Helsinki and was approved by the Central University Research Ethics Committee at the University of Oxford under two separate certificates (MSD-IDREC-C1-2014-100 and Oxford CUREC C1-2014-090).

5.3.2 Participants

Previous data suggested a likely effect size of $\eta^2 = 0.250$ (stimulation condition × neurochemical [GABA, glutamate] × hemisphere × time) (Bachtiar et al., 2018), giving a estimated total sample size of 12 (G*Power 3.1.9.2; $1\text{-}\beta = 0.95$, $\alpha = 0.05$). To allow for dropout and data loss, we therefore recruited fifteen healthy participants between 18-35 years (28 ± 3 years; 7 Female). All participants were right-handed (85.8 ± 18.8) based on the short form Edinburgh handedness questionnaire (Oldfield, 1971; Veale, 2014), did not play a musical instrument, had no known contraindications to receiving an magnetic resonance imaging (MRI) scan, and did not have a history of neurological or psychiatric disorders.

5.3.3 Experimental outline

All participants took part in two sessions. During each session participants participated in two separate experiments (figure 5.1). The only differences between sessions was in the relative force level of a visuomotor handgrip motor task that was performed with either 5% or 50% MVC. These experiments both had a within-subject repeated measures design, with the order of conditions were stratified-randomized accounting for sex.

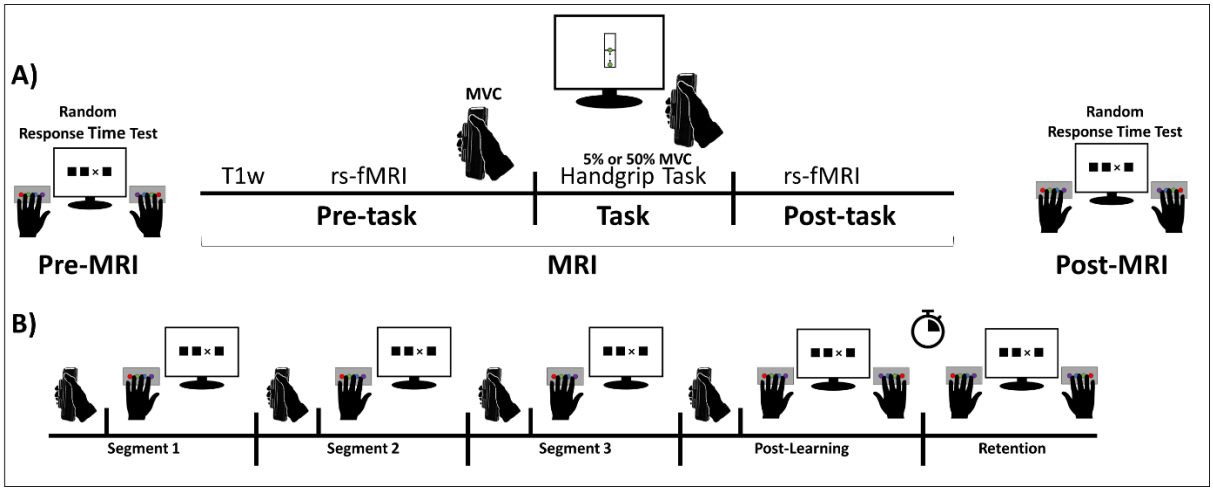

Figure 5.1 Schematic outlining the study design for **A)** Experiment one, **B)** Experiment two.

5.3.3.1 Experiment one

With experiment one, response times (RTs) of each hand were assessed before and after entering the MRI with visually-cued pseudo random block (i.e., no sequence) segments of a SRTT. During the MRI portion of the experiment, functional MRI (fMRI) scans were acquired before and after participants performed a nine-minute right handgrip motor task at 0.5 Hz with the right hand at either 5% or 50% MVC. While in the MRI, participant performed three Maximal Voluntary Contractions (MVC) with their right hand, which were used to calculate the target force for the motor task (figure 5.1A).

5.3.3.2 Experiment two

Following experiment one, the participants participated in a SRTT motor learning and CE experiment (experiment two) outside of the scanner. For experiment two, participants performed three separate segments of a motor sequence learning task with their left hand. Before each sequence learning segment participants performed two-minutes of a right 0.5 Hz handgrip force target matching task at either 5% or 50% MVC (figure 5.1B).

5.3.4 Magnetic resonance imaging acquisition

All MR data were acquired using a Siemens 3-Tesla Prisma whole-body MRI scanner with a 32-channel head array receiver coil (Siemens, Erlangen, Germany). T1-weighted MPRAGE scans were acquired at the start of the session (TR = 1900 ms, TE = 3.96 ms, TI = 912 ms, flip angle =

8°, 1 mm^3 isotropic voxels, TA = 7:21). Resting-state fMRI data was acquired (490 volumes, TR = 735 ms, TE = 39 ms, flip angle = 52°, isotropic 2.4 mm^3 with zero gap) while participants viewed a grey fixation cross on a black screen, and were asked to think of nothing in particular.

5.3.5 fMRI preprocessing

Pre-processing was carried out using single-subject independent component analysis (ICA) in MELODIC Version 3.15, part of FSL (FMRIB's Software Library, *www.fmrib.ox.ac.uk/fsl*); (Jenkinson et al., 2012). Standard preprocessing steps were performed, including removal of non-brain tissue (BET; (Smith, 2002)), removal of the initial two volumes, motion correction (MCFLIRT; (Jenkinson et al., 2002)), high-pass temporal filtering at 0.01Hz, and distortion correction with the implementation of field-maps.

Following single-subject MELODIC pre-processing, FMRIB's ICA-based Xnoiseifier (ICA-FIX) was used to automatically denoise the data (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014). The UK Biobank training-weights file (UKBiobank.RData) was used with a threshold value of 20, and 0.01Hz high-pass filtered motion confound cleanup. Following the automated denoising, all components were manually inspected to ensure accuracy before the cleaned data were smoothed with a 5mm full-width half maximum (FWHM) kernel (sigma = 2.12).

Individual resting state fMRI scans were first registered to the respective T1 structural scan using boundary-based registration as implemented in FMRIB's Linear Image Registration Tool (FLIRT; (Jenkinson and Smith, 2001; Jenkinson et al., 2002)), and then to MNI152 space using non-linear registration (FNIRT; (Andersson et al., 2007a, 2007b)).

5.3.6 fMRI analysis

Resting state functional connectivity was assessed using two different approaches: (1) a seed-based functional connectivity approach was used to test the hypothesis that unimanual handgrip contractions lead to changes in iM1 functional connectivity; and (2) a region of interest (ROI) node-based functional connectivity analysis was carried out to investigate interhemispheric homologous relationships between the M1 and SMA.

5.3.6.1 Seed-based functional connectivity

A functional group M1 hand area mask was used (Weinrich et al., 2017), and the mean timeseries of the right iM1 was extracted for each subject and each session independently. This

was then entered as a regressor into a lower-level FEAT analysis (Woolrich et al., 2001), and task-related changes in functional connectivity of iM1 were investigated via a higher level mixed-effects analysis with z = 3.1 and $p = 0.05$ (Woolrich et al., 2004).

5.3.6.2 ROI-ROI node-based functional connectivity

To assess changes in interhemispheric functional connectivity between homologous M1-M1 and SMA-SMA motor regions, ROIs were used to extract mean timeseries from participants' preprocessed resting-state scans in standard space. The M1 ROI was defined as above. The SMA mask was defined based on a connectivity parcellation (Johansen-Berg et al., 2004). The mean timeseries from within each region was extracted for each participant and session separately. Using custom in-house MATLAB scripts, correlations were then calculated for each functional connectivity edge of interest (M1-M1, SMA-SMA), next a Fishers *r* to z transformation was used to convert the Pearson's *r* values to z-scores. The z-scores for each connectivity edge were then used in a 2 × 2 × 2 (condition (5%, 50% MVC) × ROI (M1, SMA) × time (pre-task, post-task)) RM-ANOVA. Breakdown analyses were run by splitting data by condition and running separate ROI × time RM-ANOVA tests.

5.3.7 Handgrip force target-matching

The handgrip motor task was performed using an MRI-compatible hand clench dynamometer (Biopac Systems Inc. Aero Camino Goleta, CA) and was implemented using in-house code (Matlab, Mathworks). Participants performed a visually cued 0.5 Hz (1 second contraction, 1 second rest) repeated target matching task at either 5% or 50% of their MVC. The target force (either 5% or 50% MVC) was displayed at 50% of the vertical axis, and participants were instructed to squeeze the dynamometer to guide a cursor up the screen until it reached the target. During experiment one, participants performed 273 handgrip contractions (nine-minute task). During experiment two, participants performed 62 contractions (two-minute task) four separate times. Participants were not instructed that the target force would differ between conditions.

5.3.8 Handgrip task analysis

The area under the curve (AUC) of the contraction force was calculated for every contraction separately, using a two second window that accounted for the one second contraction within the window of analysis. A regression line was then fitted to the AUC metric over each trial. The

beta-value for each trial was then used as a metric to quantify motor performance, whereby a beta value < 0 indicates a decrease in motor performance over time.

5.3.9 Serial reaction time task

Participants performed a visually-cued four-choice random sequence response time task before and after MRI scanning with each hand (Experiment one). The task was implemented in PsychoPy3 (Peirce et al., 2019) and participants responded via a button box in their right or left hand (4-Button Inline, HHSC-1x4-L; Current Designs Inc. Philadelphia, PA USA). The task was divided into blocks of 64 self-paced cues and participants were asked to respond to the visual cue by pressing the corresponding button on a handheld button box as quickly and accurately as possible. Each block consisted either of a 16-button sequence repeated four times (sequence blocks), or 64 cues presented in a pseudo-random order (random blocks). Different sequences were used for each session and the order was counter-balanced across the group. Before entering the MRI (Pre-MRI) and after exiting the MRI (Post-MRI) participants performed two random blocks with each hand.

For experiment two, participants performed an implicit SRTT motor training session with their left hand. This session contained three training segments, each starting with a random block, followed by four sequence blocks. Before each training segment participants performed a right handgrip force target-matching task at the same force level that was used during scanning (5% or 50% MVC) for two minutes. After the third training segment, participants performed an additional two-minute right handgrip force target-matching task, immediately followed by a Post-Learning evaluation, which included a trio of random, sequence, random blocks. Performance was re-assessed 15 minutes later with the same trio (Retention).

5.3.9.1 SRTT analysis

Incorrect button-press responses, and response times < 50 ms and > 700 ms were removed. Response times < 50 ms were considered too fast to be physiologically possible, and response times > 700 ms were considered too slow for inclusion. The median response time of the remaining correct button presses was then calculated for each block separately.

To investigate changes in RT before and after MRI brain scans and right-handgrip fatigue in experiment one, the mean of the Pre-MRI task blocks was used as a Pre-MRI baseline

measure, and the mean of the two Post-MRI random blocks was calculated and serve as a Post-MRI measure. The percent change in RT was then calculated and a condition (5% MVC, 50% MVC) × hand (left, right) RM-ANOVA was performed.

$$Percent\ change = \frac{(Post\ MRI - Pre\ MRI)}{|Pre\ MRI|} \times 100$$

Equations 5.1

For experiment two, changes in RT during SRTT motor learning were assessed by using the mean Pre-MRI RT task as a true baseline measure since it was not influenced by the nine-minute motor task performed inside the MRI during experiment one. Further, the mean of the random blocks were calculated at Post-learning and Retention. Using the mean data values of the random block RTs, a 2 × 2 × 3 (condition × hand × time) RM-ANOVA was performed, and further breakdown analyses involved separate 2 × 3 (condition × time) RM-ANOVA tests to assess the motor performance with each hand separately.

To analyze learning of the SRTT during experiment two, we calculated the median response times for each block. A 2 × 15 RM-ANOVA with factors of condition × time (Three training segments, each starting with a random block, followed by four sequence blocks) was run on the median response time data for the left sequence trained hand. To assess sequence learning during the Post-learning and Retention trios (random, sequence, random) for each hand, the median RT during the sequence block was compared to the mean of the medians for the two random blocks. For each hand, separate condition × block type (random, sequence) RM-ANOVA tests were run at Post-Learning and Retention. Statistical analyses were run using Jamovi version 1.6.9 (The Jamovi Project, 2020). Hedges g effect sizes are reported for t-tests and partial eta squared (η_p^2) effect sizes are reported for ANOVA results.

5.4 Results

5.4.1 Experiment one

5.4.1.1 50% MVC handgrip task induced fatigue

To determine if the 50% MVC condition induced motor fatigue, it was hypothesized that the total grip force would be reduced over time during our 50% MVC fatiguing condition, but not

during simple non-fatiguing movement in our 5% MVC control condition. Therefore the AUC was quantified for the force profile for each individual handgrip contraction separately, and then a line of best fit for each participant and session was calculated. There was a significant difference in slope of this fit between the 50% and 5% conditions, with 50% MVC showing significantly more decrement in performance over time than 5% (50% MVC: β = -0.912 ± 1.070; 5% MVC: β = -0.022 ± 0.107; paired t-test $t(14)= 3.211$, $p = 0.006$, g = 0.801; figure 5.2). The slope of the 50% MVC condition was significantly different from zero ($t(14)= -3.302$, $p = 0.005$, $g = -0.824$), whereas the 5% MVC condition was not, ($t(14)= -0.785$, $p = 0.446$, $g = -0.196$), suggesting a worsening performance over time in the 50% MVC condition, consistent with what would be expected during fatigue.

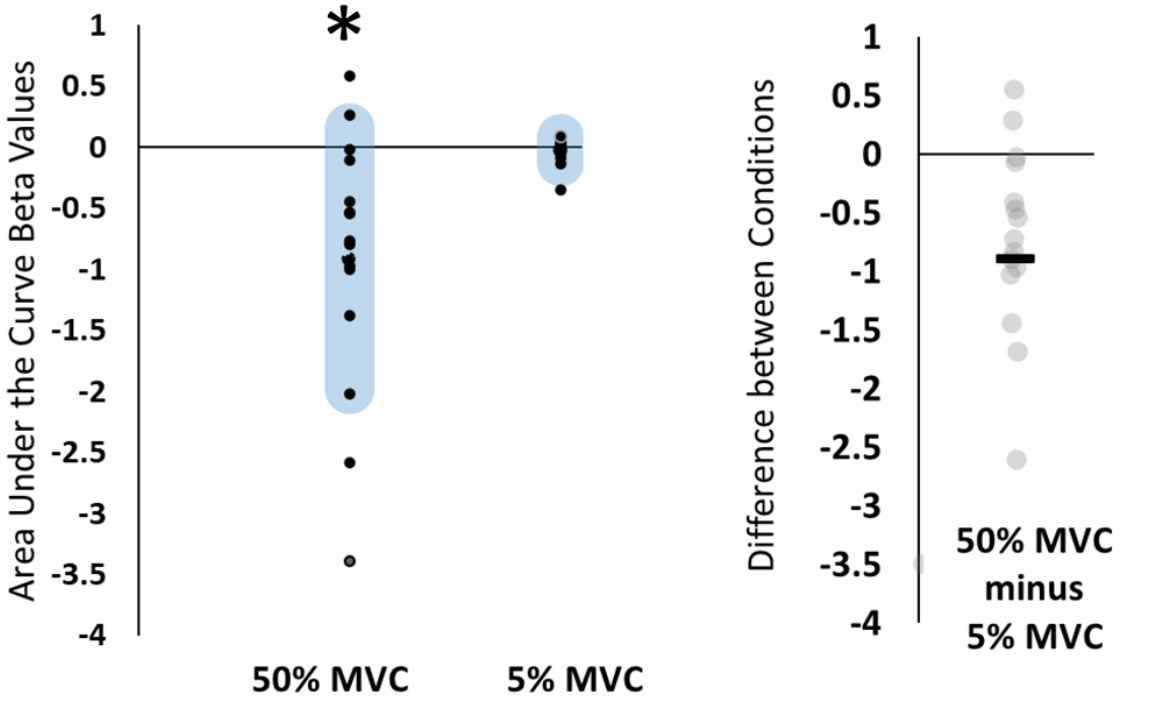

Figure 5.2 Beta values for the area under the curve of the force profile from the nine-minute right handgrip motor task in experiment one. * = 50% MVC experienced a significant decline in motor performance (one-sample t-test, $p < 0.005$).

5.4.1.2 Right hand fatigue decreased response times in the left, unfatigued hand

To investigate whether performance of a fatiguing task with the right hand would lead to changes in behaviour of the non-fatigued left hand, the change in RT after performance of the visuomotor

handgrip task between the 5% MVC and 50% MVC sessions was compared. A RM-ANOVA with one factor of condition (5%, 50% MVC) and one factor of hand (left, right) demonstrated no significant main effect of condition ($F(1,14) = 3.665$, $p = 0.076$, $\eta_p^2 = 0.207$), or hand ($F(1,14) = 2.255$, $p = 0.155$, $\eta_p^2 = 0.139$), but there was a significant condition × hand interaction, ($F(1,14) = 4.842$, $p = 0.045$, $\eta_p^2 = 0.257$). Post-hoc tests suggested that this interaction was driven by a greater decrease in RT in the left, non-fatigued hand after fatiguing contractions than after simple movement (50% MVC: -5.8 ± 7.1%; 5% MVC: 0.1 ± 6.0%, $p = 0.037$, g = 0.574; figure 5.3).

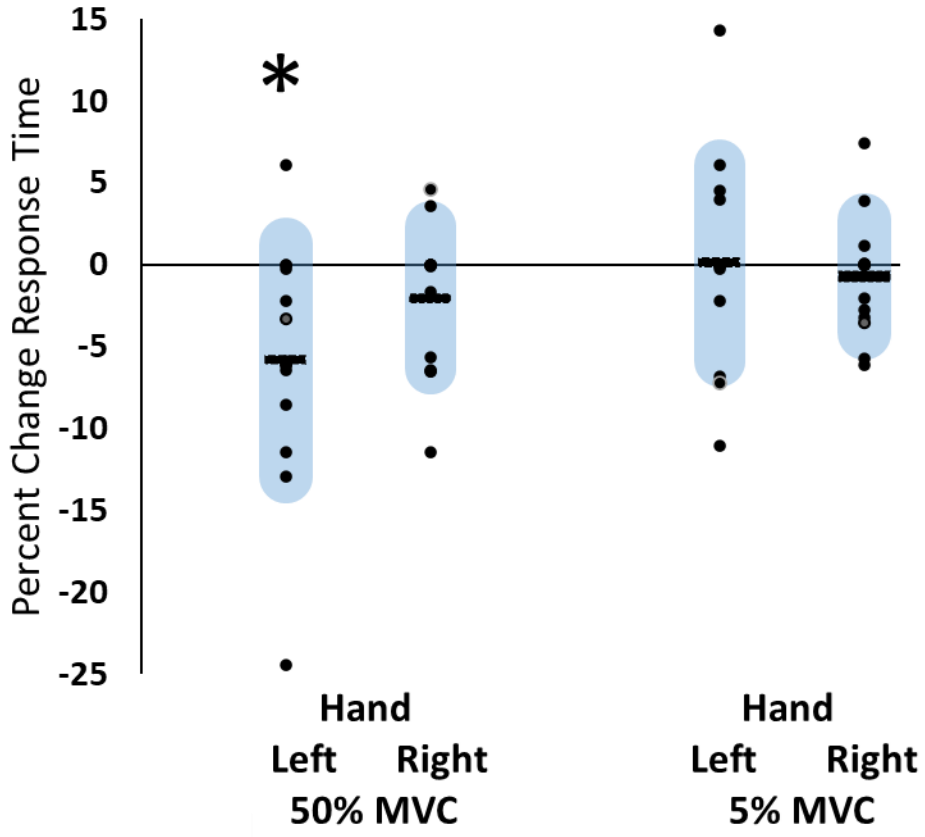

Figure 5.3 The percent change in response time for the left and right hand for the 50% MVC condition (Left) and 5% MVC condition (Right). * = significant decrease in response times ($p = 0.037$).

5.4.1.3 Response time motor performance accuracy

To further assess the motor performance quality of the left and right hand before and after the right-hand motor task, a condition × arm RM-ANOVA test was run using the percent change of

correct button presses. Based on the results of a condition × arm RM-ANOVA, the condition × arm interaction $F(1,14) = 0.015$, $p = 0.905$, $\eta_p^2 = 0.001$, and main effect of condition, $F(1,14) = 1.050$, $p = 0.323$, $\eta_p^2 = 0.070$, and arm $F(1,14) = 0.033$, $p = 0.859$, $\eta_p^2 = 0.002$ failed to reach significance, indicating that the button-press accuracy did not significantly change nor were there differences between hands or conditions (table 5.1).

Table 5.1 Mean ± standard deviation of correct button presses for Pre- and Post-MRI response time task

Condition	Hand	Pre-MRI	Post-MRI	% Change
5% MVC	Left	58.8 ± 3.1	58.7 ± 2.3	0.05
	Right	59.2 ± 2.9	59.1 ± 3.3	-0.25
50% MVC	Left	59.3 ± 3.2	58.6 ± 2.1	-0.87
	Right	59.4 ± 2.9	58.8 ± 3.5	-0.90

5.4.1.4 SMA-SMA connectivity increased after fatiguing contractions

The main objective of this study was to determine the neural correlates of fatigue-induced behavioural improvements. Therefore, to address this objective a RM-ANOVA on the functional connectivity between the left and right M1 and SMA was run, with one factor of condition (5%, 50% MVC), one factor of ROI (iSMA, cSMA, iM1, cM1), and one factor of time (Pre-Task, Post-Task). This revealed a significant three-way (condition × ROI × time) interaction ($F(1,14) = 10.154$, $p = 0.007$, $\eta_p^2 = 0.420$), as well as significant interactions for ROI × time ($F(1,14) = 4.614$, $p = 0.050$, $\eta_p^2 = 0.248$) and condition × ROI, ($F(1,14) = 4.797$, $p = 0.046$, $\eta_p^2 = 0.255$), and a significant main effect of ROI, ($F(1,14) = 118.113$, $p < 0.001$, $\eta_p^2 = 0.894$). The condition × time interaction ($F(1,14) = 0.109$, $p = 0.746$, $\eta_p^2 = 0.008$), and the main effects of condition ($F(1,14) = 0.508$, $p = 0.488$, $\eta_p^2 = 0.8035$) and time ($F(1,14) = 0.797$, $p = 0.387$, $\eta_p^2 = 0.054$) were not significant.

To understand this three-way interaction, separate ROI × time RM-ANOVA tests for the 50% and 5% conditions were run. This revealed a significant ROI × time interaction for the 50% MVC condition ($F(1,14) = 11.970$, $p = 0.004$, $\eta_p^2 = 0.461$) but not for the 5% MVC condition ($F(1,14) < 0.001$, $p = 0.997$, $\eta_p^2 < 0.001$). Follow-up tests revealed that this interaction was driven by a significant change in SMA-SMA connectivity in the 50% MVC condition ($t(14) = -2.203$, $p = 0.045$, $g = -0.550$), figure 5.4).

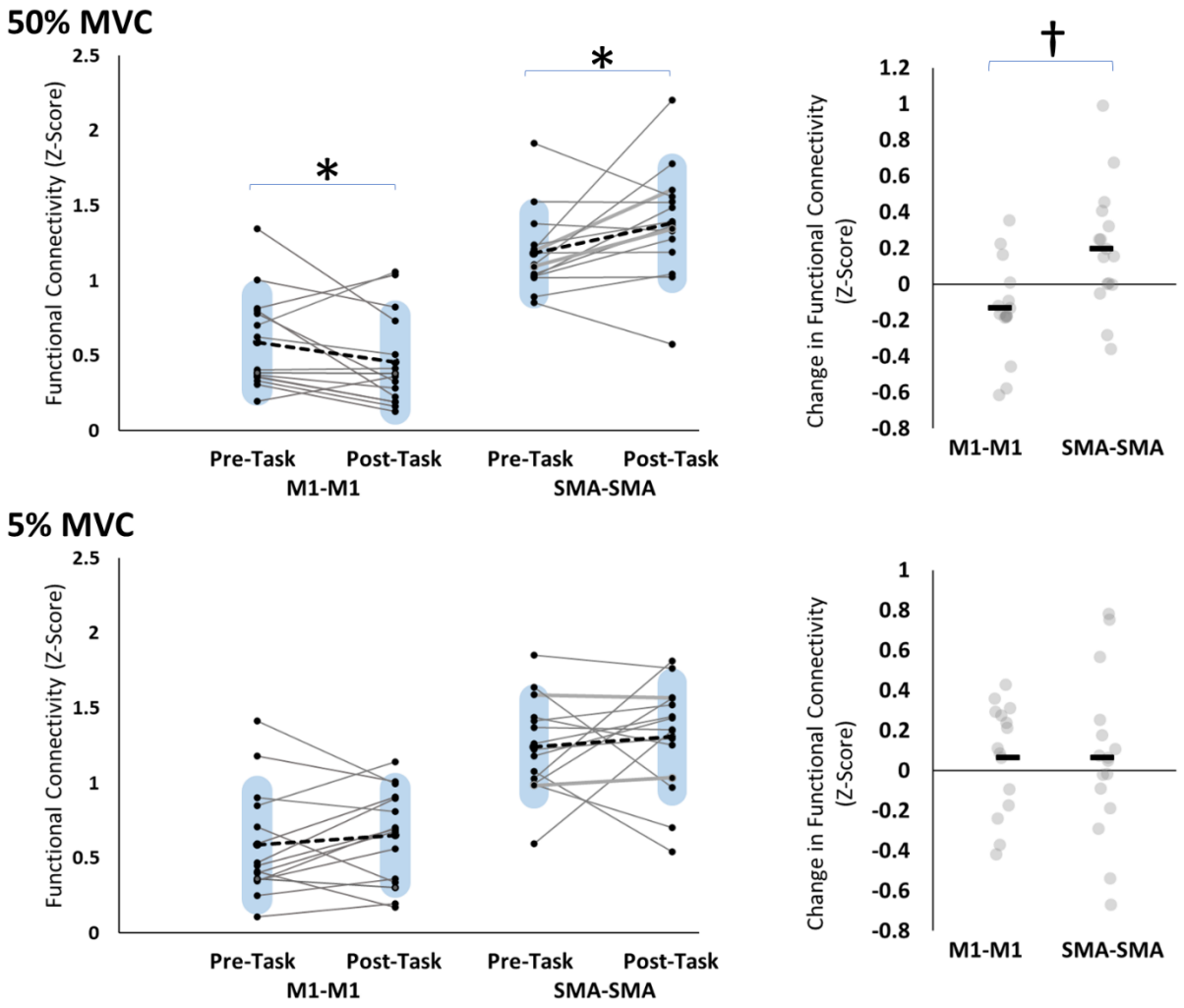

Figure 5.4 Changes in interhemispheric functional connectivity from Pre-Task to Post-Task for M1-M1 (left), and SMA-SMA (right) for the 50% MVC condition (top) and 5% MVC condition (bottom). Panels on the far right show the change score that corresponds with the Pre- Post-Task data on the left. * = significant Pre- to Post-Task change ($p < 0.05$), † = significant ROI × time interaction ($p = 0.004$).

5.4.1.5 Increased SMA-SMA functional connectivity was related to fatigue

Given that the 50% MVC task led to fatigue and to a change in SMA-SMA connectivity, a correlation analysis was carried out on these change scores to determine if these two changes were related. A negative correlation between SMA-SMA connectivity and submaximal handgrip exercise performance was observed, whereby greater fatigue, indexed by greater decrease in the force output, correlated with an increase in SMA-SMA connectivity ($r^2_{adjusted}$= 0.208, p = 0.049, β = -0.168; figure 5.5). This correlation was specific, to the change in SMA-SMA functional

connectivity, whereas the M1-M1 connectivity change did not correlate with fatigue ($r^2_{adjusted}$< 0.0001, p = 0.945, β = -0.005).

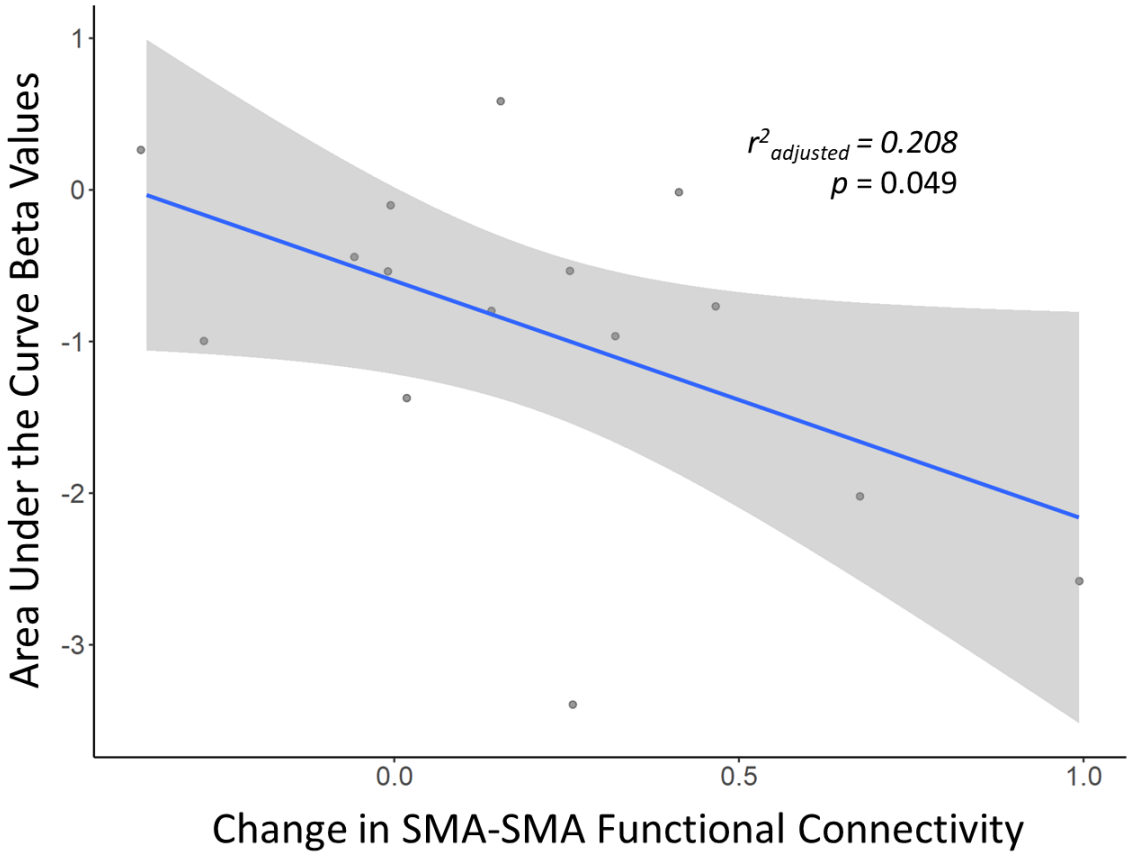

Figure 5.5 Correlation between the beta values for the area under the curve of the nine-minute handgrip task at 50% MVC and the change in SMA-SMA interhemispheric functional connectivity.

5.4.1.6 Fatigue increased functional connectivity between the iM1 and orbitofrontal cortex

Finally, to explore the whole-brain effects of our fatigue-induced behavioural improvements, a voxel-wise seed-based functional connectivity analysis with the right iM1 hand-knob (ipsilateral to the handgrip visuomotor task) was performed. There was a significant increase in functional connectivity with the right ipsilateral orbitofrontal cortex (MNI peak z-stat: x = 34, y = 26, z = -6) for the 50% MVC condition after performing the repeated handgrip task with the right hand (table 5.2). In the 5% MVC condition, there were two significant clusters of increased functional connectivity with the iM1 after movement, in the right cerebellum VI lobule (MNI peak z-stat: x = 28, y = -50, z = -26, and the left inferior temporal gyrus (MNI peak z-stat: x = -42, y = -54, z =

-4; figure 5.6; table 5.2).

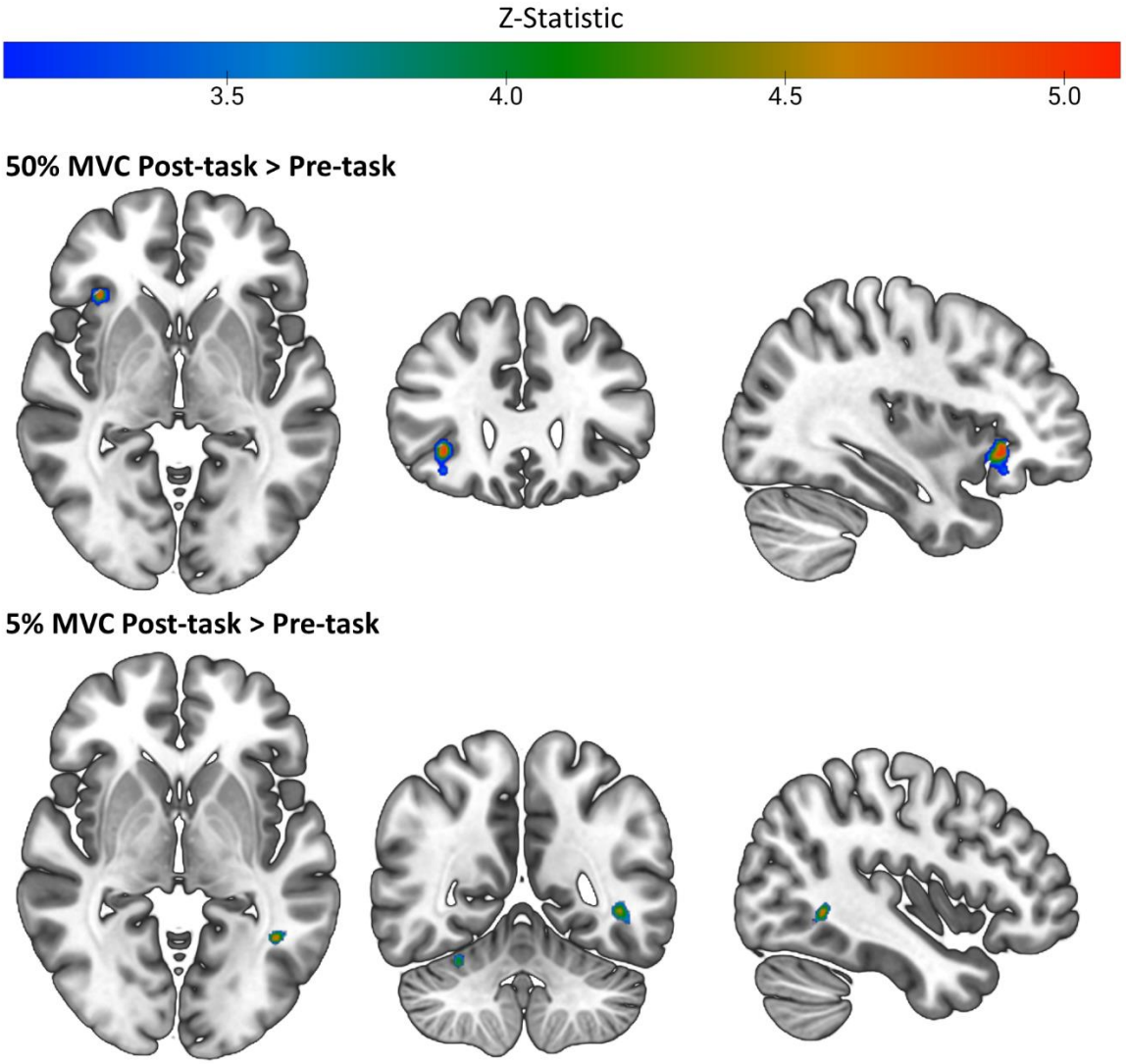

Figure 5.6 Right primary motor cortex (M1) seed-based functional connectivity results from Post > Pre-motor task contrast for the 50% MVC (top) and 5% MVC (bottom) conditions. 50% MVC map shows a significant cluster in the right orbitofrontal cortex. 5% MVC map shows significant clusters in right cerebellum VI and Left inferior temporal gyrus. Image is in radiological view (Left on right, right on left).

Table 5.2 Post > Pre-motor task rs-fMRI contrasts from right M1 seed-based analysis

50% MVC						
			MNI Coordinates (mm)			
Voxels	***p*-value**	**z-max**	**X**	**Y**	**Z**	**Label**
104	0.0348	5.12	34	26	-6	Right Orbitofrontal Cortex
5% MVC						
			MNI Coordinates (mm)			
Voxels	***p*-value**	**z-max**	**X**	**Y**	**Z**	**Label**
182	0.00187	4.44	28	-50	-26	Right Cerebellum VI
104	0.0363	4.78	-42	-54	-4	Left Inferior Temporal Gyrus

5.4.1.7 Relationship between fatigue-induced change in connectivity between iM1 and orbitofrontal cortex and the change in response times

For the right hand, the percent change in RTs from Pre-MRI to Post-MRI correlated with the change in functional connectivity between the iM1 and the right ipsilateral orbitofrontal cortex $r^2_{adjusted} = 0.353$, $p = 0.012$, $\beta = 23.976$, but did not correlate with the change in SMA-SMA ($r^2_{adjusted} = -0.030$, $p = 0.454$, $\beta = 2.604$) or M1-M1 ($r^2_{adjusted} = -0.060$, $p = 0.656$, $\beta = -2.003$) functional connectivity. However, after a manual Bonferroni correction for the multiple comparisons, the significant relationship between the percent change in RT with the right hand and the change in functional connectivity between the iM1 and right ipsilateral orbitofrontal cortex was lost (0.05/6 adjusted $\alpha = 0.008$).

5.4.2 Experiment two

5.4.2.1 Right handgrip contractions did not result in fatigue in experiment two

The beta values from each of the 4 sets of two-minute handgrip contractions during SRTT training were analyzed with a 2 × 4, condition (5%, 50% MVC) × time (Sets 1-4) RM-ANOVA. The condition × time interaction, $F(3,42) = 0.476$, $p = 0.701$, $\eta_p^2 = 0.033$, main effect of time, $F(3,42) = 0.633$, $p = 0.598$, $\eta_p^2 = 0.043$, and main effect of condition, $F(1,14) = 2.597$, $p = 0.129$, $\eta_p^2 = 0.157$ all failed to reach significance. Further, to assess if the beta values were significantly different from zero multiple one sample *t*-tests were run. Based on the one sample *t*-test results, the beta values were not significantly different from zero regardless of set or condition (table 5.3). Based on these analyses, participants were able to maintain a steady motor performance during the two-minutes of repeated handgrip contractions, and these contractions did not appear to result in any fatigue or performance decline.

Table 5.3 Experiment two - One-sample t-test against zero for AUC right handgrip contractions

Condition	Set	Result
5% MVC	1	$t(14) = -1.330$, $p = 0.205$, $g = 0.331$
	2	$t(14) = -1.891$, $p = 0.080$, $g = 0.471$
	3	$t(14) = -0.768$, $p = 0.455$, $g = 0.191$
	4	$t(14) = -1.643$, $p = 0.123$, $g = 0.410$
50% MVC	1	$t(14) = -2.067$, $p = 0.058$, $g = 0.516$
	2	$t(14) = -1.328$, $p = 0.205$, $g = 0.331$
	3	$t(14) = -0.802$, $p = 0.436$, $g = 0.200$
	4	$t(14) = -0.252$, $p = 0.805$, $g = 0.063$

5.4.2.2 Right handgrip contractions did not impact contralateral motor learning

To address the hypothesis that right-hand fatigue would lead to greater motor learning with the left hand SRTT motor training (expressed as a reduction in RTs), a RM-ANOVA with one factor of condition (5%, 50% MVC) and one factor of block (1-15) revealed a significant main effect of block ($F(1.9,26.5) = 12.035$, $p < 0.001$, $\eta_p^2 = 0.462$), indicating participants were able to learn the task. However, there was no significant main effect of condition ($F(1,14) = 0.595$, $p = 0.453$, $\eta_p^2 = 0.041$), nor condition × block interaction (Greenhouse-Geisser corrected $F(4.2,59.1) = 0.836$, $p = 0.513$, $\eta_p^2 = 0.056$), suggesting no difference in learning between the two conditions (figure 5.7).

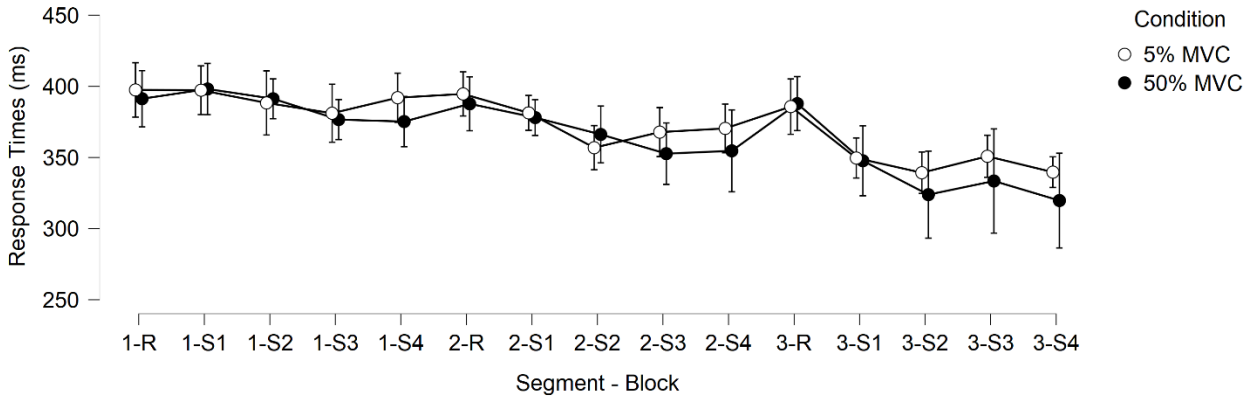

Figure 5.7 Left hand response times (ms) during the serial reaction time task motor training. First number on x-axis corresponds to training segment, R = random block, S = Sequence block. Error bars = 95% confidence intervals.

5.4.2.3 SRTT motor performance accuracy

To assess the accuracy of the left hand SRTT motor performance, the number of correct button presses was assessed for each arm with separate condition × time RM-ANOVA tests. The

separate tests were necessary given the different number of time points for each hand. For the left hand, there was a significant main effect of time, Greenhouse-Geisser corrected $F(6.4,89.1) = 3.846$, $p = 0.002$, $\eta_p^2 = 0.216$. However, the Greenhouse-Geisser corrected condition × time interaction, $F(5.8,80.9) = 1.762$, $p = 0.120$, $\eta_p^2 = 0.112$, and the main effect of condition, $F(1,14) = 0.020$, $p = 0.890$, $\eta_p^2 = 0.001$ failed to reach significance. The main effect of time for the left hand indicates that regardless of condition the performance accuracy changed over the duration of the experiment as a result of the combination of random and sequence blocks in the analysis (figure 5.8).

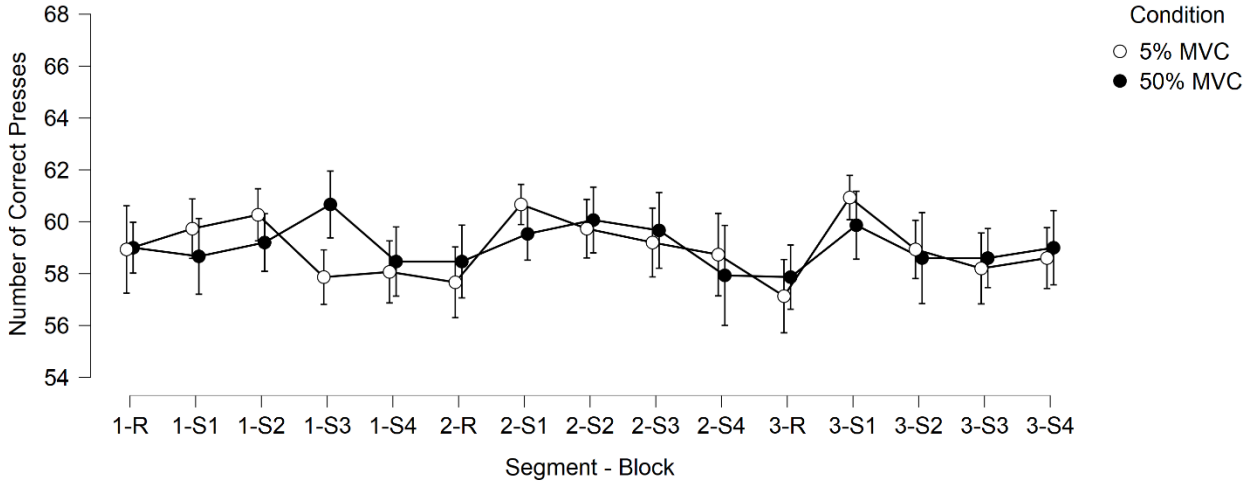

Figure 5.8 Left hand motor performance accuracy (number of correct button presses) for each block of the serial reaction time task motor training. First number on x-axis corresponds to training segment, R = random block, S = Sequence block. Error bars = 95% confidence intervals.

5.4.2.4 Fatigue resulted in greater cross-education of a SRTT at Post-Learning

Next, to determine whether the behavioural gains from the trained hand in the SRTT would transfer to the untrained hand, separate RM-ANOVA tests with one factor of condition (5%, 50%) and one factor of time (Pre-MRI, Post-Learning, Retention) were carried out using the left and right-hand random blocks data. For the left trained hand, there was a significant main effect of time ($F(2,28) = 59.94$, $p < 0.001$, $\eta_p^2 = 0.811$). However, the condition × time interaction ($F(2,28) = 1.707$, $p = 0.200$, $\eta_p^2 = 0.109$), and the main effect of condition ($F(1,14) = 0.089$, $p = 0.769$, $\eta_p^2 = 0.006$), failed to reach significance. The significant main effect of time indicates that regardless of condition, participants improved left-hand RT motor performance after left hand motor training.

For the right untrained hand, this analysis revealed a significant main effect of time ($F(2,28) = 31.613$, $p < 0.001$, $\eta_p^2 = 0.683$), suggesting that participants saw an improvement in motor performance in their right untrained hand after performing the SRTT with their left hand. However, there was no significant main effect of condition ($F(1,14) = 0.126$, $p = 0.728$, $\eta_p^2 = 0.010$), nor significant condition × time interaction, ($F(2,28) = 0.056$, $p = 0.946$, $\eta_p^2 = 0.004$). These results suggest that CE occurred in both the 5% and 50% conditions.

Finally, given there was no difference between change in random block RTs in the two conditions, the next step was to determine if right-handgrip contractions affected the ability to transfer the learned sequence. To answer this question, four separate RM-ANOVA tests were run with one factor of condition (5%, 50%) and one factor of block type (random, sequence) at the Post-Learning and Retention periods for the left and right hands.

For the trained left hand there was a significant main effect of block type at Post-Learning ($F(1,14) = 14.556$, $p = 0.002$, $\eta_p^2 = 0.510$) and Retention ($F(1,14) = 12.739$, $p = 0.003$, $\eta_p^2 = 0.476$). However, the condition × block type interactions at Post-Learning ($F(1,14) = 0.815$, $p = 0.382$, $\eta_p^2 = 0.055$) and Retention ($F(1,14) = 0.909$, $p = 0.357$, $\eta_p^2 = 0.061$), in addition to the main effect of condition at Post-Learning ($F(1,14) = 2.892$, $p = 0.111$, $\eta_p^2 = 0.171$) and Retention ($F(1,14) = 0.013$, $p = 0.909$, $\eta_p^2 = 0.001$) all failed to reach significance. These data suggest that sequence learning occurred for both conditions but no differences between conditions were observed with the left trained hand.

For the untrained right hand at Post-Learning, this analysis uncovered a significant main effect of block type, as would be expected, ($F(1,14) = 8.155$, $p = 0.013$, $\eta_p^2 = 0.368$), and a significant condition × block type interaction ($F(1,14) = 6.325$, $p = 0.025$, $\eta_p^2 = 0.311$). The main effect of condition was not significant ($F(1,14) = 1.382$, $p = 0.259$, $\eta_p^2 = 0.090$). On inspection, this interaction was driven by shorter RTs in the sequence compared to random blocks with the 50% MVC condition (figure 5.9).

For Retention, there was a non-significant condition × block type interaction ($F(1,14) = 0.780$, $p = 0.387$, $\eta_p^2 = 0.054$), and main effect of condition ($F(1,14) = 0.377$, $p = 0.549$, $\eta_p^2 = 0.026$). A significant main effect of block type was observed ($F(1,14) = 8.419$, $p = 0.012$, $\eta_p^2 = 0.376$). These data suggest that the significant difference between conditions in the untrained right hand at Post-Learning was lost at Retention, but regardless of condition, the sequence

learning was still maintained, with faster motor performance during the sequence block compared to random blocks.

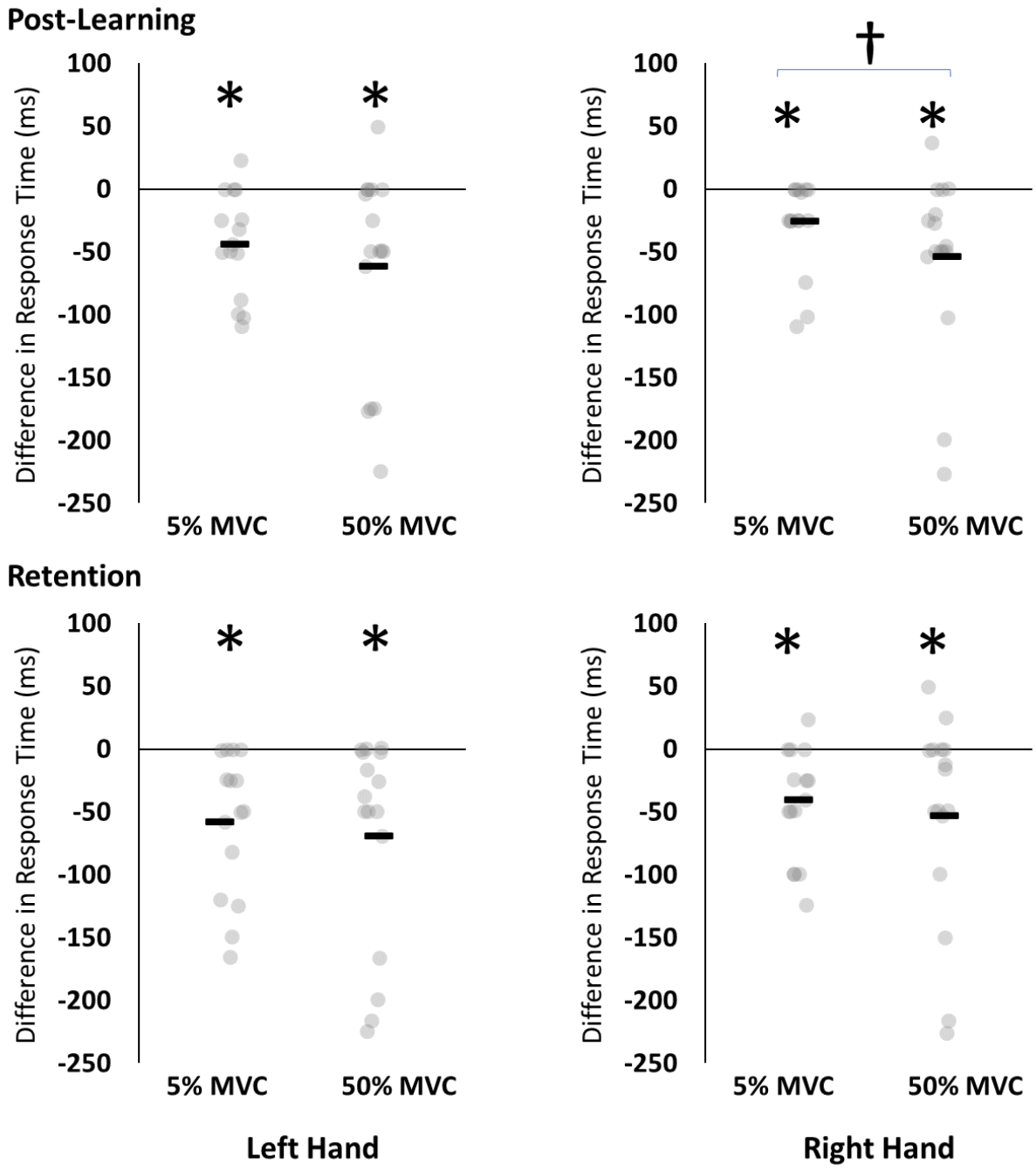

Figure 5.9 SRTT sequence learning expressed as a difference in response times between sequence and random blocks for Post-Learning (top) and Retention (bottom) for the left (left) and right (right) hands. * = significant main effect of block type ($p < 0.05$), † = significant condition × block type interaction ($p = 0.025$).

5.5 Discussion

The objective of this study was to determine how unimanual submaximal handgrip contractions at either 5% or 50% MVC impact resting-state functional connectivity and RT motor performance in both hands (Experiment one). Further, this study aimed to incorporate the unimanual right handgrip contractions into a left hand SRTT motor training paradigm to determine how the right handgrip contractions may impact the motor learning with the left hand, and CE of the trained motor skill to the right untrained hand (Experiment two).

5.5.1 Experiment one

RTs for each hand were assessed before and after participants were in the MRI. Resting-state fMRI scans were assessed before and after a nine-minute repeated submaximal handgrip motor task with the right hand at either 5% or 50% MVC. The nine-minutes of right handgrip contractions at 50% MVC resulted in a performance decline in the right hand during the submaximal contraction motor task that was not observed with the 5% MVC condition. The 50% MVC condition (-5.8 ± 7.1%) also experienced a greater percent change in RT performance compared to the 5% MVC condition (0.1 ± 6.0%) for their left hand after the right handgrip motor task (figure 5.3). However, there were no significant differences in the percent change RT performance with the right hand (50% MVC: -2.0 ± 4.3%; 5% MVC -0.7 ± 3.5%). Additionally, there were several observable changes throughout the brain after the 50% MVC right handgrip motor task that may account for the improved left-hand RTs.

5.5.1.1 Interhemispheric homologous ROI functional connectivity

The ROI node-based functional connectivity analysis examined the temporal correlates between bilateral M1 and SMA sensorimotor network nodes, and how these correlations changed after the right handgrip motor task. An examination of the interhemispheric connectivity between M1-M1 and SMA-SMA revealed that after performing the right handgrip contractions the M1-M1 functional connectivity changed differently than the SMA-SMA functional connectivity, with M1-M1 connectivity decreasing (Δ = -0.131) and SMA-SMA connectivity increasing (Δ = 0.157; figure 5.4). Of interest, this discrepancy between interhemispheric homologous ROIs may hint at a mechanistic explanation for the greater CE observed in the 50% MVC condition. Based on brain stimulation studies, greater CE is observed with a reduction in interhemispheric inhibition (Perez et al., 2007b; Camus et al., 2009; Hortobágyi et al., 2011). M1-M1 functional

connectivity reflects inhibitory processes, and therefore a reduction in M1-M1 functional connectivity likely reflects increased cortical excitability in bilateral M1 (Wahl et al., 2007). Further, the SMA contains excitatory connections (Arai et al., 2012; Shirota et al., 2012) and therefore an increase in SMA-SMA functional connectivity may also indicate a bilateral increase in excitability. The change in SMA-SMA functional connectivity was negatively correlated with the change in motor performance during the right handgrip motor task for the 50% condition. Participants that had a greater decline in handgrip motor performance during the nine-minute task, experienced a greater increase in SMA-SMA functional connectivity (figure 5.4). Previous research has identified the SMA as an important network node for CE (Perez et al., 2007a; Ruddy et al., 2017b) and specifically the interhemispheric connectivity may serve as an important pathway for which CE is mediated (Ruddy et al., 2017b). To date, Perez et al. (2007a) is the only study to investigate CE with a SRTT. Perez and colleagues found that the SMA was an important region for facilitating CE, and that inhibiting the SMA abolished CE without impacting the motor learning of the SRTT with the trained limb. The SMA has been described as a 'phylogenetically older' M1 (Goldberg, 1985) with direct connections not only onto the M1 circuitry, but onto alpha-motoneurons that innervate the hand and fingers (Maier et al., 2002). Therefore, it is plausible that the modulation of the SMA-SMA functional connectivity after right handgrip contractions at 50% MVC impacted motor performance through increase cortical excitability onto M1 and/or upper limb alpha-motoneurons, and this modulation may be one important connection that contributed to the faster RTs observed with the left, contralateral hand immediately following right hand contractions during the MRI scans.

5.5.1.2 Increased functional connectivity between ipsilateral M1 and orbitofrontal cortex after fatigue

Based on the seed-based functional connectivity analysis, the 5% MVC condition experienced an increase in functional connectivity between the iM1 (seed) and the right ipsilateral cerebellum VI lobule and the left contralateral inferior temporal gyrus. The right ipsilateral cerebellum VI lobule is known to have a right hand representation, with activation in this region observed during right hand motor tasks (King et al., 2019). The increased functional connectivity between the iM1 and the right ipsilateral cerebellum VI lobule may be ascribed to the participants learning how to perform the handgrip motor task (Spampinato et al., 2017) and modulating visuomotor errors in the motor task (Streng et al., 2018). The 5% MVC condition required low

levels of force to achieve accurate performance in the visuomotor task (target matching), where participants needed to learn how to modulate force levels to match visually cued low force targets accurately and consistently. For this study the visual display of the target position for the 5% and 50% conditions was standardized to control for differences in the visual field with the 5% and 50% conditions, but the drawback of this approach was increased sensitivity for the low force condition. Another possible explanation for the increased connectivity with the right ipsilateral cerebellum VI lobule, is an increased perception of fatigue. Svolgaaard et al. (2018) observed an increase in brain activity in the VI lobule with a non-fatiguing handgrip task that was associated with individuals perceiving motor fatigue even without evidence of physiological fatigue or performance declines.

For the 50% MVC condition, the seed-based functional connectivity analysis revealed an increased functional connectivity between the iM1 (seed) and the right ipsilateral orbitofrontal cortex. Previously, increased activation of the orbitofrontal cortex has been associated with faster RTs (Bode et al., 2018), and specifically orbitofrontal activation has been positively implicated in hand motor learning (Alves Heinze et al., 2019). The increased functional connectivity between the iM1 and the right ipsilateral orbitofrontal cortex may be reflective of the orbitofrontal cortex serving as a top-down motor control region (Ono et al., 2014) which is involved in the regulation of motor responses and error monitoring (Alves Heinze et al., 2019). Using positron emission tomography, Jackson et al. (2003) showed that right orbitofrontal cortex activation was observed in participants that experienced significant improvements in motor performance after having physically executed a motor task and also having practiced motor imagery of a motor sequence learning task. The data from the 50% MVC condition showing a cluster of increased functional connectivity between the iM1 and the right ipsilateral orbitofrontal cortex may be of functional relevance for contralateral left-hand RT improvements after executing the right handgrip motor task.

5.5.2 Experiment two

5.5.2.1 Motor learning – Random block response times and sequence learning

Performing multiple sets of two-minute repeated handgrip contractions prior to each SRTT training segment did not result in measurable handgrip motor performance decrements with the right hand. The purpose of the 50% MVC condition was to induce a state of disinhibition as a

by-product of motor fatigue (Maruyama et al., 2006, 2012; Takahashi et al., 2009), which would then be applied to augment the SRTT motor training in the contralateral hand. Nine-minutes of repeated right handgrip contractions resulted in a motor performance decline with the right hand, faster RT with the contralateral left hand, and altered functional connectivity between bilateral SMA and M1, and the iM1 and the right ipsilateral orbitofrontal cortex, both of which may contribute to the behavioural improvements in the contralateral hand (Maier et al., 2002; Jackson et al., 2003; Perez and Cohen, 2008; Ono et al., 2014; Ruddy et al., 2017a, 2017b; Alves Heinze et al., 2019). During the SRTT motor learning component, four, two-minute bouts were selected to ensure the potential priming effects were distributed throughout the SRTT motor training portion of experiment two. But the shorter two-minute bouts of repeated right handgrip contractions during SRTT motor training may have failed to induce similar cortical alterations as the nine-minute bout performed in the MRI during experiment one, and therefore did not prime the ipsilateral hemisphere enough to augment SRTT motor training with the left hand. The rationale was that fatiguing handgrip contractions would induce a state of increased cortical excitability (Benwell et al., 2006; Aboodarda et al., 2016) and decreased inhibition (Maruyama et al., 2006, 2012; Takahashi et al., 2009) within ipsilateral sensorimotor areas and this would effectively improve motor performance in the contralateral hand. This absence of motor performance decrements from the two-minute bouts may explain the lack of difference between 5% and 50% MVC conditions in the SRTT motor training with the left hand.

The SRTT motor training intervention was effective for improving RT motor performance during random blocks. Strong CE effects for RT motor performance were reported, whereby the right untrained hand improved RTs to random blocks to a similar extent as the left trained hand. There were no differences between conditions for RT motor performance assessed during random blocks. There was a difference, however, between conditions at Post-Learning with sequence learning for the right untrained hand. Given that the only difference between conditions was in the relative contraction intensity of the right handgrip task (5% vs. 50% MVC) and there were no changes in motor performance during the two-minute segments of submaximal handgrip contractions, one plausible explanation is that higher force contractions increased the cortical excitability and/or activity in sensorimotor areas within the left hemisphere after the right-hand contractions. This increased cortical excitability may have had an impact on cortical regions involved in sequence learning (Perez et al., 2007a) rather than simply motor performance

(i.e., faster RTs for random blocks). The significant interaction at Post-Learning for the right hand was a result of the 5% MVC condition having poorer sequence performance at that time-point (5% MVC: 357 ms; 50% MVC: 332 ms). However, at Retention the interaction was lost because the 5% MVC condition improved sequence motor performance to a similar level as the 50% MVC condition (5%: MVC 339 ms; 50% MVC: 327 ms). Higher contraction force with the right hand combined with left hand SRTT motor training seemed to have direct impact on the rate of right-hand sequence performance at Post-Learning, whereas the low force condition combined with left hand SRTT motor training lagged behind and did not improve to the same level of sequence performance in the untrained right hand until retention.

5.6 Limitations and future directions

The purpose of the right handgrip contractions was to cause motor fatigue which would result in cortical changes in functional connectivity and inhibition. The two-minute bouts of handgrip contractions during experiment two failed to result in measurable decrements in motor performance as an indicator of fatigue, which may explain why there were no differences between conditions in the SRTT motor learning for the trained left hand. Future studies should attempt to reproduce this SRTT experiment but with a motor task that is sure to induce a level of motor fatigue that yields decreased inhibition and increased functional connectivity within the ipsilateral cortex (Maruyama et al., 2006; Takahashi et al., 2009; Jiang et al., 2012). Conducting the handgrip task, and SRTT during fMRI brain scans may also provide better insight into the link between handgrip contractions and motor learning.

5.7 Conclusions

This study identified that right handgrip contractions at 50% MVC for nine-minutes altered functional connectivity within the sensorimotor network, specifically SMA-SMA and M1-M1 functional connectivity. In addition, an increase in functional connectivity between the iM1 and the right ipsilateral orbitofrontal cortex was observed. The interaction effects observed for functional connectivity (decrease in M1-M1 and increase in SMA-SMA) paired with the intrahemispheric connectivity between the iM1 and the right ipsilateral orbitofrontal cortex may serve as functionally relevant connections to augment motor performance in the opposite contralateral hand. Unfortunately, the handgrip contractions during SRTT motor training (Experiment two) failed to elicit motor fatigue, which may explain the lack of differences

between conditions during left hand SRTT motor training. Both hands significantly improved RT motor performance during random blocks similarly between conditions. The only difference between conditions was observed with the Post-Learning sequence block where the right non-trained hand for the 50% MVC condition exhibited faster RTs without compromising task accuracy. The improvement in RTs for the non-trained hand indicates that there was effective CE at Retention regardless of condition. The Post-Learning improvement in non-trained hand sequence performance should be interpreted with caution because the collective data from this study did not identify the specific mechanism involved.

Chapter 6
General Discussion

6.0 Introduction

Collectively, all three studies of this thesis highlight the impact of unimanual handgrip contractions on cortical modulation within the ipsilateral hemisphere. Study one (Chapter 3) first examined the cortical activation and 'on-task' functional connectivity during unimanual handgrip contractions at three different submaximal contraction forces (25%, 50% and 75% MVC). Next, a behavioural experiment was carried out to examine the muscle activity during the same tasks (Study 1, Exp 2). For study two the same experimental paradigm used in study one was then implemented in a group of participants with stroke to determine if differences in unimanual handgrip contraction force with the less-affected limb impacted cortical activation, with specific interest in the ipsilesional hemisphere. The third study investigated how submaximal handgrip contractions alter cortical resting-state functional connectivity and response time motor performance of a four button visuomotor response time task (Study 3, Exp 1). Then employed unimanual handgrip contractions to 'prime' the ipsilateral sensorimotor network in an effort to enhance motor performance and learning of a SRTT with the contralateral limb (Study 3, Exp 2).

In study one, the ipsilateral hemisphere increased parametrically both in brain activation and functional connectivity, with increasing handgrip contraction force. Study two demonstrated that the increased brain activation in the ipsilateral hemisphere with higher force handgrip contractions is replicated in participants with stroke.Whereas in study three, repeated 50% submaximal handgrip contractions were found to increase resting state functional connectivity between several cortical regions that may hint at mechanistic origins of improved response time for a button press task with the contralateral limb, after the handgrip contractions.

These data offer valuable new insight into the neural underpinnings governing unimanual handgrip force and provide support for the *Cross-Activation* hypothesis of CE. All three brain imaging experiments in this thesis highlight ipsilateral/ipsilesional cortical changes in activation (Study 1, Exp 1; Study 2) and functional connectivity with unimanual handgrip contractions during task (Study 1, Exp 1) and at rest (Study 3, Exp 1). As indicated in chapter two, the *Cross-Activation* hypothesis suggests that a duplicate motor engram is stored in the ipsilateral hemisphere after bouts of unilateral motor training. Although these data do not provide evidence

of a motor engram, they demonstrate ipsilateral activation and functional connectivity patterns that are likely to precede motor engram formation that may subserve improvements in motor function of the contralateral untrained limb.

6.1 Summary of findings

6.1.1 Chapter three - Study one

Study one covered two experiments that determined the cortical brain activity and functional connectivity with parametric increases in right handgrip contraction force levels, and the peripheral correlates of the handgrip motor task. In study one, experiment one, participants underwent MRI brain scans on two separate days. Each day the experimental paradigm remained the same. Data between sessions were combined and brain activation and 'on-task' functional connectivity were examined during repeated submaximal handgrip contractions at 25%, 50%, and 75% MVC. Between hemispheres, the cM1 exhibited greater activation across all three conditions, but the cM1 activation did not scale (i.e., further increase) with increasing contraction force. Similar levels of activation were observed across the three conditions. This finding was somewhat surprising given that cM1 cortical activation is often found to scale with output force (Keisker et al., 2009; Derosière et al., 2014). However, this is not always the case. Dettmers et al. (1995) observed that brain activity in the cM1 exhibits a rapid increase as contraction force increases with low level contractions (0 – 10% MVC), but increases beyond 10% MVC were not accompanied with similar increases in brain activation. Rather, the brain activation in the cM1 began to plateau. The findings from study one, experiment one support the findings from Dettmers et al. (1995) given the lack of differences in cM1 brain activation between the three contraction force levels. These data suggest that an increase in motor output during unilateral contractions cannot be fully explained by increases in cM1 brain activation.

An intriguing finding of study one, was that activation of the iM1 did reveal parametric scaling with increases in contraction force. The functional relevance of iM1 brain activation during unilateral movements is poorly understood (Kobayashi et al., 2003; Jankowska and Edgley, 2006; Perez and Cohen, 2008), and the neural origin of the signal as primarily excitatory (Perez and Cohen, 2008) or inhibitory (Kobayashi et al., 2003) remains unknown.

To investigate the functional connectivity of the sensorimotor network between conditions, an ICA network level analysis approach was used, and ROI specific information was

extracted to determine how changes in the iM1 and cM1 contributed to changes in overall sensorimotor network strength. With this approach, both cM1 and iM1 were found to increase functional connectivity network strength in concordance with the parametric increases in handgrip force. To our knowledge this is the first time that the 'on-task' functional connectivity was assessed during unimanual handgrip contractions. Functional connectivity is often measured at rest, whereas measuring it during a motor task provides valuable insight into the neural activity that governs the task itself. The increased functional connectivity provides insight into how the sensorimotor network modulates with different handgrip force levels, and specifically, how both the cM1 and iM1 change in relation to the entire network. Overall, these data demonstrate that the iM1 parametrically increases in brain activation and functional connectivity, but the mechanistic role of the iM1 remains unknown.

Neuro-mechanistic observations are difficult to obtain in humans using non-invasive MRI approaches. MRI derived BOLD signal is a measure of the ratio between oxy- and deoxyhemoglobin, which provides an indication of active metabolic processes (Glover, 2011; Golkowski et al., 2017b). Given that neuronal excitability and inhibition are both active processes, an examination of the BOLD signal is unable to definitively differentiate between the two (Arthurs and Boniface, 2002). The metabolic process for excitatory (glutamate) and inhibitory (GABA) neurotransmission differs, where GABA reuptake and synthesis can occur directly at the axon terminal/bouton (Fish et al., 2011), and glutamate synthesis occurs in the astrocyte (Schousboe et al., 2014). This means that inhibition has a lower metabolic demand and is less likely to be reflected in the BOLD signal. Research has supported this notion, concluding that the BOLD signal from fMRI reflects primarily excitatory rather than inhibitory neuronal activity (Sotero and Trujillo-Barreto, 2007). Yet, even with the BOLD signal likely to primarily reflect excitability, the BOLD signal can still be influenced by inhibition. Therefore, BOLD signal derived measures of functional connectivity also fail to differentiate between active inhibitory or excitatory neural processes. An increase in functional connectivity indicates an improved coupling between cortical regions. Improved coupling may indicate improved facilitative responses (i.e., excitability or activation), but it may also suggest improved inhibitory control (Wahl et al., 2007; Cincotta and Ziemann, 2008; Harper et al., 2018).

Data from study one experiment two was used to complement the brain imaging findings from experiment one in study one. In experiment two of study one, the identical paradigm was

implemented, but rather than using MRI to collect functional brain scans, peripheral surface-based EMG was used to record muscle activity in the wrist flexors (FCR muscle groups) of each hand. These data indicated that while the right, active hand displayed parametric increases in muscle activity during the right-hand contractions at 25%, 50% and 75% MVC, the muscle activity in the left, non-active hand did not differ between conditions, and the muscle activity during the contractions was also not significantly different from resting-baseline noise in the EMG signal. Therefore, it is likely that the observed brain activity and connectivity in experiment one was not a result of contralateral hand muscle activity during right hand contractions (i.e., mirror activity) but this cannot be ruled out because brain and muscle activation were not collected simultaneously.

6.1.3 Chapter four – Study two

In chapter four a group of 11 stroke survivors performed repeated submaximal handgrip contraction with their less-affected limb at 25%, 50%, and 75% of their MVC in a randomized order during fMRI brain scans. These data were then compared to the session one data from the first study in this thesis where a group of healthy participants completed the same visuomotor tasks (Chapter 3, Study 1, Exp 1). Just like in the first study (Study 1, Exp 1), unimanual handgrip contractions at higher forces resulted in increased ipsilesional brain activation in the M1. However, in contrast to the first study, an increase in 'on-task' functional connectivity was not observed in the ipsilesional hemisphere as was seen in the ipsilateral hemisphere in healthy participants. These findings are in stark contrast from what would have been expected based on the interhemispheric competition model, where a motor paradigm that promotes contralesional hemispheric brain activation should have suppressed brain activation in the ipsilesional hemisphere (Kinsbourne, 1974; Murase et al., 2004; Bütefisch et al., 2008; Grefkes et al., 2008; Nowak et al., 2009; Hordacre and Goldsworthy, 2018). Yet, these data provide a possible hint at a mechanistic basis for which CE is effectively administered in stroke survivors where improvements in motor function of the impaired limb are observed (Dragert and Zehr, 2013; Urbin et al., 2015; Sun et al., 2018; Dehno et al., 2021).

6.1.2 Chapter five – Study three

In chapter five, two experiments were presented; one that investigated the impact of repeated handgrip contractions on response time, and cortical resting-state functional connectivity within

the sensorimotor regions, and a second behavioural experiment that determined the impact of right handgrip contractions on sequence learning and response time motor performance with the trained left, and untrained right hand.

In experiment one of study three, right handgrip contractions at 50% MVC were found to modulate cortical resting-state functional connectivity in a number of relevant regions that may contribute to improved motor performance in the left contralateral hand. After handgrip contractions at 50% MVC there was an increased functional connectivity between the right M1 and the right orbitofrontal cortex, and a significant ROI × time interaction whereby the interhemispheric connectivity between bilateral M1 and the interhemispheric connectivity between bilateral SMA changed differently. M1-M1 functional connectivity decreased slightly whereas SMA-SMA increased after 50% MVC handgrip contractions. The orbitofrontal cortex has been previously implicated with motor control and is suggested to serve as a top-down motor control region which is involved in regulating motor responses and monitoring of errors (Ono et al., 2014; Alves Heinze et al., 2019). In experiment one, repeated right handgrip contractions at 50% MVC were shown to increase the intrahemispheric connectivity between the right M1 and the right orbitofrontal cortex. In line with previous observations that unilateral motor activity resulting in motor fatigue results in ipsilateral cortical modulation in the form of increased excitability (Aboodarda et al., 2016) and decreased inhibition (Maruyama et al., 2006, 2012; Takahashi et al., 2009), performing handgrip contractions with the right hand to fatigue may have contributed to the faster response times in the left contralateral hand as a result of the increased connectivity between the right M1 and the right orbitofrontal cortex. The significant interaction effects for interhemispheric connectivity between M1-M1 and between SMA-SMA after the 50% MVC contractions with the right hand is of potential relevance for CE. M1-M1 and SMA-SMA connectivity are two pathways that have been suggested as mechanistically relevant for CE (Lee and Carroll, 2007; Ruddy and Carson, 2013; Ruddy et al., 2017b; Manca et al., 2018). Specifically, the increased SMA-SMA connectivity after fatiguing unimanual handgrip contractions may enhance the interhemispheric regulatory control over M1 to aid in movement planning and preparation (Goldberg, 1985). These adjustments could manifest in improved motor performance or learning in an untrained limb.

With study three, experiment two, participants performed a sequence motor learning paradigm using a SRTT with the left hand, after performing two-minute bouts of repeated right

handgrip contractions at either 5% or 50% MVC, that were interspersed throughout the SRTT motor training, for a total of eight minutes of right handgrip contractions. The aim was to extend the findings from study three experiment one into augmenting motor performance and learning in the left hand via cortical modulation with similar right unimanual handgrip contractions that were observed in study three experiment one. Unfortunately, the dispersion of the handgrip contractions into two-minute segments failed to induce motor fatigue, with no observable changes in handgrip motor performance within or between the different segments. As a result, the left-hand motor performance and learning did not differ between the two conditions (5% and 50% MVC). There was however a significant difference between conditions at post-learning for the CE effect, where the 50% condition exhibited greater motor performance with the right hand immediately following the left-hand motor training. This result was no longer present at the retention testing phase, with the 50% MVC condition reducing their response times with sequence blocks sooner, whereas the 5% MVC condition did not achieve the same level of performance until retention. It is possible that 50% contractions augmented the rate of learning by some unknown neural mechanism. However, a more reasonable conclusion is to interpret this finding with caution. The findings from the two experiments in study three do not provide direct insight into the origins of this improvement, because the rationale was that unimanual contractions resulting in motor fatigue would cause ipsilateral cortical priming to augment motor performance with the contralateral limb (Maruyama et al., 2006, 2012; Takahashi et al., 2009), but there was no significant difference between conditions in the trained left hand.

6.2 Synthesis of findings

The purpose of this thesis was to i) investigate the neural correlates of the sensorimotor network with parametrically increasing unimanual handgrip contractions in healthy and stroke-impaired individuals, and ii) determine the effect of handgrip motor fatigue on resting-state cortical activity in the sensorimotor network and motor performance and learning in the contralateral hand. Collectively, all three studies identifed ipsilateral cortical modulation of brain activation Study 1, Exp 1; Study 2), on-task functional connectivity (Study 1, Exp 1; Study 2) and resting-state functional connectivity (Study 3, Exp 1) that are present with either high-force (Study 1, Exp 1; Study 2) or fatiguing (Study 3, Exp 1) unimanual handgrip contractions. To address the second purpose of the thesis, the first experiment in study three identified contralateral motor

performance improved after unimanual fatiguing contractions. In addition, the second experiment in study three found that the 50% MVC condition had an improved rate of transfer with a learned motor skill back to the fatigued hand.

There are some notable similarities and differences between the three studies with regards to how the handgrip motor task was implemented. In study one and two, the handgrip contractions at the three different submaximal contraction intensities (25%, 50%, 75% MVC) were performed in five sets of five contractions, with each contraction held for 1.65 seconds with equal rest between each contraction and 16.5 seconds rest between each set. In study three, the contractions were performed at either 5% or 50% MVC at a much faster rate of 0.5 Hz (one second on one second off) but for either nine minutes (Study 3, Exp 1) and two minutes (Study 3, Exp 2). In study one and two, greater iM1 activation was observed with the higher force 75% MVC condition, yet increased functional connectivity with the same high-force condition was only observed in study one. In study three, 50% MVC was the highest force level utilized in the experiments. It is likely that in study three the 50% MVC condition still effectively resulted in high levels of ipsilateral brain activation during the task (not measured in study three) due to the fatiguing component of the 50% MVC condition (Aboodarda et al., 2016, 2019).

A notable difference between study one and two was in the approach to analyzing the functional connectivity data. In study one, an ICA network level approach was taken, and ROI values represented their contribution to the overall sensorimotor network connectivity. Whereas in study two this was not the case. The ICA network level analysis failed to be replicated in the 11 stroke survivors, whereby a reliable sensorimotor network could not be easily identified during the quality control pre-processing steps. This was likely due to the heterogeneous size and location of lesions. Based on this, an ROI timeseries analysis was carried out using Pearson's *r* correlations to assess the functional connectivity between ROIs. Given there was a significant condition × time interaction for the ipsilesional/ipsilateral M1-SMA functional connectivity in study two, where neurologically intact participants appear to display an ipsilateral increase in functional connectivity, the differences in analysis approach did not likely impact the interpretation where increased connectivity was observed in the neurologically intact participants with both analysis approaches.

Together these studies demonstrate that cortical activity can be altered and/or augmented similarly with high-force and fatiguing lower force contractions. In a similar line of reasoning to

support the notion of matched neural adaptations with different muscle contraction methods, there is evidence from the exercise physiology literature that supports similar neuromuscular adaptations (e.g. muscle size and strength) with high-force and fatiguing low force contractions (Mitchell et al., 2012; Morton et al., 2016). Based on the cellular level mechanisms of neuroplasticity involved with neuroplasticity and 'rewiring the brain' (Chapter two, Section 2.1), and the concept that functional connectivity is an indirect method to measure changes in the functional connections throughout the brain, these thesis data provide insight into how higher force muscle contractions and/or lower force fatiguing contractions may result in functionally relevant neuroplasticity within the ipsilateral hemisphere that supports preferential training adaptations with the contralateral limb.

6.3 Advances in theoretical knowledge

This thesis advances our understanding of cortical functional connectivity during submaximal handgrip contractions. Previous studies have investigated cortical activation (Dettmers et al., 1995; Thickbroom et al., 1998; Dai et al., 2001; Van Duinen et al., 2008) and excitability (Hess et al., 1986; Stedman et al., 1998; Tinazzi and Zanette, 1998; Muellbacher et al., 2000; Hortobágyi et al., 2003; Perez and Cohen, 2008; Vercauteren et al., 2008; Hendy et al., 2017) with unimanual motor tasks, but for the first time, this thesis investigated the 'on-task' functional connectivity and determined that with higher force handgrip contractions, not only does cortical activation in the iM1 parametrically modulate in neurologically intact participants (Study 1, Exp 1) and participants with stroke (Study 2), but as does the strength of functional connectivity in the iM1 with the remainder of the sensorimotor network bilaterally in neurologically intact individuals (Study 1, Exp 1). An increase in functional connectivity is an indirect indication of functional relevance between cortical regions and these data may hint at a functionally relevant modulatory mechanism to contribute to higher force motor output.

Previous work has identified a role for the orbitofrontal cortex in response time motor performance (Bode et al., 2018), hand motor learning (Alves Heinze et al., 2019), and motor control (Ono et al., 2014). However, for the first time, this thesis identified that fatiguing submaximal handgrip contractions (50% condition, study 3, Exp 1) with the right hand modulates resting-state functional connectivity between the ipsilateral orbitofrontal cortex and

the iM1. Further, the increase in motor performance of the left, non-fatigued hand may be reflective of this increased modulation within the ipsilateral hemisphere.

The SMA is a brain region that has strong relevance to unimanual motor performance related to sequence learning (Mushiake et al., 1991), decision making under time-constraints (Forstmann et al., 2008) and motor memory recall of a previously learned motor task (Mushiake et al., 1991). As mentioned in chapter five (Study 3) of this thesis, the SMA has direct connections not only onto the M1, but also onto the alpha-motoneurons that innervate the hand and fingers (Rouiller, 1996; Maier et al., 2002; Chainay et al., 2004) and has been described as a "phylogenetically older" M1 (Goldberg, 1985). Among the cortical regions within the sensorimotor network, the SMA is also known to have the densest transcallosal white matter projections between homologous pairs (Ruddy et al., 2017a). The accumulation of previous literature regarding the SMA, it is unsurprising that the region has also been identified as a potentially relevant cortical region within the sensorimotor network for CE (Perez et al., 2007a; Ruddy et al., 2017b). Study three found an increase in interhemispheric connectivity between bilateral homologous SMA that correlated with the amount of motor performance decrements (i.e., fatigue) in the 50% condition. Given the potential relevance of SMA-SMA functional connectivity for interlimb transfer effects, an increase in functional connectivity may prove to be meaningful. Therefore, fatiguing unimanual handgrip contractions may provide a means to augment motor performance and CE effects through preferential increases in functional connectivity between iM1 and ipsilateral orbitofrontal cortex, and between the SMA in each hemisphere respectively.

6.4 Practical applications of thesis

The use of high-force muscle contractions is commonly believed to result in greater neuromuscular adaptations, expressed as an increase in strength (Beneka et al., 2005). Further, literature has suggested that unilateral motor training with higher force muscle contractions also result in better CE effects (Urbin et al., 2015; Pelet and Orsatti, 2021), however the neural underpinnings that govern CE have remained elusive (Ruddy and Carson, 2013; Hendy and Lamon, 2017; Manca et al., 2018). In chapters three and four of this thesis, the findings of increased brain activation with higher force handgrip contractions in addition to the observed increase in 'on-task' functional connectivity within the sensorimotor network in bilateral

hemispheres (Study 1, Exp 1) may hint at the potential mechanism that account for greater CE effects with higher force (Urbin et al., 2015; Pelet and Orsatti, 2021) and/or more fatiguing contractions (Fariñas et al., 2019). Although it remains to be determined if the ipsilateral hemisphere is acting in an excitatory or inhibitory capacity, the BOLD signal from fMRI primarily reflects excitatory rather than inhibitory neuronal activity (Sotero and Trujillo-Barreto, 2007). The increased oxygen uptake/metabolic activity and the increased coupling are likely to reflect excitatory processes, and therefore may facilitate neuroplastic adaptations that preferentially facilitate the interlimb transfer of a trained motor task.

Study two provides a hint at the mechanisms for which CE can be effectively implemented in stroke recovery interventions. If promoting use-dependent neuroplasticity in the ipsilesional hemisphere is the ultimate goal in participants with stroke to ensure good motor recovery with the more-affected limb, then high-force motor training with the less-affected limb is one such method to achieve this.

In study three, nine-minutes of repeated submaximal handgrip contractions at 50% MVC with the right hand resulted in motor performance decline measured by the linear slope of the AUC for each contraction throughout the nine-minutes. After the handgrip motor task with the right hand, the response times with the left hand significantly improved (i.e., faster and maintained accuracy). These findings suggest that unimanual handgrip fatigue may result in 'contralateral priming' whereby the motor performance is enhanced in the opposite, non-fatigued limb. Despite the fact that these data (Study 3) were collected in healthy young adults, they may have an important translational relevance for post-stroke survivors undergoing neuromotor rehabilitation to combat hemiparesis in an impaired limb. A common and efficacious method of post-stroke rehabilitation is constraint-induced movement therapy - an approach that constrains the less-affected limb during motor rehabilitation, forcing the individual to try and use their more-affected limb in achieving the motor tasks (Dromerick et al., 2000; Grotta et al., 2004; Hakkennes and Keating, 2005). Performing fatiguing unimanual handgrip contractions with the less-affected limb prior to the conventional constraint-induced movement therapy with the more-affected limb, may 'prime' the affected cortical hemisphere and more-affected limb to enhance motor performance and functional outcomes from the therapy.

6.5 Limitations and future research

Collectively studies one and three utilized separate experiments to investigate cortical, peripheral and/or behavioural measures. To strengthen inferences, future research should utilize experimental paradigms that combine the behaviour and/or peripheral measures of muscle activity with MRI based measures of cortical activity; although it is difficult.

MRI based metrics of brain activity are indirect measures of brain activation and are substantially limited in their temporal resolution based on the repetition time (TR; the time it takes to scan one brain volume). The TR of the present investigations were 1.65 seconds for study one and two and 0.735 seconds for study three. Although the third study had a substantially faster TR, all three studies are limited relative to real-time neuronal activity, with human neurons capable of firing rates ~200 Hz (Crone et al., 2006). Future work could utilize alternative methods of recording brain activity with a temporal resolution better able to capture the true temporal dynamics of the brain. Magnetoencephalography (MEG) and Electroencephalography (EEG) are two methods that can be used to gain temporal information throughout the entire brain with MEG, or on the cerebral cortical surface with EEG.

For study two, the healthy participants were a convenience sample taken from study one (Session 1 of Exp 1) that were not age matched controls. Based on this limitation, any between group differences in brain activation and functional connectivity must be made with caution. It is currently unclear if the between group differences in the functional connectivity strength and the modulation of connectivity between conditions is due to cortical alterations resulting from the stroke, or whether the differences are driven by the large age-difference between groups (~32 years). Future research should control for this by recruiting healthy age-matched controls. Finally, age related differences and changes in ipsilateral cortical activation and modulation with parametric increases in force output is not currently known and follow up research may consider investigating this to gain a greater understanding of how age-related changes in the nervous system impact the observed cortical patterns of activation and functional connectivity in this thesis. Two other notable limitations with study two are heterogenous sample of stroke survivors with respect to lesion size (table 4.1) location (figure 4.1), time post-stroke (table 4.1) and functional capacity (table 4.1). Further, obtaining quality brain scans during relatively high-force motor tasks is not easy to achieve, and high levels of motion or task correlated motion renders data unusable (Makowski et al., 2019). This limitation resulted in data loss for the high-force

75% MVC condition. However, the cost-benefit trade off for researching brain dynamics during high-force motor tasks is important and worth continuing to study even with substantial data loss. Future work should aim to expand on the findings from study two through multi-site data collections in order to bolster participant recruitment to offset data loss, while improving the capacity to screen for a more homogenous group of stroke survivors.

In the second experiment in study three, the right handgrip contractions failed to induce a measurable state of motor fatigue, and the experiment failed to detect differences between conditions (5%, 50% MVC) on the left hand SRTT motor learning paradigm. Future work should aim to replicate this experiment with a definitively fatiguing motor task to determine if the fatiguing contractions augment motor learning in the opposite non-fatigued limb.

6.7 Conclusions

These novel thesis studies investigated the neural correlates of unimanual motor behaviour during handgrip contractions (Study 1, Exp 1; Study 2) and how resting-state functional connectivity changes after repeated submaximal handgrip contractions resulting in fatigue and/or motor performance declines with the gripping hand (Study 3, Exp 1). Further, the thesis presents novel findings of an increase in functional connectivity within the iM1 during parametric increases in right unimanual handgrip contractions (Study 1, Exp 1) that was not replicated in a group of stroke survivors (Study 2). In addition to augmented motor performance in a visuomotor response time task in a left hand after performing nine-minutes of submaximal right handgrip exercise, cortical modulation was observed. Resting-state functional connectivity between the iM1 and the right orbitofrontal cortex increased, in combination with a differential modulation of interhemispheric connectivity for bilateral SMA (i.e., increased) and bilateral M1 (i.e., decreased). These data hint at possible mechanisms and functional connections that influence contralateral improvements in motor performance after fatiguing unimanual motor tasks.

While these thesis experiments nicely compliment pre-existing literature, they also identify a crucial need for future research to bridge-the-gap in the current knowledge of motor performance changes and neural mechanisms that govern them. The intriguing possibility that high-force and fatiguing handgrip contractions not only increase bilateral brain activation and functional connectivity, but also augment acute contralateral motor performance, offers great

potential to enhance neuromotor rehabilitation in individuals recovering from stroke or other unilateral injuries.

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Appendix A: Study one, experiment one ethics certificate

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB) 27-Jan-2021

Certificate of Approval Amendment

Ethics Number: 01-125

Principal Investigator: Ronald Borowsky Department: Department of Psychology

Locations Where Research Activities are Conducted: Royal University Hospital, Saskatoon, Canada

Student(s): Alice Li
Chelsea Ekstrand
Joshua Neudorf
Justin Andrushko
Shaylyn Kress

Funder(s): College of Arts and Science
Natural Sciences and Engineering Research Council of Canada
Office of the Vice-Provost, Faculty Relations

Sponsor:

Title: Functional Magnetic Resonance Imaging (fMRI) and Electroencephalography (EEG)

Protocol Number:

Approved On: 24-Jan-2021

Expiry Date: 15-Jan-2022

Approval Of: Addition of Student Researcher Justin Andrushko

Acknowledgment Of:
* TCPS2 Core Certificate of Completion for Justin Andrushko
* Reviewed with COVID-19 safety considerations in mind

Review Type: Delegated Review

IRB Registration Number:

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board (Bio-REB) has reviewed the above-named project.The project is acceptable on scientific and ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this project, and for ensuring that the authorized project is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved project.

FIRST TIME REVIEW AND CONTINUING APPROVAL

The University of Saskatchewan Research Ethics Boards review above minimal projects at a full-board (face-to-face) meeting. If a project has been reviewed at a full board meeting, a subsequent project of the same protocol may be reviewed through the delegated review process. Any research classified as minimal risk is reviewed through the delegated (subcommittee) review process. The initial Certificate of Approval includes the approval period the REB has assigned to a study. The Status Report form must be submitted within one month prior to the assigned expiry date. The researcher shall indicate to the REB any specific requirements of the sponsoring organizations (e.g. requirement for full-board review and approval) for the continuing review process deemed necessary for that project.

REB ATTESTATION

In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Part 4 of the Natural Health Products Regulations and Part C Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. Members of the Bio-REB who are named as investigators, do not participate in the discussion related to, nor vote on such studies when presented to the Bio-REB. This approval and the views of this REB have been documented in writing. The University of Saskatchewan Biomedical Research Ethics Board is constituted and operates in accordance with the current version of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2 2018).

Digitally Approved by Dr. Gordon McKay, Ph.D.
Chair, Biomedical Research Ethics Board
University of Saskatchewan

Appendix B: Study one, experiment two ethics certificate

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB) 13-Aug-2020

Certificate of Re-Approval

Ethics Number: 16-154

Principal Investigator: Jonathan Farthing Department: College of Kinesiology

Locations Where Research Activities are Conducted: University of Saskatchewan, Canada

Student(s): Doug Renshaw
Justin Andrushko
Lauren Gleed

Funder(s): Natural Sciences and Engineering Research Council of Canada

Sponsor:

Title: Neural Mechanisms of Sparing Effects in Humans

Protocol Number:

Approval Effective Date: 06/08/2020

Expiry Date: 06/08/2021

Acknowledgment Of: none

Review Type: Delegated Review

Meeting Date: 09/09/2020

IRB Registration Number: Not Applicable

* This study, inclusive of all previously approved documents, has been re-approved until the expiry date noted above

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board (Bio-REB) has reviewed the above-named project.The project is acceptable on scientific and ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this project, and for ensuring that the authorized project is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved project.

FIRST TIME REVIEW AND CONTINUING APPROVAL

The University of Saskatchewan Research Ethics Boards review above minimal projects at a full-board (face-to-face) meeting. If a project has been reviewed at a full board meeting, a subsequent project of the same protocol may be reviewed through the delegated review process. Any research classified as minimal risk is reviewed through the delegated (subcommittee) review process. The initial Certificate of Approval includes the approval period the REB has assigned to a study. The Status Report form must be submitted within one month prior to the assigned expiry date. The researcher shall indicate to the REB any specific requirements of the sponsoring organizations (e.g. requirement for full-board review and approval) for the continuing review process deemed necessary for that project.

REB ATTESTATION

In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Part 4 of the Natural Health Products Regulations and Part C Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. Members of the Bio-REB who are named as investigators, do not participate in the discussion related to, nor vote on such studies when presented to the Bio-REB. This approval and the views of this REB have been documented in writing. The University of Saskatchewan Biomedical Research Ethics Board is constituted and operates in accordance with the current version of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2 2018).

Digitally Approved by Dr. Gordon McKay, Ph.D.
Chair, Biomedical Research Ethics Board
University of Saskatchewan

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Appendix C: Saskatchewan MRI safety screening form

Saskatchewan Health Authority

Patient Label

NAME: _____

HSN: _____

D.O.B.: _____

❑ RUH ❑ SCH ❑ SPH ❑ Other _____

MEDICAL IMAGING

MRI OUTPATIENT SAFETY SCREENING QUESTIONNAIRE

Page 1 of 2

❑ OP ❑ IP ❑ GA

Booked date: _____ **Time:** _____ ❑ AM ❑ PM

Office use only: Require physiological monitoring, sedation, analgesia, or direct nursing care? ❑ Yes ❑ No
Meets NSF risk criteria and may require serum creatinine testing? See over. ❑ Yes ❑ No

Yes	No	
		Have you had a previous MRI examination? If yes, when? _____ Where? _____
		Have you had any abdominal, chest, or heart surgery or procedures? If yes, did you have a colonoscopy or gastroscopy? If yes, did they snare, biopsy, or clip anything in the bowel or stomach? Do you have a cardiac pacemaker, pacemaker leads, coronary artery stent, vessel coils or filters, cardiac defibrillator, prosthetic heart valve, etc. implanted in your body? Please list/describe: _____
		Have you had any head, neck, spine, or brain surgery or procedures? If yes, do you have intracranial aneurysm clip, cochlear implants, intra-ventricular drain, valve or VP shunt (adjustable?), brain/spine stimulator, etc.? Please list/describe: _____
		Have you had any orthopedic devices such as metal rods, pins, or screws implanted in your body? If yes, please list/describe: _____
		Have you had any other surgery or procedures? Please list/describe: _____ If so, did they use any metallic clips, sutures, staples, etc.? ❑ Yes ❑ No
		Do you have electronic pumps, electrodes, prosthesis, or other devices implanted or attached to or near your body? Example: IUD, diabetic pump, pain pump, ear (cochlear) or eye implant, etc. Please list/describe: _____
		Have you ever had a known foreign body in your eye or felt something strike your eye during welding, grinding, metalworking, etc. and could not confirm you or your physician removed it successfully? If yes, explain: _____
		Have you ever had any metal or shrapnel pierce/enter your body from a motor-vehicle accident, industrial accident, or war injury? If yes, explain: _____
		Are you claustrophobic? If yes: - Will you require sedation for the procedure? ❑ Yes ❑ No ❑ Don't know - Will you supply your own from your family doctor? ❑ Yes ❑ No
		Are you experiencing significant pain, which could make this test difficult for you? Will you require an analgesic (pain medication)? If yes, please take it 30 minutes before or have it available at your appointment time.
		Can you walk? (ambulatory, cane, walker, walk with assistance, wheelchair, need mechanical lift)
		Can you lay flat on your back without moving, with only a thin pillow for a minimum of 30 minutes?
		Do you have any dentures, hearing aids, or a wig? If so, they must be removed prior to the MRI scan.
		Is there any chance you might be pregnant? If yes, when is your due date? _____ or LMP _____
		Do you have any body piercing(s) or tattoos?
		Do you use any trans-dermal medication patches or silver nitrate dressings? If so they must be removed prior to scanning.

What is your height? _____ ❑ Feet/Inches ❑ Meters | **What is your weight?** _____ ❑ Pounds ❑ Kg

Do you have any other concerns or comments about having an MRI scan? _____

This questionnaire was completed by: ❑ Patient ❑ Mother ❑ Father ❑ Sibling ❑ Guardian ❑ Nurse ❑ Other _____

If completed in person: **Patient/Guardian signature:** _____ **Date:** _____

Technologist/nurse signature: _____ **Date:** _____

PHONE SCREENED OUTPATIENTS ONLY: Do you know how to get here and where to park? ❑ Yes ❑ No ❑ N/A **OVER**

Form #102676 (Saskatoon Area) 11/2018 Category: Assessments/Histories Original – Health Record Copy – Scanned into PACS

MRI OUTPATIENT SAFETY SCREENING QUESTIONNAIRE

Patient Label

NAME: _____

HSN: _____

D.O.B.: _____

NSF RISK ASSESSMENT – FOR GADOLINIUM ENHANCED MRI EXAMINATIONS ONLY

Patients with significant renal (kidney) disease may be at an increased risk of developing NSF (nephrogenic systemic fibrosis), a serious but rare condition resulting in fibrosis of the skin, muscles, and internal organ. Exposure to MRI contrast (gadolinium) has been implicated in the development of NSF.

Yes	No	N/A	
			Have you ever been told you have protein in your urine or gout?
			Do you have a history of renal (kidney) disease or serious injury to the kidneys?
			Have you had a previous reaction to MR IV contrast?
			Are you diabetic?
			Do you have a history of hypertension (high blood pressure)?
			Have you ever been on kidney dialysis?

If you answered yes to any of the above questions you will need to have a serum creatinine level blood test obtained within a three (3) month period prior to your gadolinium enhanced MRI. It will be important for the result to be available for the radiologist on the day of the MRI to determine if it is safe to administer gadolinium. You may already have these results as part of a recent routine blood test and, if so, we will access those results. If not, we will arrange to have this done either at the hospital or a clinic of your choice. If you are already onsite, we will arrange for this to be done here today, before your MRI.

FOR OFFICE USE ONLY	
Lab Results	
Date of specimen collection:	
Serum Creatinine (µmol/L)	Reference range – adult male 60-104 µmol/L
	Reference range – adult female 45-90 µmol/L
eGFR (mL/min/1.73m²)	

Appendix D: Study one, Participant data collection sheet

Sub-###
Order:

1. ## % MVC
2. ## % MVC
3. ## % MVC

Left Hand		Right Hand	
MVC 1		**MVC 1**	
MVC 2		**MVC 2**	
MVC 3		**MVC 3**	

Start MVC's with _____ Hand First

Age (yrs)
Height (cm)
Mass (Kg)

Have you had coffee or other sources of caffine in the last 24 hours? Yes | No
If you answered yes, please provide detail on the activity below

Have you participated in any physical activity in the last 24 hours? Yes | No
If you answered yes, please provide detail on the activity below

Appendix E: Study two, ethics certificate

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB) 07-Jul-2021

Certificate of Re-Approval

Ethics Number:	16-157
Principal Investigator:	Jon Farthing Department: College of Kinesiology
Locations Where Research Activities are Conducted:	College of Kinesiology, Canada Saskatoon City Hospital, Canada Royal University Hospital, Saskatoon, Canada
Student(s):	Justin Andrushko
Funder(s):	Royal University Hospital Foundation
Protocol Number:	X-Ed-Stroke01
Sponsor:	University of Saskatchewan
Title:	Clinical Application of Cross-education During Stroke Rehabilitation
Approval Effective Date:	24-Jun-2021
Expiry Date:	24-Jun-2022
Acknowledgment Of:	*Bio 16-157 Re-Approval Memo
Review Type:	Delegated Review
IRB Registration Number:	

* This study, inclusive of all previously approved documents, has been re-approved until the expiry date noted above

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board (Bio-REB) has reviewed the above-named project.The project is acceptable on scientific and ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this project, and for ensuring that the authorized project is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved project.

FIRST TIME REVIEW AND CONTINUING APPROVAL

The University of Saskatchewan Research Ethics Boards review above minimal projects at a full-board (face-to-face) meeting. If a project has been reviewed at a full board meeting, a subsequent project of the same protocol may be reviewed through the delegated review process. Any research classified as minimal risk is reviewed through the delegated (subcommittee) review process. The initial Certificate of Approval includes the approval period the REB has assigned to a study. The Status Report form must be submitted within one month prior to the assigned expiry date. The researcher shall indicate to the REB any specific requirements of the sponsoring organizations (e.g. requirement for full-board review and approval) for the continuing review process deemed necessary for that project.

REB ATTESTATION

In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Part 4 of the Natural Health Products Regulations and Part C Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. Members of the Bio-REB who are named as investigators, do not participate in the discussion related to, nor vote on such studies when presented to the Bio-REB. This approval and the views of this REB have been documented in writing. The University of Saskatchewan Biomedical Research Ethics Board is constituted and operates in accordance with the current version of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2 2018).

Digitally Approved by Dr. Gordon McKay, Ph.D.
Chair, Biomedical Research Ethics Board
University of Saskatchewan

Appendix F: Chedoke McMaster stroke assessment

Saskatoon Health Region

☐ RUH ☐ SCH ☐ SPH ☐ Other _____

Date: _____

Signature: _____

Chedoke-McMaster Stroke Assessment
IMPAIRMENT INVENTORY: STAGE OF RECOVERY OF ARM AND HAND

ARM and HAND: Start at Stage 3. Starting position: sitting with forearms in lap or supported on a pillow in a neutral position, wrist at 0° and fingers slightly flexed. Changes from this position are indicated by underlining. Place an X in the box of each task accomplished. Score the highest Stage in which the client achieves at least two Xs.

	ARM	HAND
1	not yet Stage 2	not yet Stage 2
2	resistance to passive abduction or elbow extension	positive Hoffman
	facilitated elbow extension	resistance to passive wrist or finger extension
	facilitated elbow flexion	facilitated finger flexion
3	touch opposite knee	wrist extension > ½ range
	touch chin	finger/wrist flexion > ½ range
	shoulder shrugging > ½ range	supination, thumb in extension: thumb to index finger
4	extension synergy, then flexion synergy	finger extension, then flexion
	shoulder flexion to 90°	thumb extension > ½ range, then lateral prehension
	elbow at side, 90° flexion: supination, then pronation	finger flexion with lateral prehension
5	flexion synergy, then extension synergy	finger flexion, then extension
	shoulder abduction to 90° with pronation	pronation: finger abduction
	shoulder flexion to 90°: pronation then supination	hand unsupported: opposition of thumb to little finger
6	hand from knee to forehead 5x5 in 5 sec	pronation: tap index finger 10X in 5 sec
	shoulder flexion to 90°: trace a figure 8	pistol grip: pull trigger, then return
	arm resting at side of body	pronation: wrist and finger extension with finger abduction
7	clap hands overhead, then behind back 3X in 5 sec	thumb to finger tips, then reverse 3X in 12 sec
	shoulder flexion to 90°: scissor in front 3X in 5 sec	bounce a ball 4 times in succession, then catch
	elbow at side, 90° flexion: resisted shoulder external rotation 3 seconds	pour 250 ml. from 1 litre pitcher, then reverse
	STAGE OF ARM	**STAGE OF HAND**

Appendix G: Fugl Meyer stroke assessment

FUGL-MEYER ASSESSMENT OF PHYSICAL PERFORMANCE

Motor Function Upper Extremity				
TEST	ITEM	SCORE		SCORING CRITERIA
		Pre	Post	
I. Reflexes	Biceps			0-No reflex activity can be elicited
	Triceps			2-Reflex activity can be elicited
II. Flexor Synergy	Elevation			0-Cannot be performed at all
	Shoulder retraction			1-Performed partly
	Abduction (at least 90^0)			2-Performed faultlessly
	External rotation			
	Elbow flexion			
	Forearm supination			
III. Extensor Synergy	Shoulder add./int. rot.			0-Cannot be performed at all
	Elbow extension			1-Performed partly
	Forearm pronation			2-Performed faultlessly
IV. Movement combining synergies	Hand to lumbar spine			0-No specific action performed 1-Hand must pass anterior superior iliac spine 2-Performed faultlessly
	Shoulder flexion to 90^0, elbow at 0^0			0-Arm is immediately abducted, or elbow flexes at start of motion 1-Abduction or elbow flexion occurs in later phase of motion 2-Performed faultlessly
	Pronation/supination of forearm with elbow at 90^0 & shoulder at 0^0			0-Correct position of shoulder and elbow cannot be attained, and/or pronation or supination cannot be performed at all 1-Active pronation or supination can be performed even within a limited range of motion, and at the same time the shoulder and elbow are correctly positioned 2-Complete pronation and supination with correct positions at elbow and shoulder
V. Movement out of synergy	Shoulder abduction to 90^0, elbow at 0^0, and forearm pronated			0-Initial elbow flexion occurs, or any deviation from pronated forearm occurs 1-Motion can be performed partly, or, if during motion, elbow is flexed, or forearm cannot be kept in pronation 2-Performed faultlessly
	Shoulder flexion 90-180^0, elbow at 0^0, and forearm in mid-position			0-*Initial* flexion of elbow or shoulder abduction occurs 1-Elbow flexion or shoulder abduction occurs during shoulder flexion 2- Performed faultlessly
	Pronation/supination of forearm, elbow at 0^0 and shoulder between 30-90^0 of flexion			0-Supination and pronation cannot be performed at all, or elbow and shoulder positions cannot be attained 1-Elbow and shoulder properly positioned and pronation and supination performed in a limited range 2-Performed faultlessly
VI. Normal reflex activity	Biceps and/or finger flexors and triceps (This item is only included if the patient achieves a maximum score on all previous items, otherwise score 0)			0-At least 2 of the 3 phasic reflexes are markedly hyperactive 1-One reflex is markedly hyperactive, or at least 2 reflexes are lively 2-No more than one reflex is lively and none are hyperactive

TEST	ITEM	SCORE		SCORING CRITERIA
VII. Wrist	Stability, elbow at 90^0, shoulder at 0^0			0-Patient cannot dorsiflex wrist to required 15^0 1-Dorsiflexion is accomplished, but no resistance is taken 2-Position can be maintained with some (slight) resistance
	Flexion/extension, elbow at 90^0, shoulder at 0^0			0-Volitional movement does not occur 1-Patient cannot actively move the wrist joint throughout the total ROM 2-Faultless, smooth movement
	Stability, elbow at 0^0, shoulder at 30^0			0-Patient cannot dorsiflex wrist to required 15^0 1-Dorsiflexion is accomplished, but no resistance is taken 2-Position can be maintained with some (slight) resistance
	Flexion/extension, elbow at 0^0, shoulder at 30^0			0-Volitional movement does not occur 1-Patient cannot actively move the wrist joint throughout the total ROM 2-Faultless, smooth movement
	Circumduction			0-Cannot be performed 1-Jerky motion or incomplete circumduction 2-Complete motion with smoothness
VIII. Hand	Finger mass flexion			0-No flexion occurs 1-Some flexion, but not full motion 2-Complete active flexion (compared with unaffected hand)
	Finger mass extension			0-No extension occurs 1-Patient can release an active mass flexion grasp 2-Full active extension
	Grasp I - MCP joints extended and proximal & distal IP joints are flexed; grasp is tested against resistance			0-Required position cannot be acquired 1-Grasp is weak 2-Grasp can be maintained against relatively great resistance
	Grasp II - Patient is instructed to adduct thumb, with a scrap of paper inter-posed			0-Function cannot be performed 1-Scrap of paper interposed between the thumb and index finger can be kept in place, but not against a slight tug 2-Paper is held firmly against a tug
	Grasp III - Patient opposes thumb pad against the pad of index finger, with a pencil interposed			0-Function cannot be performed 1-Pencil interposed between the thumb and index finger can be kept in place, but not against a slight tug 2-Pencil is held firmly against a tug
	Grasp IV - The patient should grasp a can by oppos-ing the volar surfaces of the 1st and 2nd digits.			0-Function cannot be performed 1-A can interposed between the thumb and index finger can be kept in place, but not against a slight tug 2-Can is held firmly against a tug
	Grasp V - The patient grasps a tennis ball with a spherical grip or is instructed to place his/her fingers in a position with abduction position of the thumb and abduction flexion of the 2nd, 3rd, 4th & 5th fingers			0-Function cannot be performed 1-A tennis ball can be kept in place with a spherical grasp but not against a slight tug 2-Tennis ball is held firmly against a tug
IX.Coordination/ Speed- Finger from knee to nose (5 repetitions in rapid succession)	Tremor			0-Marked tremor 1-Slight tremor 2-No tremor
	Dysmetria			0-Pronounced or unsystematic dysmetria 1-Slight or systematic dysmetria 2-No dysmetria
	Speed			0-Activity is more than 6 seconds longer than unaffected hand 1-(2-5.9) seconds longer than unaffected hand 2-Less than 2 seconds difference
Upper Extremity Total				**Maximum = 66**

Motor Function - Lower Extremity				
TEST	ITEM	SCORE		SCORING CRITERIA
		Pre	Post	
I. Reflex Activity	Achilles			0-No reflex activity can be elicited 2-Reflex activity can be elicited
	Patellar			
II. A. Flexor Synergy (in supine)	Hip flexion			0-Cannot be performed at all 1-Partial motion 2-Full motion
	Knee flexion			
	Ankle dorsiflexion			
II. B. Extensor Synergy (in side lying)	Hip extension			0-Cannot be performed at all 1-Partial motion 2-Full motion
	Adduction			
	Knee extension			
	Ankle plantar flexion			
III. Movement combining synergies (sitting: knees free of chair)	A. Knee flexion beyond 90°			0-No active motion 1-From slightly extended position, knee can be flexed, but not beyond 90° 2- Knee flexion beyond 90°
	B. Ankle dorsiflexion			0-No active flexion 1-Incomplete active flexion 2-Normal dorsiflexion
IV. Movement out of synergy (standing, hip at 0°)	A. Knee flexion			0-Knee cannot flex without hip flexion 1-Knee begins flexion without hip flexion, but does not reach to 90°, or hip flexes during motion 2-Full motion as described
	B. Ankle dorsiflexion			0-No active motion 1-Partial motion 2-Full motion
V. Normal Reflexes (sitting)	Knee flexors Patellar Achilles (This item is only included if the patient achieves a maximum score on all previous items, otherwise score 0)			0-At least 2 of the 3 phasic reflexes are markedly hyperactive 1-One reflex is markedly hyperactive, or at least 2 reflexes are lively 2-No more than one reflex is lively and none are hyperactive
VI. Coordination/speed - Sitting: Heel to opposite knee (5 repetitions in rapid succession)	A. Tremor			0-Marked tremor 1-Slight tremor 2-No tremor
	B. Dysmetria			0-Pronounced or unsystematic dysmetria 1-Slight or systematic dysmetria 2- No dysmetria
	C. Speed			0-Activity is more than 6 seconds longer than unaffected side 1-(2-5.9) seconds longer than unaffected side 2-Less than 2 seconds difference
Lower Extremity Total				Max = 34
Total Motor Score (UE + LE)				Max = 100

Sensation				
TYPE OF SENSATION	AREA	SCORE		SCORING CRITERIA
		Pre	Post	
I. Light Touch	Upper Arm			0-Anesthesia 1-Hyperesthesia / dysesthesia 2-Normal
	Palm of Hand			
	Thigh			
	Sole of Foot			
II. Proprioception	Shoulder			0-No Sensation 1-75% of answers are correct, but considerable difference in sensation relative to unaffected side 2- All answers are correct, little or no difference
	Elbow			
	Wrist			
	Thumb			
	Hip			
	Knee			
	Ankle			
	Toe			
Total Sensation Score				Maximum = 24
Total Motor and Sensory Score				Maximum = 124
Comments	**Pre:**			
	Post:			

Appendix H: Stroke demographic and medical history questionnaire

Subject ID: _____
Date: _____ /_____ /_____

DEMOGRAPHIC AND MEDICAL HISTORY QUESTIONNAIRE

Name of Family Physician: (if known) _____
Clinic: _____

Which of the following best describes your place of residence? (Check off all that apply)

☐ House ☐ Apartment or condo ☐ Senior residence ☐ Other

☐ Live alone ☐ Live with another adult

For the following questions,

Please fill in the blanks, circle or check your response

1. Are you experiencing any of the following symptoms today or have experienced them within the last few days?

Symptoms		
1. Dizziness when getting up from a chair or bed?	YES	NO
2. Any Light-headedness	YES	NO
3. Chest pain	YES	NO
4. Shortness of breath	YES	NO
5. Nausea or vomiting	YES	NO
6. Fainting	YES	NO
7. Blurring of vision	YES	NO
8. Extreme fatigue	YES	NO
9. Muscle weakness	YES	NO
10. Muscle Cramping	YES	NO
11. Unusual or severe pain of any kind	YES	NO
12. Any other symptoms or concerns you are worried about (please explain to staff present)	YES	NO

2. Have you ever been diagnosed as having any of the following conditions? (check off all that apply)

Conditions		Approximate year of onset
• Heart Attack	☐	
• Transient Ischemic Attack	☐	
• Angina (chest pain)	☐	
• High blood pressure	☐	
• Stroke	☐	
• Peripheral Vascular Disease	☐	
• Diabetes	☐	
• Neuropathies (problems with sensation)	☐	
• Respiratory Disease	☐	
• Parkinson's Disease	☐	
• Multiple Sclerosis	☐	
• Polio/Post Polio Syndrome	☐	
• Epilepsy/Seizure	☐	
• Other neurological conditions	☐	Describe:
• Any other balance disorders	☐	Describe:
• Osteoporosis	☐	
• Rheumatoid Arthritis	☐	
• Other arthritic conditions	☐	Describe:
• Uncorrected Visual problems	☐	
• Inner ear problems/ear infections	☐	
• Cancer	☐	
• Joint Replacement	☐	
• Cognitive condition	☐	
• Any other health problems	☐	

3. Do you require eyeglasses? *(Circle one)* YES NO

4. Do you require a hearing aid? *(Circle one)* YES NO

5. Do you currently smoke? *(Circle one)* YES NO

6. If you consume alcohol, how much do you typically consume per day? _____
per week? _____

7. Have you required emergency medical care or hospitalization in the past 2 years? *(Check one)*

YES ☐ NO ☐ NOT SURE ☐

If YES, explain why_____

8. List all prescription medications that you currently take (include any hormonal replacement therapy (HRT), birth control pills, glucocorticoids (cortisol or hydrocortisone) and any medications for osteoporosis or other health conditions

Name	Dosage	For what reason

9. List all over-the-counter medications that you currently take. (Pain killers, antacids, allergy pills and hydrocortisone creams or supplements (vitamins) are all examples of over-the-counter medications)

Name	Dosage	For what reason

10. Have you ever had a wrist fracture? *(Check one)*

YES ☐ NO ☐ NOT SURE ☐

If yes, please indicate the body site and date:

Left or Right *(Circle one)* Date: (mm/yy) _____/_____

If yes, did you require surgery:

If yes, please indicate if you have had physiotherapy:

Where: _____

Number of treatment sessions: _____

What type of therapy: exercise instruction, stretching, hands on "mobilizations", other (describe) _____

_____

11. Have you ever had any other broken bones or stress fracture? *(Check one)*

YES ☐ NO ☐ NOT SURE ☐

If yes, please indicate the bone and date:

Bone _____

Left or Right (Circle one) Date: (mm/yy) _____/_____

12. Has your father or mother fractured his/her hip? *(Check one)*

YES ☐ NO ☐ NOT SURE ☐

13. Have you ever been treated for or diagnosed with arthritis or other joint or bone disease? *(Check one)*

YES ☐ NO ☐ NOT SURE ☐

If yes, please explain: _____

14. Have you menstruated in the past 12 months? *(Check one)*

YES ☐ NO ☐ NOT SURE ☐

If yes, have you menstruated in the past 3 months? *(Check one)*

YES ☐ NO ☐ NOT SURE ☐

15. If yes, has the length of your cycle become less predictable in the past year? *(Check one)*

YES ☐ NO ☐ NOT SURE ☐

If no, when did you stop menstruating? Date: (mm/yy) _____/_____

16. Have you used any female hormones in the preceding 3 months? *(Check one)*

YES ☐ NO ☐ NOT SURE ☐

If yes, please fill in the question #9.

17. Have you had your uterus (hysterectomy) or both ovaries removed**?** ? *(Check one)*

YES ☐ NO ☐ NOT SURE ☐

Thank-you for completing this questionnaire!

Appendix I: Study three, ethics certificate

MEDICAL SCIENCES INTERDIVISIONAL RESEARCH ETHICS COMMITTEE

Research Services, University of Oxford, Wellington Square, Oxford, OX1 2JD
Tel: +44(0)1865 616577 Fax: +44(0)1865 280467
ethics@medsci.ox.ac.uk

CONFIDENTIAL

7th February 2019

Dr Charlotte Stagg
FMRIB Centre
University of Oxford
John Radcliffe Hospital
Oxford

Dear Dr Stagg,

Amendment of Research Ethics Application

Ref No: MSD-IDREC-C1-2014-100

Study Title: Investigating motor learning using 3T/7T MRI

Thank you for submitting a request for an amendment to the above study, which has been reviewed on behalf of the Medical Sciences Interdivisional Research Ethics Committee (IDREC).

I am pleased to inform you that your request to:

- add a new researcher (Mr Justin Andrushko)

has been judged as meeting appropriate ethical standards, on the basis of the information provided to the IDREC.

Please do not hesitate to contact me if you have any queries.

Yours Sincerely

Dr. Helen Barnby-Porritt
Research Ethics Manager, Medical Sciences

Appendix J: Study three, Oxford MRI safety screening form

3T VOLUNTEER MRI SCREENING FORM

Please carefully check the following. Some items can interfere with MR examinations and may be hazardous to your safety. Clearly mark your answer with a circle and add any relevant information. To ensure your safety we must ask for your biological sex, weight and height. Your answers will be kept strictly confidential.

Volunteer name _____ **Sex** _____

Date of birth _____ **Weight** _____ **kg** **Height** _____ **cm**

IF YOU HAVE ANY QUESTIONS THEN PLEASE ASK US BEFORE YOUR SCAN		
Do you have a heart pacemaker or pacing wires?	YES	NO
Have you had any heart surgery (e.g. coronary stent, PFO closure)?	YES	NO
Have you had any surgery to your head including eyes / ears / brain?	YES	NO
Have you had any surgery to your neck or spine?	YES	NO
Do you have any implanted devices (e.g. aneurysm clip, hydrocephalus shunt, nerve stimulator, cochlear implant, mesh)?	YES	NO
Have you had any operations involving metallic pins / plates / screws / wires?	YES	NO
Have you had any surgical procedures or endoscopy in the last 6 weeks? (Please write below)	YES	NO
Have you **ever** had any other surgical procedures of any kind? (Please write below)	YES	NO
Have you **ever** sustained any injuries involving metal to the eyes or other part of the body (e.g. from drilling, grinding or welding)?	YES	NO
Have you ever had a serious accident or injury (e.g. road traffic accident, industrial accident, explosion injury, shooting injury or shrapnel injury?)	YES	NO
Have you ever had a fit or blackout, or do you suffer from epilepsy or diabetes?	YES	NO
Do you have any of the following (if yes please circle): Body piercing, eye makeup, coloured contact lenses; Hearing aid, wearable medical device (e.g. drug pump, glucose monitor); Tattoos (including cosmetic); Dentures, dental braces, dental implant, dental bridge; Medicated skin patch (e.g. pain, HRT, nicotine, contraceptive); Artificial limb, prosthesis, splint, brace or support		
FOR WOMEN OF CHILDBEARING AGE: Do you have an IUD (coil)?	YES	NO
FOR WOMEN OF CHILDBEARING AGE: Could you be pregnant?	YES	NO
Are you wearing any clothing, including underwear, that contains metallic threads or has been silver impregnated (e.g. anti-microbial)?	YES	NO
Do you understand that this is a research scan and is not useful for diagnosis?	YES	NO
Have you removed your jewellery, hairgrips, hearing aids, watch, spectacles, keys and coins?	YES	NO

Volunteer / Guardian signature _____ **Date of study** _____

Screened by _____ **Signature** _____ **Consent sighted** _____

IMPORTANT: NO METAL OBJECTS TO BE TAKEN INTO THE MAGNET ROOM

Version: Aug 2018

Notes

For scans using contrast agent only: *(please ask a member of staff if you don't know whether your scan will involve contrast agent)*		
Have you had MR contrast agent before? (please leave blank if unknown)	YES	NO
Are you aware of any problems with your kidneys?	YES	NO
Do you have any allergies to medications? If yes please give details	YES	NO
Are you currently breast-feeding?	YES	NO

Appendix K: Study three, data collection notes

During Data Collection Notes

P0##_Session#
F3T_2019_004_0##
Date, June ##, 2019
Time: 7:15am – 11:15am
Session Details: *Low* or *High* and Sequence *1* or *2*

PreMRI
SRTT_Baseline (LL RR)…
GoNoGo (Session 1 = Left Index finger
session 2 = Right Index finger,
version 1 or 2, this should match the sequence)...

In bore time:

The radiographer typically checks in with participant for the first scan (localizer)

Once this finished (14 seconds), I like to then check in with the participant and let them know about the next scan. Essentially, I tell them that for the next scan they can keep watching the movie and it will be about 5 minutes.

Typically, each time I speak with the participant, I ask
i. if they are doing alright
ii. let them know what is coming next (e.g., task, short scan, set up scans, etc.)
iii. What they should be doing during that time (e.g., instructions)
iv. How long that step will be, and I always round generously down…
v. Finally, clarify they are happy with the instructions and to continue

1. T1 (play movie)…
   Once this finished, I then check in and say that we have about 3 minutes of set up, feel free to watch the movie.

   We set the field of view and have 3 shims before the RS is ready to start.

2. Resting State (show fixation)…

   After this scan, we can play movie for the fieldmap and setup.
   I would check in and say we have 5 minutes of "set-up scans"

3. Placement Screenshot…
4. Field map (play movie)…
5. MRSI set up (play movie)…

a. Check Adjust Vol Dims:

b. FWHM:

After this, we run a short MRSI scan (Uzay_csi_slazer_metab). I then check in with the participant and give them instructions on the ”rest task”.

Something like, this next scan is the rest task, for this, please keep your eyes open, soft gaze on the fixation cross and try your best to stay awake. It should be about 6 minutes, does that sound alright?

6. Pre MRSI x2 (show fixation)…

I then check in here and remind them of the MVC task details.

7. MVC L…[# of the accepted attempts]

REMEMBER TO START EMG!

1. EMG…
2.

Instructions for next MVC/check in

Switch Cable…

8. MVC R…[# of the accepted attempts]

a. EMG…

After this, I then tell them that we have a short scan and then the task will begin. I then give the task instructions and rough time of task and ask if that sounds alright….

9. Localizer…
10. MRSI Task x2 …

Instructions for MVC/check in

11. MVC R…[# of the accepted attempts]

a. EMG…

Switch Cable…

12. MVL L…[# of the accepted attempts]

a. EMG…

Stop and Save EMG…

After this, I then tell them that we have a short scan and then the will begin the resting task, instructions and rough time of scan.

13. Localizer (could play short movie clip)…
14. MRSI Post x2 (fixation)…

    I check in here to see how they are doing. Give encouragement that we are nearly at the end and let them know about the next resting task, instructions and rough time of scan

15. Resting State Post (Fixation)…
16. Field map (movie)…
    Let them know it is now finished and will be in to take them out in a moment.

Out of bore:
Transfer Twix and screenshot…

Post MRI Session

17. SRTT Baseline (LL RR)…
18. MVC Right…
19. Motor Task_1 at ##%...
20. SRTT_PostMRI_1 (seq #)…
21. Motor Task_2…
22. SRTT_PostMRI_2 (seq #)…
23. Motor Task_3…
24. SRTT_PostMRI_3 (seq #)…
25. Motor Task_4…
26. Probe 1 (seq #)…
27. 15 Timer minutes…
28. GonoGo (#### hand, version #, match seq #)…
29. Handedness (only once)…
30. Probe 2 (seq #)…

Appendix L: Edinburgh handedness inventory – short form

Edinburgh Handedness Inventory - Short Form

Please indicate your preferences in the use of hands in the following activities or objects:

	Always right	Usually right	Both equally	Usually left	Always left
Writing	☐	☐	☐	☐	☐
Throwing	☐	☐	☐	☐	☐
Toothbrush	☐	☐	☐	☐	☐
Spoon	☐	☐	☐	☐	☐

Scoring:

For each item: Always right = 100; Usually right = 50; Both equally = 0; Usually left = -50; Always left = -100

To calculate the Laterality Quotient add the scores for the four items in the scale and divide this by four:

Writing score	
Throwing score	
Toothbrush score	
Spoon score	
Total	
Total ÷ 4 (Laterality Quotient)	

Classification:	Laterality Quotient score:
Left handers	-100 to -61
Mixed handers	-60 to 60
Right handers	61 to 100