Characterizing interneuronal networks in the motor cortex after stroke using transcranial magnetic stimulation

by

ANJANA RAJENDRAN

BSc., The University of British Columbia, 2021

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Neuroscience)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

June 2024

© Anjana Rajendran, 2024
The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, the thesis entitled:

Characterizing interneuronal networks in the motor cortex after stroke using transcranial magnetic stimulation

submitted by Anjana Rajendran in partial fulfilment of the requirements for the degree of Master of Science in Neuroscience

Examining Committee:

Dr. Lara Boyd, Professor, Physical Therapy, UBC

Supervisor

Dr. Fidel Vila-Rodriguez, Associate Professor, Psychiatry, UBC

Supervisory Committee Member

Dr. Jason Neva, Assistant Professor, Kinesiology, University of Montreal

Supervisory Committee Member

Dr. Jean-Sébastian Blouin, Professor, Kinesiology, UBC

Additional Examiner
ABSTRACT

Previous research has linked the contribution of interneuronal networks in the primary motor cortex (M1) to motor planning, preparation and execution. The non-invasive recruitment of distinct networks in M1 can be indexed by measuring motor evoked potentials (MEP) during directional transcranial magnetic stimulation (TMS). Stimulation in the anterior-posterior (AP) direction results in longer latency MEP responses as compared to stimulation in the posterior-anterior (PA) direction. Importantly, corticospinal tract (CST) activation is measured through pulses in the lateral-medial (LM) direction. Variance in response latency is attributed to the synaptic distance between interneurons and the CST neurons. The purposes of this study were 1) to map interneuronal network activation in the contralesional and ipsilesional hemispheres in individuals with chronic stroke (> 6 months) and, 2) characterize the relationship between network activation and motor function post-stroke. Single pulse TMS was delivered bilaterally in the LM, PA and AP directions over the motor cortex in individuals with chronic stroke (n=21). Network onset times were indexed by subtracting mean MEP onset times in the LM direction from the AP and PA directions. The Wolf-Motor Function test (WMFT) quantified arm motor function in both upper limbs (n=20). Results demonstrate a significant difference between AP-LM and PA-LM MEP onset latencies difference in both hemispheres (β = 1.87, CI = 1.29 - 2.45, p<0.001). A significant interaction between hemisphere and stimulation network was also found (β = -0.84, CI = -1.58 - -010, p = 0.026), with AP-LM MEP onset latency difference being longer in the contralesional hemisphere. The AP-LM MEP onset latency difference between hemispheres explained 21% of the variance in WMFT performance between limbs; this was not the case for PA-LM MEP onset latency differences. Findings suggest the AP-LM onset latency, reflective of CST activation onset times from excitatory transsynaptic inputs, is altered post-stroke, with larger imbalances in AP-LM MEP onset across hemispheres relating to worse motor
function after stroke. These data illustrates a new relationship between interneuron pools in the human motor cortex and motor function after stroke.
LAY SUMMARY

I examined how different networks in the brain are involved in movement after a stroke, specifically focusing on changes between the two hemispheres of the brain. I used a technique called transcranial magnetic stimulation (TMS) to activate different networks within the motor cortex, each having different implications for motor behaviours. I found that in individuals with stroke, there were differences in the onset timing of different networks between the two sides of the brain. Specifically, motor function after stroke was related to the activity in one of the brain networks in the motor cortex that is thought to be more highly connected with other brain regions. These findings shed light on how the brain changes after a stroke and introduces the possibility of a unique marker in the brain for outcomes post stroke, though future experiments testing the predictive value of these different networks are warranted.
PREFACE

This thesis contains a research experiment I, Anjana Rajendran, conducted under the supervision of Dr. Lara Boyd, with guidance from Dr. Jason Neva and Dr. Fidel Vila-Rodrigues as part of my master’s thesis. The initial framework for the study was ideated by Dr. Lara Boyd and later narrowed to align with my research interests and feasibility.

The collection, analysis, and writing of this manuscript were principally conducted by me, with involvement from Dr. Lara Boyd at each stage. This thesis will be submitted for publication as a multi-authored manuscript in peer-reviewed journals. Ethical review and approval for this thesis was performed by UBC Clinical Research Ethics Board (H22-01720-A004).
# TABLE OF CONTENTS

Abstract............................................................................................................................................. iii

Lay Summary .................................................................................................................................... v

Preface............................................................................................................................................. vi

Table of Contents .......................................................................................................................... vi

List of Tables .................................................................................................................................... ix

List of Figures ................................................................................................................................... x

List of Abbreviations ....................................................................................................................... xi

Acknowledgements .......................................................................................................................... xiii

1 Introduction................................................................................................................................. 1

1.1 The Problem: Stroke in Canada ............................................................................................... 1

1.2 Transcranial Magnetic Stimulation ......................................................................................... 3

1.3 The Origin of Indirect Waves ................................................................................................. 4

1.4 Modulation of I-waves ............................................................................................................ 6

1.5 MEP Responses: Indexing I-waves ....................................................................................... 7

1.6 Interneuronal Circuits ............................................................................................................ 8

2 Study Aim and Hypothesis.......................................................................................................... 12

3 Methods........................................................................................................................................ 14

3.1 Participants .............................................................................................................................. 14

3.2 Experiment 1: Assessing MEP Onset times in a stroke affected brain ................................. 17

3.2.1 Independent and Dependent measures .............................................................................. 17

3.2.2 Data Analysis ....................................................................................................................... 18

3.3 Experiment 2: Assessing M1 onset time and clinical motor function ................................. 19

3.3.1 Independent and Dependent measures .............................................................................. 19

3.4 Statistics .................................................................................................................................. 19

3.4.1 Experiment 1 ......................................................................................................................... 19

3.4.2 Experiment 2 ......................................................................................................................... 20

4 Results.......................................................................................................................................... 22

4.1 Comparison of MEP onset times between hemispheres ....................................................... 22

4.2 Quantifying MEP onset and WMFT relationship ................................................................. 23
5 Discussion.................................................................................................................................26
5.1 Experiment 1: AP-LM MEP Onset changes between hemispheres after stroke ........26
5.2 Experiment 2: AP-LM MEP Onset explains variance in motor function after stroke .30
5.3 Limitations ..........................................................................................................................32
6 Conclusion .............................................................................................................................35
References ..................................................................................................................................36
Appendix A: Consent Form ........................................................................................................51
Appendix B: TMS Screening Form ...........................................................................................61
LIST OF TABLES

Table 1: Participant characteristics and onset time .................................................................16
LIST OF FIGURES

Figure 1: Interneuronal activation through directional TMS and MEP outputs .......................... 11
Figure 2: Summary of participant schedule and measurements .............................................. 15
Figure 3: Comparison of network onset time between hemispheres ...................................... 23
Figure 4: Relationship between PA-LM onset time and WMFT performance .......................... 24
Figure 5: Relationship between AP-LM onset time and WMFT performance .......................... 25
Figure 6: Proposed alternate AP pathway .................................................................................. 30
LIST OF ABBREVIATIONS

AP: Anterior to Posterior

BAI: Beck’s Anxiety Index

cTBS: Continuous Theta Burst Stimulation

CST: Corticospinal Tract

D-wave: Direct wave

ECR: Extensor Carpi Radialis

EMG: Electromyography

EPSP: Excitatory Post-Synaptic Potential

FMA-UE: Fugl-Meyer Assessment for Upper Extremity

GABA: Gamma-aminobutyric acid

iTBS: Intermittent Theta Burst Stimulation

I-wave: Indirect Wave

LICI: Long interval intracortical inhibition

LM: Lateral to Medial

LMER: Linear Mixed Effects Regression

MoCA: Montreal Cognitive Assessment

M1: Primary Motor Cortex

MEP: Motor Evoked Potential

MNI: Montreal Neurological Institute

MRI: Magnetic Resonance Imaging

NHSS: National Institute of Health Stroke Scale

PA: Posterior to Anterior
QIDS-SR: Self-Reported Quick Inventory of Depressive Symptomology

RMT: Resting Motor Threshold

RST: Reticulospinal tract

SICI: Short Intracortical Inhibition

rTMS: Repetitive Transcranial Magnetic Stimulation

TMS: Transcranial Magnetic Stimulation

WMFT: Wolf-Motor Function Test
ACKNOWLEDGEMENTS

I would like to express my gratitude to all the students, faculty, and mentors at UBC that inspired me to pursue neuroscience research and shaped my love of science over the last six years. I especially thank Dr. Lara Boyd for giving me incredible opportunities for personal and professional growth as a scientist – your mentorship was pivotal in my decision to pursue a Ph.D. and I’m excited to keep learning with you at the Brain Behaviour Lab.

I thank the members of my committee Dr. Jason Neva and Dr. Fidel Vila-Rodrigues for their expertise and guidance in formulating this master’s project. Thank you to Dr. David Cunningham for guiding me through processing and learning new tools to analyze TMS data and Dr. Justin Andrushko for providing critical support and answering my many questions.

I could not have done this work without the support of all my colleagues (and their dogs) at the Brain Behaviour Lab. A special thanks to Dr. Ronan Denyer, Cristina Rubino, Beverley Larssen, Shie Rinat, Julia Dahlby, Christy Jones, Tamara Koren, and Jordan Brocato for helping me with recruitment, data collection and lending a listening ear when I needed it – you all inspire me to be a better scientist every day!

Amma, Achan, and Sanjay, thank you for always encouraging my academic pursuits and believing in me. Thank you, Shreyas, for your unwavering support at every step along the way.

Finally, I want to express my deepest gratitude to every participant and their families who shared their stories with me and agreed to participate in our research. None of this work is possible without you.
1 INTRODUCTION

1.1 THE PROBLEM: STROKE IN CANADA

Stroke remains one the leading causes of death and disability globally, with recent reports stating that the risk of incurring a stroke increases after age 70 (GBD Stroke Collaborators, 2021). In Canada, recent estimates claim that there is an occurrence of stroke approximately every five minutes and the total number of strokes continue to grow (Holodinsky et al., 2023). Impairment from stroke is an enormous burden on the global healthcare system, with estimates suggesting that people who experienced a stroke in Canada are more likely to seek out care for stroke related mobility and associated cognitive issues than those who did not experience a stroke (Einstad et al., 2021; Feigin et al., 2014; Obempe et al., 2019). Combined with an increasingly aging population in Canada (Canadian Institute for Health Information, 2017), the need to address stroke related care and reduce the burden of long-term disability for which no long-term care strategies have been implemented is a pressing need (Bernhardt et al., 2016). The investigation into potential biomarkers is a promising avenue which hopes to address the heterogeneity in stroke recovery, a path often punctuated by the enigmatic nature of the recovering stroke brain (Boyd et al., 2017). Current understanding of how the brain recovers after stroke suggests that the majority of the behavioural processes and rapid cell-level changes occur within the first three months post-stroke, with an earlier plateau for recovery of paretic upper limb motor control but not function (Bernhardt et al., 2017; Cortes et al., 2017). Motor function has been reported to increase for up to one year following stroke, lending support to the idea that the dynamic and changing stroke brain has behavioural consequences (Ingwersen et al., 2021).
After stroke, the brain exhibits various properties of plasticity especially as it relates to communication with the corticospinal tract. For instance, previous work has found that in chronic stroke survivors with substantial damage to the corticospinal tract (CST), activity in the reticulospinal tract (RST) is upregulated indicating evidence of a compensatory mechanism in recovery that exists in more severe paretic phenotypes; CST integrity can give insight into impairment in mild-moderate chronic stroke survivors (Choudhury et al., 2019). Additionally, increased ipsilateral connectivity from the stroke-unaffected hemisphere to the paretic arm has not been shown to relate to motor function, indicating the contralateral pathway is the best marker of motor function (Hammerbeck et al., 2019). This is supported by work finding that characteristics of the motor evoked potential (MEP) such as its presence and amplitude is a marker for upper motor paresis three months post-stroke (Bembenek et al., 2020). Importantly in the chronic stage of stroke recovery, classified as six months post stroke (Bernhardt et al., 2017), worsening motor function has been linked to an observed imbalanced interhemispheric inhibition where the stroke unaffected hemisphere exhibits greater inhibition onto the stroke affected hemisphere (Xu et al., 2019). Several reviews have linked gamma-aminobutyric acid (GABA) transmission post-stroke to motor learning and functional recovery with the overall understanding that decreased GABA is linked to better function (Paik and Yang, 2014; Paperella et al., 2023). Calls for GABA transmission and receptor availability to be considered as a recovery biomarker brings into question the role of interneuronal activation and whether this property could inform motor behaviour during recovery. Interneurons are a class of cells within the central nervous system that are integral in relaying and modulating both inhibitory and excitatory information within cortical circuits (Batista-Brito et al., 2020). In the motor cortex (M1), a site of importance for learning and execution of movement, interneuron are pivotal in
relaying information from different cortical layers and modulating neurotransmitter release (Papale & Hooks, 2018). Changes in synaptic connectivity of the interneurons are observed during motor skill learning and have been thought to underlie mechanisms driving cortical plasticity, a heightened phenomenon after stroke (Alia et al., 2017).

These findings, linking changes in the brain, specifically in interneuron communication, to changes in behaviour post-stroke provide evidence for using TMS to characterize how M1 and its communication to upper limbs after stroke have changed. Previous work has also suggested through stimulation and imaging techniques that pre-motor areas and surrounding inputs to M1 through alternate pathways are involved in producing movement (Sandrini and Cohen, 2013). Considering the dynamic nature of interneuron circuits, their relationships to the CST, and the heterogenous nature of stroke recovery, interneuronal responses after stroke could be an important metric to evaluate the range of behavioural implications owing to unique physiology in motor performance.

1.2 TRANSCRANIAL MAGNETIC STIMULATION

Transcranial Magnetic Stimulation (TMS) is a widely used form of non-invasive brain stimulation. It varies from being used as a clinical intervention as seen in treatment of depression (Rizvi & Khan, 2019) or in enhancing upper limb mobility (Hoyer & Celnik, 2011), to a tool for measuring neurophysiological excitability (Ortega-Robles et al., 2023). TMS works by inducing an electrical field on the brain tissue through the application of a perpendicular magnetic field which crosses the skull and excites underlying cortical tissue (Barker et al., 1985). The response from this stimulation over the motor cortex can be measured through electromyography (EMG) recorded over the peripheral muscle. Specifically, following stimulation of the motor cortex, the EMG response is seen as motor-evoked potentials (MEP) which reflects the sum of inputs to the
CST (DiLazzaro and Rothwell, 2014). The strength of the response reflects activation of spinal alpha motor neurons which, upon sufficient stimulation of M1, indirectly reflect activity from the CST (Wasserman, 2002). Cortical stimulation affects axons running underneath the coil meaning an MEP represents firing of both inhibitory and excitatory signals of M1 but also to and from other structures in the brain that are connected to the motor cortex (Anderson et al., 2010; DiLazzaro and Rothwell, 2014; Rothwell, 1997; Seibner et al., 2022; Zeimann, 2020). MEP responses from TMS applied over M1 can be leveraged to evaluate how cortical responses are relayed to the associated muscles.

1.3 THE ORIGIN OF INDIRECT WAVES

Electrical stimulation of the CST in animals informs how responses from the cortex manifest at the level of the spinal cord. Patton and Amassian provided evidence through recordings from the CST in non-human primates and cats that stimulation of motor neurons resulted in discharges that consisted of an initial volley, termed a direct (D-) wave, which is linked to excitation of the corticospinal neurons, followed by subsequent indirect (I-) waves which arise from synaptic activation of corticospinal neurons from cortical interneurons (1954). These I-waves can be classified as early, termed I1, referring to the volley following the D-wave, and late, termed I2-I5, which occur regularly at intervals of 1-1.5ms (Shimazu et al., 2004). Animal studies using pharmacological and surgical techniques further parsed the origin of these CST responses. For instance, the D-wave persisted after cortical injury in the motor cortex and were a stable response independent to the state of the cortex in both cats and monkeys unlike the I-waves which varied based on state and integrity of the motor and sensory cortices in the animals (Patton and Amassian 1954). The D-wave also persisted after administering barbiturate anesthesia in the animals whereas I-waves did not (Patton and Amassian, 1954), lending support
to the cortical origins of I-waves. Additionally, using muscimol injections, a GABA-A receptor agonist, in M1 of non-human primates, late, but not early I-waves were abolished. This established that late I-wave discharges arise from connections within the cortex and revealed that late and early I-waves stem from different networks (Adrian and Moruzzi, 1939; Patton and Amassian, 1954; Shimazu et al., 2004).

In humans, studies using TMS and transcranial electrical stimulation reaffirmed these findings. Studies with electrodes placed in the epidural space of the spinal cord established that that after stimulation over M1, the output from the CST, a D-wave followed by volleys of I-wave activity, was similar to those seen in animal studies and had comparable origins (Di Lazzaro et al., 2001, 2004, 2012; Rothwell et al., 1994; Zeimann, 2020). Importantly, the response from a monophasic suprathreshold pulse of TMS is described from cervical spinal cord recordings. Using high intensity TMS with the coil orientation in a lateral-medial (LM) direction, pyramidal tract neuronal axons can be indirectly stimulated leading to the preferential recruitment of a D-wave. Changing the coil orientation to posterior-anterior (PA) direction preferentially recruits early I-waves with latency approximately 1 ms later than the D-wave which is linked to activation of excitatory monosynaptic cortical connections projecting to the pyramidal tract neurons in cortical layer V (Di Lazzaro et al, 2004; 2012; Di Lazzaro & Rothwell, 2014; Di Lazzaro and Zeimann, 2013; Hannah, 2020). Changing the coil direction so that it runs in an anterior to posterior (AP) direction over M1 resulted in volleys that have a longer latency than the PA induced response (Di Lazzaro et al., 2001, 2017) and preferentially recruits late I-waves which are representative of polysynaptic activation of the corticospinal neurons from cortical layer II/III and other cortical structures (Di Lazzaro & Zeimann, 2013). While early I-waves are easily modulated by changes in muscle activation state increasing cortical excitability and less so
by drugs inhibiting cortical excitability, late I-wave modulation is seen after both changes in cortical excitability. Together, this provides evidence that different interplays of cells orchestrate the production and modulation of I-waves (Di Lazzaro et al., 2018) and TMS allows for an insight into these different cortical circuits.

1.4 MODULATION OF I-WAVES

The interneuronal circuitry mediating late I-waves is thought to be primarily underpinned by GABA releasing neurons in the cortex, an important differentiator when comparing mechanisms behind early and late I-waves (Di Lazzaro et al., 2012). Paired pulse TMS where a subthreshold conditioning stimulus is delivered preceding a suprathreshold test stimulus can probe excitability of interneuronal circuits within M1, as in the case of measures such as short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI) (Hanajima et al., 1998; Kujirai et al., 1993; Di Lazzaro et al., 2012). The mechanisms of SICI and LICI are through the activation of inhibitory GABA-A and GABA-B receptor modulated projections respectively (Florian et al., 2008). This is reflected in the CST as a diminished I-wave response, with little effect on the early (Nakamura et al., 1997). Thus, the preferential recruitment of late I-wave can be attributed to the activation of these inhibitory connections in M1 that do not dictate recruitment of early I-waves (Di Lazzaro et al., 2008, 2012). Supporting this idea, administration of lorazepam, a positive allosteric modulator of GABA-A receptors, further suppressed late I-waves indicating that the polysynaptic circuit is mediated by GABA whereas the monosynaptic circuit that underpins early I-waves does not (Di Lazzaro et al., 2012; Kujirai et al., 1993).

Repetitive TMS (rTMS) paradigms also uniquely modulate the activity of I-waves. Following low frequency rTMS (<1 Hz), which is implicated in synaptic plasticity there is reduced excitability of late I-waves (Di Lazzaro et al., 2008a; Godfrey et al., 2021). Yet low frequency
rTMS has little effect on early I-waves (Di Lazzaro et al., 2010). Subthreshold rTMS in the form oftheta burst stimulation (TBS) has varying effects on I-wave modulation. Intermittent TBS (iTBS) typically excites the cortex and following this stimulation, the late I-wave response is heightened but no changes are observed in the cervical spinal recordings of the early I-wave. (Di Lazzaro et al., 2008b). In contrast, continuous TBS (cTBS) reduces cortical excitability. After cTBS only the early I-wave is attenuated and later I-waves are less affected. This may be explained by differences in which cells are targeted by the stimulation with cTBS dampening the direct excitatory inputs to corticospinal neurons without impact on inputs with more synaptic distance from the CST (Di Lazzaro et al., 2005). The differential response following iTBS and cTBS provide more evidence to the suggest that the volleys of responses observed from the CST stem from different groups of cells within the motor cortex.

1.5 MEP RESPONSES: INDEXING I-WAVES

Importantly, MEP responses from TMS follow the same pattern of onset times and variability patterns as the interneuronal circuits being stimulated. Outlined by Aberra et al., an integrated computational model showed that TMS delivered over the motor cortex in the AP direction produced MEP onset times 2-3 ms later than MEPs produced from PA oriented stimulation (2020). This shift in onset time is reflective of the origins of these responses where PA stimulation preferentially recruitscorticomotor neurons monosynaptically whereas AP stimulation preferentially recruits pre-motor pyramidal cells, the main projection neurons in the cortex (Takata et al., 2021). More rostral inputs to M1 may produce a slower response latency reflective of the polysynaptic pathway as compared to the monosynaptic path (Aberra et al., 2020, Hamada et al., 2013). MEP onset can index I-wave onset latency as the times from each
different direction is representative of the duration each activated group of cells takes to relay inputs to the CST (Figure 1.0).

Relating the onset time of each network relative to LM stimulation can give insight to the variability of I-wave recruitment. Stimulation of a muscle holding a mild contraction in healthy individuals showed that while PA-LM MEP onset times had relatively less variability between participants, AP-LM MEP onset times varied, in some participants more than 4 ms (Hamada et al., 2013, Volz et al., 2015). Shorter AP-LM onset times correlated with greater functional connectivity, indicative of less synaptic distance between premotor and motor regions (Volz et al., 2015).

Results from these studies provide context for consideration of individual cortical circuit variability when interpreting MEP onset values. MEP onset of AP-LM, depends on the variability in excitation of mono and polysynaptic pathways with greater values meaning more recruitment of cells connecting to the CST whereas smaller values may suggest more direct pathways, with fewer connecting cells. The PA-LM onset time was not correlated with functional connectivity, indicating less variability of cells underlying the early I-waves (Volz et al., 2015).

1.6 INTERNEURONAL CIRCUITS

Given the mutable properties of I-waves and their origins in the cortex, specifically in M1, it can be extrapolated that AP and PA-induced MEPs are susceptible to change through behaviour. Recent work investigating the impact of acute cycling exercise on the untrained upper limb through stimulation of these circuits found that while the PA circuit remained unchanged, the AP circuit showed increased excitability and reduced inhibition. This was unsurprising given the role of inhibitory neurotransmitters in modulating the early and late I-waves (Neva et al.,
Similarly, aging may also impact these circuits differentially. SICI is increased in older adults but only when measuring from the AP circuit, suggesting a reduced connection to other cortical elements recruited with AP stimulation that was associated with aging (Sale et al., 2016). Given the individualistic nature of the response from these networks with factors such as physical exercise and age influencing the physiological response, work investigating heterogeneity in responses to cTBS and iTBS found that differences in MEP latency from stimulation determined the likelihood of response and after-effect predictability as opposed to the intrinsic plasticity of the neurons themselves (Hamada et al., 2013). Longer AP-LM onset times, indicate a greater likelihood of preferential I-wave recruitment and are associated with a higher likelihood of exhibiting the expected direction of change in cortical excitability post iTBS and cTBS (Hamada et al., 2013). The underlying principle driving this body of work is that motor outputs can be considered an analog of network activation from different cell groups in the motor cortex; each with different communication pathways that can be accessed as different therapeutic targets.

Recent work found that excitability of the early but not late network was increased during motor imagery, perhaps reflecting the role played by interneurons within M1 in fine-tuning movement preparation and preventing unwarranted movement during mental representation (Neige et al., 2022). The study also showed an increase of inhibition of the PA mediated as compared to the AP circuit during motor imagery, corroborating inhibition of the early network and not late suggesting involvement of the pre-motor structures involved in the task. However, the task-dependent nature and preferential excitability of these networks may be crucial in changing motor behavior, yet the consequences for these networks after brain injury have yet to be explored.
The exact role of both the PA and AP network in M1 is still under investigation. Work from Hamada et al., have shown that each network may be involved in different forms of learning, with PA induced networks showing no dependency on cerebellar activity whereas the nature of AP inputs to premotor and cerebellar areas suggest the AP network is involved in model-based learning (2014). The cerebellar connections modulated by PA and AP networks differently when a simple motor sequence and a cognitively demanding skill task were compared; PA network responses were shown for both tasks whereas the AP network responses were only sensitive to the skilled task which was explained as involvement from pre-motor areas (Spampinato, 2020; Spampinato et al., 2020). Understanding the differing roles of these networks provides the understanding that I-wave responses are a representation of motor output from these different networks that may represent an individual’s ability to move and learn. Understanding the dynamic and interconnected nature of the neurons within and connected to M1 lead to conceptualizing I-waves, at least in part, as a marker of learning and movement.

By linking changes in the brain to changes in behaviour post stroke provide evidence for using TMS to characterize how M1 and its communication to upper limbs after stroke have changed. Involvement from pre-motor areas and surrounding inputs to M1 through alternate pathways are involved in the production of movement (Sandrini and Cohen, 2013). The dynamic nature of I-wave origins, the networks’ relationships to the CST, and the heterogenous nature of stroke recovery, interneuronal responses after stroke is an important metric to evaluate motor performance, one reflective of the range in behavioural implications owing to unique physiology.
Figure 1: Interneuronal activation through directional TMS and MEP outputs. A schematic showing the targets and outputs of directional TMS stimulation. Each coil orientation is colour coded to show what pathway in the brain is preferentially recruited, what the CST output is and what the origins are, and the motor output measurement from EMG.
2 STUDY AIM & HYPOTHESIS

This thesis examines how interneuronal activation in the motor cortex changes between hemispheres in individuals who have experienced a stroke and how this property relates to clinical motor function.

I-wave onset is a variable property, reflecting interneuronal pathways in M1. They can be indexed by directional TMS with MEP onset times elicited in the PA and AP onset times reflective of the monosynaptic and polysynaptic pathway in M1 to the CST respectively. These network onset times can be compared to MEP onset times elicited in a LM direction to represent early, PA-LM, and late, AP-LM, I-wave onset times (Di Lazzaro et al., 2012; Hamada et al., 2013; Volz et al., 2015).

Unique interneuron recruitment within the motor cortex is a well-documented response to directional TMS (Aberra et al., 2020; Hamada et al., 2013), and each network is sensitive to different motor tasks (Hamada et al., 2014). Probing these networks in a stroke-affected brain will give valuable insight into how injury and recovery impact neuronal network properties and how they communicate to the CST. Stroke recovery, which results in a wide spread of outcomes, is known to have functional and structural changes reflected in motor behaviour. To our knowledge, interneuronal activity, reflective of I-wave variability, has not been examined in the recovering stroke brain.

Given individual variability in MEP responses onset from directional TMS, the role of neurotransmitters in modulating these networks, and considering the large-scale physiological changes in the mechanisms behind relaying motor messages to the CST after stroke, studying
interneuronal activation in the motor cortex is an important goal to fully understand the mechanisms behind the recovering stroke brain.

Overall, this study takes an exploratory approach with the aim of characterizing how neurobiology, specifically interneuronal activation changes in the motor cortex after stroke and how it may impact upper limb motor function.

Given the novelty of the work, we hypothesized that there would be differences in I-wave onset, measured through MEP onset latency from M1 between hemispheres. Specifically, the AP-LM onset will be different in the contralesional hemisphere as compared to the ipsilesional hemisphere in the chronic stage of stroke, given the previously observed variability in onset times.

Additionally, we hypothesize that the greatest variance in measures of clinical upper limb motor function in the chronic stage of stroke will be explained by measures of MEP onset latency. AP-LM onset latency may be more explanatory of variability in behaviour as PA-LM onset is less variable between individuals.
3 METHODS

3.1 Participants

30 participants (8 females), at least six months post-stroke participated in the experiment. Written consent in accordance with the University of British Columbia Ethical Review Board was obtained prior to participation and individuals were screened for contraindication to TMS procedures (See Appendix A-C).

Participants were excluded if they experienced any head trauma or a diagnosis of epilepsy, any history of seizure, neurodegenerative or musculoskeletal disorders, pregnancy or a Fugl-Meyer Assessment for Upper Extremity (FMA-UE) score of less than 15, above mild on the Self-Reported Quick Inventory of Depressive Symptomology (QIDS-SR) or Beck’s Anxiety Index (BAI), or if they were less than six months post-stroke. Each participant completed two sessions: a TMS session to measure MEP onset times from both hemispheres as well as a clinical measurement session to measure FMA-UE, Wolf-Motor Function Test (WMFT), National Institute of Health Stroke Scale (NIHSS), Montreal Cognitive Assessment (MoCA), BAI, and the QIDS-SR (Figure 2.0). The experimental protocol was completed in the Brain Behaviour Lab at the University of British Columbia. If an MEP could not be elicited from the ipsilesional hemisphere, the participant was excluded from the current study. The contralesional hemisphere was used as a point of comparison for measurements from the ipsilesional hemisphere.

Of the total 30 participants who consented to the study, 21 (5 female, mean age 71.48 ± 11.15) showed an MEP on the ipsilesional hemisphere and were included in the analysis for experiment 1 (Table 1.0). One outlier was removed for experiment two through inter-quartile range outlier detection, and a reported muscular disorder confounding WMFT performance. The
participant sample size was based largely on feasibility and was estimated based on previous work measuring MEP onset with medium to large effect sizes where reported (Neige et al., 2022; Pisa et al., 2021; Volz et al., 2019).

Figure 2: **Summary of participant schedule and measurements.** Outline of study tasks for each day of visit. Order of visit days and time between visit days varied based on participant, physiotherapist, and research assistant availability.
<table>
<thead>
<tr>
<th>Participant</th>
<th>Age</th>
<th>Sex</th>
<th>MoCA</th>
<th>FM</th>
<th>WMFT Rate*</th>
<th>RMT&lt;sub&gt;LM&lt;/sup&gt;</th>
<th>RMT&lt;sub&gt;PA&lt;/sub&gt;</th>
<th>RMT&lt;sub&gt;AP&lt;/sub&gt;</th>
<th>LM Onset*</th>
<th>PA Onset*</th>
<th>AP Onset*</th>
<th># of trials*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ipsi Contra</td>
<td>Ipsi Contra</td>
<td>Ipsi Contra</td>
<td>Ipsi Contra</td>
<td>Ipsi Contra</td>
<td>Ipsi Contra</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>M</td>
<td>26</td>
<td>51</td>
<td>21.04</td>
<td>69 62 64 60</td>
<td>94 74</td>
<td>20.90 18.36</td>
<td>22.27</td>
<td>18.96</td>
<td>22.66</td>
<td>21.14 16 20</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>F</td>
<td>27</td>
<td>60</td>
<td>3.98</td>
<td>60 58 61 47</td>
<td>80 71</td>
<td>19.16 19.20</td>
<td>20.14</td>
<td>19.90</td>
<td>21.60</td>
<td>20.53 20 20</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>M</td>
<td>21</td>
<td>48</td>
<td>33.27</td>
<td>67 51 61 45</td>
<td>73 53</td>
<td>23.30 17.01</td>
<td>24.53</td>
<td>20.05</td>
<td>24.11</td>
<td>24.08 20 20</td>
</tr>
<tr>
<td>4</td>
<td>78</td>
<td>M</td>
<td>25</td>
<td>60</td>
<td>5.99</td>
<td>65 59 53 54</td>
<td>64 56</td>
<td>19.46 18.90</td>
<td>19.55</td>
<td>19.51</td>
<td>20.58</td>
<td>20.76 20 20</td>
</tr>
<tr>
<td>5</td>
<td>74</td>
<td>F</td>
<td>28</td>
<td>59</td>
<td>6.52</td>
<td>92 85 86 75</td>
<td>97 85</td>
<td>20.09 18.10</td>
<td>20.88</td>
<td>20.19</td>
<td>23.20</td>
<td>21.18 20 20</td>
</tr>
<tr>
<td>7</td>
<td>65</td>
<td>F</td>
<td>25</td>
<td>41</td>
<td>27.43</td>
<td>71 65 65 67</td>
<td>90 83</td>
<td>19.55 17.16</td>
<td>19.79</td>
<td>18.38</td>
<td>21.53</td>
<td>20.66 20 20</td>
</tr>
<tr>
<td>8</td>
<td>66</td>
<td>M</td>
<td>25</td>
<td>61</td>
<td>-2.41</td>
<td>58 59 58 53</td>
<td>69 66</td>
<td>17.41 17.25</td>
<td>20.04</td>
<td>18.75</td>
<td>19.89</td>
<td>18.66 20 20</td>
</tr>
<tr>
<td>9</td>
<td>72</td>
<td>M</td>
<td>25</td>
<td>50</td>
<td>33.40</td>
<td>83 65 64 63</td>
<td>66 68</td>
<td>20.76 17.60</td>
<td>21.79</td>
<td>19.23</td>
<td>23.78</td>
<td>24.36 20 20</td>
</tr>
<tr>
<td>10</td>
<td>64</td>
<td>M</td>
<td>26</td>
<td>44</td>
<td>40.39</td>
<td>65 48 60 48</td>
<td>72 51</td>
<td>20.40 20.36</td>
<td>25.15</td>
<td>22.29</td>
<td>24.26</td>
<td>23.65 20 20</td>
</tr>
<tr>
<td>11</td>
<td>75</td>
<td>M</td>
<td>24</td>
<td>39</td>
<td>20.79</td>
<td>37 49 38 40</td>
<td>50 53</td>
<td>21.13 17.38</td>
<td>23.94</td>
<td>20.8</td>
<td>24.20</td>
<td>21.83 20 20</td>
</tr>
<tr>
<td>12</td>
<td>85</td>
<td>F</td>
<td>30</td>
<td>61</td>
<td>8.11</td>
<td>47 66 44 44</td>
<td>58 58</td>
<td>17.28 15.01</td>
<td>19.76</td>
<td>17.70</td>
<td>22.55</td>
<td>20.67 20 20</td>
</tr>
<tr>
<td>13</td>
<td>77</td>
<td>M</td>
<td>26</td>
<td>40</td>
<td>4.95</td>
<td>52 75 41 59</td>
<td>49 77</td>
<td>22.05 20.91</td>
<td>24.13</td>
<td>20.95</td>
<td>27.34</td>
<td>23.71 19 20</td>
</tr>
<tr>
<td>14</td>
<td>83</td>
<td>M</td>
<td>27</td>
<td>61</td>
<td>17.15</td>
<td>65 51 55 55</td>
<td>67 62</td>
<td>18.22 20.21</td>
<td>19.85</td>
<td>20.26</td>
<td>23.66</td>
<td>22.29 20 20</td>
</tr>
<tr>
<td>15</td>
<td>78</td>
<td>M</td>
<td>28</td>
<td>65</td>
<td>4.034</td>
<td>52 54 49 50</td>
<td>68 54</td>
<td>22.23 19.69</td>
<td>22.08</td>
<td>22.08</td>
<td>22.87</td>
<td>25.86 19 20</td>
</tr>
<tr>
<td>16</td>
<td>67</td>
<td>M</td>
<td>29</td>
<td>51</td>
<td>19.92</td>
<td>63 41 60 44</td>
<td>71 56</td>
<td>25.41 18.98</td>
<td>21.71</td>
<td>27.21</td>
<td>26.20</td>
<td>24.46 20 20</td>
</tr>
<tr>
<td>17</td>
<td>81</td>
<td>M</td>
<td>26</td>
<td>60</td>
<td>5.67</td>
<td>81 46 72 40</td>
<td>77 58</td>
<td>24.88 17.63</td>
<td>19.78</td>
<td>25.77</td>
<td>25.18</td>
<td>20.20 15 20</td>
</tr>
<tr>
<td>18</td>
<td>86</td>
<td>M</td>
<td>25</td>
<td>62</td>
<td>1.83</td>
<td>51 40 45 39</td>
<td>56 57</td>
<td>20.88 18.65</td>
<td>23.08</td>
<td>20.80</td>
<td>23.03</td>
<td>21.50 18 20</td>
</tr>
<tr>
<td>19</td>
<td>81</td>
<td>M</td>
<td>30</td>
<td>57</td>
<td>8.23</td>
<td>60 46 51 38</td>
<td>78 48</td>
<td>20.65 16.30</td>
<td>20.30</td>
<td>19.20</td>
<td>21.20</td>
<td>20.55 20 20</td>
</tr>
<tr>
<td>20</td>
<td>59</td>
<td>F</td>
<td>28</td>
<td>58</td>
<td>2.12</td>
<td>60 50 56 54</td>
<td>67 59</td>
<td>18.64 17.19</td>
<td>19.75</td>
<td>17.75</td>
<td>21.26</td>
<td>19.09 17 20</td>
</tr>
<tr>
<td>21</td>
<td>59</td>
<td>M</td>
<td>28</td>
<td>53</td>
<td>3.03</td>
<td>37 37 34 33</td>
<td>38 44</td>
<td>21.39 18.94</td>
<td>22.97</td>
<td>22.21</td>
<td>25.15</td>
<td>23.54 19 20</td>
</tr>
</tbody>
</table>

Table 1: Participant characteristics and onset times

*Reported as a difference in rate between the non-paretic and paretic arm; # of movement/min
*Reported as % output on stimulator. Pulses were delivered at 110% RMT for AP/PA and 150% RMT for LM. Max output delivered if value exceeded 100%
*Reported in ms
*Number of accepted trials included in the onset time mean calculation. Trials were excluded if MEP did not exceed 0.05mV or if excessive noise was present
3.2 Experiment 1: Assessing MEP Onset times in a stroke affected brain

I-wave onset indexed by directional TMS induced MEPs was measured in both the ipsilesional and contralesional hemispheres to examine how interneuronal activation changes between hemispheres.

To facilitate stereotaxic navigation of the TMS coil for precise stimulation delivery, a high-resolution anatomical a T1-weighted anatomical magnetic resonance imaging (MRI) scan from the participant was used if available. In the case of two participants for whom a scan was unavailable, participants were calibrated to a Montreal Neurological Institute (MNI) template brain to allow accurate neuro-navigation. The images acquired in the session were imported to BrainSight (Rogue Research Inc., Montreal, CA), to register both the coil and participant location in stereotaxic space. Brainsight was used to ensure consistency in TMS application over the same target on the motor cortex within sessions. In order to convert the images into MNI space, the anterior and posterior commissures were identified in each individual’s MRI, and bounding boxes were set around the cortex. Single monophasic TMS pulses were delivered through the MagStim BiStim device through one unit with a D70mm Alpha Flat coil (MagStim, Oxford, UK).

3.2.1 Independent and Dependent Measures

The Extensor Carpi Radialis (ECR) motor hotspot was marked on Brainsight, defined as the stimulated site producing the strongest and most consistent response with MEPs greater than 0.05mV. Participants were seated in a semi-reclined chair, with armrests and feet planted on the ground or footrest. Resting motor threshold (RMT) was defined as the stimulator output at which five out of 10 trials yielded an MEP positive response above 0.05 mV measured through surface
EMG. 20 pulses at 110% RMT were applied in the PA and AP directions, and 20 pulses were applied at 150% RMT in the LM direction over M1 for both hemispheres. PA currents were produced by positioning the coil handle 45 degrees postero-laterally from the central sulcus over M1. AP currents were induced by rotating the coil 180° in the same place so that the coil handle was pointed antero-medially over M1 and LM currents were induced by holding the coil perpendicular to the longitudinal fissure over M1. All stimulation parameters were within published safety standards (Rossi et al., 2020). EMG data were collected at 4kHz through AD Instruments LabChart EMG and exported to MATLAB compatible format through LabChart (AD Instruments, Sydney, AU). Inspection was used to reject noisy trials and the MEP onset time in the remaining trials was analyzed offline using the TMS Analysis Toolbox (Cunningham et al., 2021). The average onset time was calculated for each stimulation direction. Each participant had at least 15 trials per direction, per hemisphere, included in mean calculations. Onset time average and stroke hemisphere information was used for statistical analysis.

Early and late I-wave onset time was indexed by subtracting LM from PA and AP current induced MEP onset times respectively. The difference in I-wave onset time was calculated as the ipsilesional hemisphere onset times subtracted from the contralesional hemisphere.

3.2.2 Data Analysis

MEP onset times were calculated using the TMS Analysis Toolbox (Cunningham et al., 2021), where an MEP was defined by the algorithm as activity exceeding 0.5 standard deviation of baseline activity, occurring at least 10ms after stimulation. Trials without an MEP exceeding 0.05mV were discarded. Trials for which the algorithm failed to recognize an MEP were corrected manually through visual inspection.
3.3 Experiment 2: Assessing M1 onset time and clinical motor function

The relationship between M1 TMS onset times and clinical motor scores in the upper motor extremities was examined. The mean PA-LM and AP-LM MEP onset times in both hemispheres from Experiment 1 was used.

3.3.1 Independent and Dependent Measures

A WMFT to quantify motor control in both limbs was administered by a physiotherapist. These tests are regularly performed in the Brain Behavior Lab for multiple tests and scheduled in accordance with participant and assessor availability. All timed items in the WMFT were averaged to calculate an overall rate measured by number of movements per minute (Hodics et al., 2012). The difference in WMFT for each participant was calculated as the overall rate in the impaired arm subtracted from the overall rate in the impaired arm.

3.4 Statistics

3.4.1 Experiment 1

A linear mixed effects regression (LMER) model was used to evaluate the relationship between stimulated hemisphere (ipsilesional, contralesional) and the onset time of each stimulated network (PA-LM, AP-LM). Prior to analysis, data was assessed for normality with Shapiro Wilk’s tests, which were insignificant, meaning no deviation from a normal distribution or homogeneity in variances. Given the violation of independence in observations and the nested structure, a LMER was chosen as it is robust against the assumptions made by parametric tests, and accounts for random variation due to individual participants, included as multiple intercepts which is prudent when considering heterogenous presentation of stroke and recovery from stroke.
The model included a fixed effect of an interaction term between hemisphere and stimulated network being PA-LM or AP-LM, predicting that onset time is likely going to differ based on the levels of these factors. The fixed effects model was compared using AIC values to models with random effects which included participant or participants with participants crossed with both repeated measures of hemisphere and stimulated network. Lower values indicate better fit, and the latter model was chosen between AIC = 306.75 and AIC = 296.60.

A significant interaction effect would indicate that the onset times from each inter-neuronal network are being influenced differently based on the hemisphere. If an interaction effect was present, a post-hoc pairwise comparison of the estimated marginal means was conducted to determine statistically significant differences between each combination of hemisphere and stimulated network.

3.4.2 Experiment 2

A linear regression model was used to test if the difference of PA-LM and AP-LM onset times, reflecting I-wave onset, between the contralesional and ipsilesional hemispheres explained the difference of WMFT rate between the non-paretic and paretic arm. Prior to analysis, data were assessed for normality with Shapiro Wilk’s tests, which gave a slight departure from normality (W = 0.88493, p-value = 0.022) however due to the need to assess the magnitude and strength of the relationship between onset time and motor control, with the considerably small deviation from normality, linear regression was chosen as the best statistical test (Schneider et al., 2010). Additionally, this test would inform consideration of onset time as a predictor of motor control, beyond establishing correlation. Significance would indicate that an increase in
onset time difference between hemispheres would be associated with an increase in the WMFT rate difference as hypothesized.

All statistical analyses were performed using R (Bates et al., 2015; R Core Team, 2021).
4 RESULTS

4.1 Experiment 1

The LMER results showed that there was a simple main effect of stimulated network on onset time ($\beta = 1.87$, CI = 1.29 - 2.45, $p<0.001$) as well as a simple main interaction effect of stimulated network on each hemisphere ($\beta = -0.84$, CI = -1.58 - -0.10, $p = 0.026$) (Figure 3.0). To further understand the main effect of stimulated network on each hemisphere, estimated marginal means were calculated. The AP-LM onset time was significantly greater than the PA-LM onset times in the contralesional ($\mu = -1.87 \pm 0.29$, $p<0.0001$) and ipsilesional hemisphere ($\mu = -1.03 \pm 0.29$, $p = 0.0053$). Importantly, the AP-LM onset was significantly greater in the contralesional hemisphere as compared to the ipsilesional hemisphere ($\mu = -1.18 \pm 0.43$, $p<0.045$). The marginal means analysis allowed testing for significantly different onset times for each hemisphere and stimulation parameter. AP-LM means being longer than PA-LM means have been documented (Aberra et al., 2020; Sakai et al., 1997, Hamada et al., 2013) therefore replication of this pattern lends support to stimulation parameters and methods having targeted separate interneuronal networks.
Figure 3: Comparison of network onset time between hemispheres. Results from experiment 1 showing mean onset time from preferential stimulation of each network in each hemisphere, calculated as AP-LM and PA-LM. Individual points represent individual data.

4.2 Experiment 2

Results from the linear regression analysis showed that difference in preferential activation of early network onset time, measured as the LM MEP subtracted from the PA MEP onset time, between the ipsilesional and contralesional hemispheres, did not explain the variance
between WMFT rates in the nonparetic arm as compared to the paretic arm \(F(1,18) = 0.37, p=0.55\) (Figure 4.0).

Figure 4: **Relationship between PA-LM onset time and WMFT performance.** Results from experiment 2 showing difference PA-LM MEP onset times in the ipsilesional hemisphere subtracted from PA-LM MEP onset times in the contralesional hemisphere, indexing preferential activation of early I-waves onset, against performance on the WMFT measured as the difference between non-paretic and paretic arms.

Results from another linear regression, analyzing the preferential activation of late network onset, average LM MEP subtracted from the average AP MEP, showed that the 21% of the variance in the difference between WMFT rates between non-paretic and paretic arms is
explained by the difference in AP-LM onset time between the ipsilesional and contralateral hemispheres ($F(1,18) = 4.74, p = 0.043$) (Figure 5). Results were significant, indicating that for every 1.96 increase in AP-LM difference between hemispheres, there is a one unit increase in the WMFT rate difference between arms.

Figure 5: **Relationship between AP-LM MEP onset time and WMFT performance.** Results from experiment 2 showing differences in AP-LM MEP onset times in the ipsilesional hemisphere subtracted from AP-LM MEP onset times in the contralateral hemisphere, indexing preferential activation of late I-waves onset, against performance on the WMFT measured as the difference between non-paretic and paretic arms.
5 DISCUSSION

Indexing I-waves through directional TMS elicited MEPs is a non-invasive method to assess the influence of different interneuronal circuits on CST output (Di Lazzaro et al., 2012). Past work established that the differences in AP-LM MEP onset time is longer and more variable as compared to the difference in PA-LM MEP onset times; these differences are inferred as representative of unique interneuronal connections between M1 and nearby cortical structures (Hamada et al., 2013, Volz et al., 2014). In this thesis, this property was explored in the stroke brain, which was previously unexamined. How the onset time of AP-LM and PA-LM MEPs may change after stroke between hemispheres is important to understand as these differences likely indicate the time it takes for interneuronal circuits to activate cortical spinal neurons from the motor cortex. The relationship between the AP-LM and PA-LM MEPs and motor function after stroke was also explored. The results indicate that AP-LM MEP onset time may be an important marker that is altered in the ipsilesional hemisphere by stroke and that the changes in onset time are related to motor function. Future work that employs a longitudinal design will be required to confirm this idea. In summary, this work points to two important discoveries regarding the activation of interneuronal circuits after stroke and are discussed in detail below.

5.1 EXPERIMENT 1: AP-LM MEP ONSET CHANGES BETWEEN HEMISPHERES AFTER STROKE

The results show that when comparing onset times between hemispheres, AP-LM MEP onset time is longer than PA-LM MEP onset time within each hemisphere regardless of stroke. Further, AP-LM MEP onset time was significantly shorter in the ipsilesional hemisphere. Results
lend support to the hypothesis that interneuronal network activation of the CST is altered in the ipsilesional hemisphere as compared to the contralesional hemisphere.

The longer AP-LM MEP onset time affirms that we were successful in preferentially activating a separate network than those producing PA-LM MEPs. These results align with the prediction that differences would be seen in the AP-LM onset time given its variability between individuals (Hamada et al. 2013). The same pattern is not observed in PA-LM onset times. Previous work found that the PA-LM onset times are unchanged after modulation of cortex excitability through iTBS (Volz et al., 2019). The PA-LM onset times observed in the current study suggest little change to the interneuronal, monosynaptic recruitment of early I-waves is seen after stroke. Previous work characterizing PA-LM onset found that there was little variability observed between individuals (Volz et al., 2014). The ability to evoke an MEP illustrates integrity of this pathway to the CST neurons, meaning that assessing interneuronal networks through directional TMS from individuals with severe motor impairment after stroke may not be possible non-invasively (Boyd et al., 2017; Hordacre et al., 2021). Thus, the PA-LM onset times that were observed in the current study suggest little change to preferential early I-wave recruitment in this sample.

AP-LM onset times are attributed to the distance between cortical structures outside of the motor cortex, and CST neurons (Opie et al., 2022; Opie and Semmler., 2021, Zeimann et al., 2020). The homogeneity in the pattern of AP-LM onset being greater than PA-LM in both hemispheres suggests that connections within and from outside the motor cortex intact after stroke and both groups of neurons continue to relay motor messages from the cortex to the corticospinal neurons. Given an MEP positive response in the lesioned hemisphere signals CST integrity after stroke (Hordacre et al., 2021), longer AP-LM MEP onset times confirm the
proposed mechanism of activation of corticospinal neurons through activation of pyramidal neurons projecting from the outer layers of the cortex (Di Lazzaro & Zeimann, 2013).

Interestingly, AP-LM MEP latency was shorter in the ipsilesional hemisphere as compared to the contralesional side. This difference could be attributed to the changes in interneuronal excitability after stroke, given that the pathway can be activated in both hemispheres. Though not explored in a stroke brain before, previous work assessing PA-LM and AP-LM onset times associated with a grasping task found increased onset times in individuals with spinal cord injury as compared to healthy controls. The finding suggests an increase in excitability after injury for both neuronal populations, but specifically the AP network had longer onset times after power grip as compared to the precision grip (Jo and Perez, 2019). Important to note is there was a significant change in the excitability of these networks after an injury impacting motor function, akin to the results observed in the current study. The differences between the results of the current study in individuals with stroke and past work testing MEP onset time in individuals with spinal cord injury may be attributed to differences in population and method. After stroke the mechanisms generating I-waves are more likely to impacted by damage to the cortex. Comparison of results between the current study and the work by Jo and Perez (2019) speak to the susceptibility of AP-LM onset times to change depending on injury state. The current study tested interneuronal networks at rest, whereas the use of a grip task was used to engage the networks before stimulation in individuals with spinal cord injury which has been shown to influence MEP onset latency (Rothwell et al., 1997). Interestingly, the AP-LM MEP onset was different for different types of grip tasks (Jo and Perez, 2019), giving more support for this interneuronal activation showing specificity to different motor tasks after injury, in this case, at a point downstream of the cortex. Nonetheless, AP-LM and PA-LM MEPs had
similar characteristics in both injury groups. While the mechanisms generating these MEPs may be similar, the results of this study suggest a change within interneuronal circuits are implicated in generating the MEPs after stroke.

Changes in AP-LM MEP onset time after stroke could also be related to inputs from other structures to M1. When considering the inputs from pre-motor areas, Volz et al. proposed a parallel model where AP circuits not only had multiple synaptic connections with the CST but also the presence of some direct pathways, similar to the monosynaptic PA pathway (2015) (Figure 6.0). This was shown with shorter AP-LM onset times correlating to higher functional connectivity, which provides evidence of shorter pathways within the motor cortex to pre-motor areas (Volz et al., 2015). Individuals with stronger connections to pre-motor areas may show shorter AP-LM onset times because of unique neurobiology (Volz et al., 2015). This connection would result in an earlier onset of the late I-wave and may not be distinguishable on the level of the MEP, introducing variances in onset differences between individuals and between hemispheres. Other data continue to support late I-waves being recruited through multiple connections to M1. For example, measuring MEP onset time from each hemisphere while participants hold a voluntary contraction in the muscle being stimulated results in AP-LM onset latencies that differ from PA-LM and indicates a polysynaptic pathway is recruited. As functional connectivity and the modulation of interneurons changes in the cortex after stroke (DeFilipe et al., 2013; Li et al., 2014) separating the components of the I-wave response may be an important step in understanding how networks communicate within M1 after stroke.
Fig 6: **Proposed alternate AP pathway.** Schematic outlining alternative direct connections to the M1 from pre-motor areas that are not reflective of a multi synaptic pathway, with shorter distances to travel.

### 5.2 EXPERIMENT 2: AP-LM MEP ONSET EXPLAINS VARIANCE IN MOTOR FUNCTION AFTER STROKE

Given that AP and PA circuits have been implicated in different forms of learning and motor action (Hamada et al., 2014), the current results provide evidence for consideration of these network responses as potential markers of motor function after stroke. The change in PA-LM MEP onset times between hemispheres did not explain a significant amount of variance in motor function differences between the upper limbs. The difference in AP-LM MEP onset time between hemispheres, however, did significantly explain variance in motor function between hemispheres.
The difference of the PA-LM MEP onset between hemispheres is not related to motor function, which given the previous results in experiment 1 was not surprising as PA-LM MEP onset did not differ between the two hemispheres. Together, the similarity in PA-LM onset times and the change in WMFT scores between arms indicates that the PA circuit, which has been thought of as a facilitator for model free learning and may participate in reduction of erroneous movements that occurs early in learning (Hamada et al., 2014; Spampinato et al., 2020). These data suggest that PA-LM onset times is not a potential marker for identifying variance in function after stroke.

AP-LM MEP onset time did explain a significant proportion of the variance in motor function, which aligns with the hypothesis. This result supports investigation of late I-wave recruitment as potential marker of motor function after stroke, especially given its role in the past as a predictor of response to rTMS (Hamada et al., 2013), a paradigm through which motor behaviour can be changed. Previous findings linked AP-LM onset time to hand dexterity in individuals with progressive multiple sclerosis, supporting the idea that in time-based tasks assessing motor function, is dependent on the integrity of the polysynaptic intracortical pathway (Pisa et al., 2020). The AP network has been shown to optimize cognitive load in learning more complex tasks (Spampinato et al., 2020), which speaks to the network’s connectivity to other structures namely the cerebellum and the pre-motor cortex (Spampinato, 2020). Specifically, preferential activation of the polysynaptic, interneuronal, AP circuit at rest elicits responses from cells involved in both inhibitory and excitatory communication (Zeimann et al., 1998). TMS paradigms such as SICI and LICI exploring the inhibitive inputs to the motor cortex have found dysregulation of the excitatory and inhibitory inputs after stroke. The CST output of these measures reflect changes activation in interneuronal circuits, and thus changes in recruitment of
excitatory and inhibitory inputs from ipsilesional hemisphere would likely change MEP characteristics (McDonell and Stinear, 2017; Opie and Semmler, 2021).

The results of the current study suggest that the reduction in AP-LM onset differences between hemispheres, with larger difference values indicating that the ipsilesional hemisphere has an earlier onset time, could be a marker of dysfunction in the efficacy of communication of intracortical circuits in and to M1. A recent review by Paperella et al., found evidence of GABA as a marker of motor function and recovery after stroke (2023), specifically linking lower levels of GABA transmission to better function. This in combination with increased inhibition in the chronic stage after stroke (Joy et al., 2021; Motahariania et al., 2021; Xu et al., 2019) suggests that interneuronal network disruption impacts the circuits connecting M1 to other motor structures. Using paired pulse TMS investigation into the effects GABA could be a future avenue of investigation given that several reviews have documented lower levels of intracortical inhibition in M1 in connection to motor function (Blicher et al., 2015; McDonald and Stinear, 2017). While more research on interneuronal network dysfunction after stroke is required to provide concrete mechanistic evidence, our results suggest that the changes in activation of the late network post stroke are related to paretic arm function.

5.3 LIMITATIONS

One limitation and caution in interpreting these results lies in CST output of the polysynaptic network being measured from stimulation strength calculated from RMT. Previous work has found that MEP onset latency is significantly longer between participants, varying ~4 ms but decreased when the strength of stimulation is increased, during stimulation of a muscle at rest as compared to an active muscle (Day et al., 1987, Rothwell et al., 1997). This discrepancy
in onset time is attributed to the time for excitatory post synaptic potential (EPSP) summation to occur to elicit a response from the motor neuron. If the initial EPSP is not of sufficient size then the temporal summation of multiple EPSPs from interneuronal pools result in the motor neuron firing and the subsequent CST activation (Rothwell et al., 1997). This results in both the shift in latency and an increase in stimulation output necessary to activate the corticomotor neurons at rest from AP stimulation (Rothwell et al., 1997). RMT was chosen due to feasibility in the stroke-impaired population for this study meaning that reported onset times are not a direct indicator of I-wave onset per se rather, an analog to capture likelihood of successful recruitment, (i.e. a longer AP-LM MEP onset time would indicate the better likelihood of the participant being able to recruit a late I-wave). It is important to acknowledge that the MEP output may be contaminated by other network inputs, however, the results suggest that preferential activation of each circuit was successful. Owing to the use of a within subject design and re-thresholding RMT for each coil direction, variability in interpretation of the MEP was reduced but the inclusion of a variable EPSP value addition to latency must be considered. Using AMT rather than RMT in future iterations of the work in this population is likely to amplify the variance in AP-LM onset time and better represent late I-wave onset time since measures will be taken in an active system. This will reduce the time taken for stimulation to recruit the intended target, and will lower the amount of stimulation delivered, allowing more focal stimulation of M1 (Ngomo et al., 2012).

The current study adopted an explorative approach to understanding the viability of investigating movement after stroke through the lens of TMS and as such did not control for multiple factors that may influence MEP onset times and inputs to each neuronal network. First and foremost, stroke lesion was not accounted for in this iteration of the study. Stroke location
and severity present different paretic phenotypes in upper motor limb recovery (Shelton and Reding, 2001) suggesting that structures connected to M1 and excitability patterns in the cortex may vary, undoubtedly influencing I-wave onset. However, the motor severity of individuals in the study was relatively similar, as this factor is related to lesion location we indirectly controlled for this factor. Severity of motor impairment and lesion location do limit the applicability of directional TMS, as individuals with substantial damage to the CST will not show a response to stimulation, making MEP analysis impossible (Smith and Stinear., 2016).

An important consideration in interpreting the results of the current study is understanding the background of the participants themselves. Evidence of increased connectivity between premotor, motor and supplementary motor areas was seen after physical therapy and mental practice (Bajaj et al., 2015). Physical therapy post stroke and exercise levels were not measured in the current study; however, exercise can influence the activity of AP and PA networks (Neva et al., 2021). Additionally, cortical responses are altered with age with previous work showing that the temporal characteristics of AP-LM onset are delayed in older populations (Opie et al., 2018). Given the relatively large range of participant age in the current study, interpretation of onset values may not be generalizable to all stroke populations, and more work is required to better understand interactions between excitability, age and CST output.
6 CONCLUSION

Motor action is contingent on effective communication between circuits and this thesis suggests that stroke interrupts this process. Preferential recruitment of AP and PA interneuronal networks through directional TMS, reflective of I-wave onset, found that AP-LM MEP onset times were changed after stroke and could be a marker of motor function in stroke. Previously, AP-LM MEP onset time has been linked to predictions of responses after rTMS (Hamada et al., 2013) and given the recent work using rTMS as an intervention in stroke research (Dioniso et al., 2018; Fisicaro et al., 2019) finding markers to characterize the response in individuals based on neurobiology of the recovering stroke brain is a high priority. Future work should consider continuing to contribute to building profiles of recovering stroke brains across time post stroke to examine how properties such as network onset time change and are influenced by factors such as age, sex, exercise training, and novel stimulation techniques such as rTMS. These data will be crucial to determine whether the characterization of interneuronal pools may be biomarkers of motor function recovery following stroke.
REFERENCES


Holodinsky, Jessalyn K., Patrice Lindsay, Amy Y. X. Yu, Aravind Ganesh, Raed A. Joudi, and Michael D. Hill. “Estimating the Number of Hospital or Emergency Department Presentations

42


Rossi, Simone, Andrea Antal, Sven Bestmann, Marom Bikson, Carmen Brewer, Jürgen Brockmöller, Linda L. Carpenter, et al. “Safety and Recommendations for TMS Use in Healthy Subjects and


https://doi.org/10.1016/s0165-0270(97)02242-5.


https://doi.org/10.1093/brain/110.5.1173.


https://doi.org/10.1007/BF02454139.


[https://doi.org/10.1002/ana.25452](https://doi.org/10.1002/ana.25452).


APPENDIX A: Consent Form

PARTICIPANT INFORMATION

Title of Study: Exploring I-Waves: A Path to Personalized Stroke Recovery

Principal Investigator: Lara Boyd, PT PhD, Professor, Department of Physical Therapy, Brain Behaviour Laboratory, Faculty of Medicine, University of British Columbia

Co-Investigators: Anjana Rajendran, Department of Physical Therapy, Graduate Program in Neuroscience, Brain Behaviour Laboratory, Faculty of Medicine, University of British Columbia

Study team members: Jordan Brocato, Research Coordinator, Brain Behaviour Lab, Faculty of Medicine, University of British Columbia

Cristina Rubino, MSc, Department of Physical Therapy, Brain Behaviour Lab, Faculty of Medicine, University of British Columbia.

Ronan Denyer, BA, Department of Physical Therapy, Brain Behaviour Lab, Faculty of Medicine, University of British Columbia.

Julia Dahlby, PT, Department of Physical Therapy, Brain Behaviour Lab, Faculty of Medicine, University of British Columbia.

Beverley Larssen, PT MSc, Department of Physical Therapy, Brain Behaviour Lab, Faculty of Medicine, University of British Columbia.

Shie Rinat, PT MSc, Department of Physical Therapy, Brain Behaviour Lab, Faculty of Medicine, University of British Columbia.

You are being invited to take part in this research study because you have experienced a stroke and are between the ages of 30 and 90 years. We will be testing 40 individuals with stroke for this study. The results of this study will provide information for the advancement of stroke rehabilitation strategies that may improve quality of life for Canadians with stroke.

Participation is Voluntary: You do not have to participate in this research study. It is important that before you make a decision to participate, you read the rest of this form. Please read the following form carefully and ask questions if anything is not clear. This consent form will tell you about the study, why the research is being done, what will happen during the study and the possible risks, benefits, and discomforts.

If you wish to participate, you will be asked to sign this form. If you do decide that you would like to participate, you are still free to withdraw from the study at any time and without giving any reasons for your decision. If you do not wish to participate, you do not have to provide any
reason for the decision nor will you lose the benefit of any medical care to which you are entitled or presently receiving.

Please take time to read the following information carefully and to discuss it with your family, friends and doctor before you decide.

Background
The number of people impacted by the aftereffects of stroke is increasing. However, there have not been changes in rehabilitation programs to address the needs of these individuals. We have not been able to customize these programs to address individual concerns. This means that there are more people living with a disability after a stroke. There is an urgent need for better understanding of how an individual’s brain changes after stroke and how it impacts movement of the body, known as motor function.

Brain stimulation can be a useful tool in assessing how well the brain can communicate to your muscles and by measuring how fast muscle activity begins after stimulation is given can be used to create a potential biomarker for predicting motor function after stroke. Biomarkers are measurements of brain function that can be taken in humans and are useful in making predictions about which therapy may best help a patient. For example, body temperature is a biomarker of fever.

Purpose
In the proposed work we will build individual profiles of brain excitability in stroke survivors. To understand how excitability changes after stroke we will assess two groups: one in the late subacute (3-6 months) and the other in the chronic phase post stroke (greater than or equal to months). With this data, we can advance our understanding of stroke recovery biomarkers.

This research is urgently needed to rapidly advance recovery of function after stroke. In sum, this work will provide a stepping stone for future exploration of biomarkers for motor function after stroke. Ultimately this will allow for tailored rehabilitation based off an individual’s brain’s intrinsic properties and increase our knowledge of neurobiology.

Who Can Participate in this Study?
You have been identified because you have had a stroke within the last three to six months or experienced a stroke 6 or more months ago and are between the ages of 30 and 90. If you agree to take part in the study, Dr. Boyd or her associates will determine if you have any condition that will prevent you from being in the study. This will be completed through screening your medical chart and through a brief interview. Screening should take no more than 15 minutes.

If you are pregnant, claustrophobic (have a fear of enclosed or narrow spaces) or if you do not meet the MRI or brain stimulation criteria you will not be able to participate.

Who Should Not Participate in this Study?
You should not participate in this study if you have another neurological condition such as history of head trauma, major psychiatric diagnosis, neurodegenerative disorder or substance abuse. If you are younger than 30 or older than 90 you should not participate in this study.

**What does the study involve (Procedures)?**

If you are eligible and decide to participate in this study, you will undergo assessment sessions at two time-points: your first visit which can be anytime post 3-months stroke, and a second visit shortly after depending on your schedule. On the first visit you will complete a magnetic resonance imaging (MRI) scan and a transcranial magnetic stimulation (TMS) safety screening. On the second visit you will complete a battery of clinical assessments and an evaluation using TMS. The total time to participate will be approximately 4 hours.

**Figure 1: Timeline from stroke to completion of study.**

Each assessment session will follow a consistent format.

You will first undergo a **3T Magnetic Resonance Imaging** (MRI) scan at the University of British Columbia. The MRI will last about one hour. The MRI uses a large magnetic field to non-invasively take pictures of your brain.

On a separate day you will undergo a **clinical assessment** that will take no longer than one hour. During this time, you will complete standardized questionnaires and tests of arm and leg function, and your cognition.

- Questionnaires will be used to determine your perceived level of impairment, activity limitations, and participation restrictions.
- Tests of your arm will require you to attempt simple tasks such as moving your arm or hand, or picking up a cup or holding a pen.
- Tests of non-motor impairments will require you to answer questions that relate to your level of fatigue, cognition, memory and comprehension.
- We will also collect information about you that includes your age at time of stroke, the date of your stroke, current medication list and ask you about the inclusion/exclusion criteria.

You will also undergo a **transcranial magnetic stimulation assessment session** at the University of British Columbia. To some individuals, transcranial magnetic stimulation feels like light tapping on the head. We use electromyography (EMG) to measure the activity of a target.
muscle. EMG uses electrodes that can sense the electrical activity of your muscle when placed on the skin. We will place these electrodes on your forearm to measure the activity of the muscle when we deliver stimulation at various locations over your skull. We will continue to deliver stimulations until we find the hotspot, or area that achieves the greatest response in the target muscle (recorded via EMG). The location will be recorded and monitored in real-time using a monitor and the images from your MRI. All of our stimulation parameters fall within published safety standards and have previously been used in the Brain Behaviour lab. This session will last about 1 hour and will be completed post your MRI scan.

Additionally, you will be doing selected motor tasks to better characterize your upper motor function on the lab’s Kinarm robot. You will do tasks involving your both your arms to control a frictionless lever to complete tasks such as a visually guided reach, moving your hand to projected targets, object hit, moving your hand to hit projected balls as they move from the top to the bottom of the screen, and ball on bar, where you use both arms to manipulate a projected bar with a ball in the centre of it to the position of another projected target. This session will last about 30 minutes.

Figure 2. The Kinarm robot.

In sum, this study involves 2 visits comprised of three evaluations sessions and will take between 3 to 4 hours.

What Are Possible Harms and Side-Effects of Participation
There are some potential discomforts and risks to your health and well being if you agree to be a subject in this research. These procedures will be conducted according to published safety standards by Dr. Boyd or her associates who have completed procedural and safety training. Dr. Boyd or her associates have discussed this research with you and have described them as follows:

**MRI:** There is very little known risk associated with undergoing an MRI scan. MRI is used routinely in hospitals around the world. A small number of people may find lying still inside the MR scanner uncomfortable and stressful. If this occurs then you will be brought out of the scanner and the scan stopped. Some people are also uncomfortable being in small places (i.e.,
claustrophobia). Because the MRI scanner is a small space you may also be uncomfortable lying inside it. If you do feel this way you will be brought out of the scanner and the scan will be halted. The MRI also makes loud noises that you may find uncomfortable but we will use ear plugs to protect your hearing. You will not be able to participate in the MRI component of this study if you have any metal or surgical implants that may be affected by the strong magnetic fields used in the MRI process or may cause tissue damage associated with dislodging the metal and/or for the objects to become heated during the scan and cause a burn.

You **CANNOT** participate if you have any of the following:

- Retained wires from an electronic implant that has been removed (i.e. pacemaker wires not attached to a pacemaker)
- Cardiac pacemaker or defibrillator
- Metal in eye or orbit
- Ferromagnetic aneurysm clip
- Pregnancy
- Makeup tattoos that are not designed to fade over time
- Stainless steel intrauterine device (IUD)

Depending on the individual situation, you **MAY NOT** be able to participate if you have/had any of the following:

- Artificial heart valve
- Ear or eye implant
- Brain aneurysm clip
- Implanted electronic device (i.e. drug infusion pump, electrical stimulator)
- Coil, catheter, or filter in any blood vessel
- Orthopedic hardware (artificial joint, plate, screw, rod)
- Shrapnel, bullets, or other metal fragments
- Surgery, medical procedure or tattoos (including tattooed eyeliner) in the last six weeks
- Other metallic prostheses

If you have any of the above or if there are possible safety issues that arise during the MRI screening process, your individual case will be reviewed by the UBC Hospital MR Technologist and/or Radiologist. A decision will be made regarding your participation in the study. In many cases, an operative report may be required to assess the nature of the implants in your body.

**Incidental Findings:** As this will **NOT** be a medically indicated examination, there will be no formal review of the scans and no report will be made. The MRI scan being done is designed to answer research questions, not examine you medically. This MRI scan is not a substitute for one a doctor would order. It may not show problems that would be picked up by a medical MRI scan. However, if we believe that we have found a medical problem in your MRI scan, we will ask a doctor who is trained in the reading of MRI scans, a radiologist, to help us review the images. If the radiologist thinks that there may be an abnormality in your MRI scan that requires follow-up, we will contact you and with your permission, contact your family physician and help
him or her obtain the appropriate follow-up for you. No information generated in this study will become part of your permanent medical record. However, if the study detects an abnormality in your MRI scan and further follow-up is required, then this information may become part of your record.

Please provide:

Your full name:_________________________  Phone number:__________________
Family Doctor/Medical Clinic:_____________________________

Transcranial magnetic stimulation: Safety standards for the application of TMS have been developed and will be followed by trained operators during this study to minimize the risk. In accordance with these standards, the TMS machine will always be run at a rate and a frequency that are known to be safe.

A member of the study team has discussed this research with you and has described the risks as follows:

- There is a potential risk of provoking a seizure in people with a history of seizures (e.g. epilepsy), you will not be eligible to participate in this study if you have such history. The risk of seizure induction is less than 1% in individuals without epilepsy.
- There is a risk of headache, scalp pain, toothache or scalp numbness associated with TMS. Each of these side effects are transient (ie., does not last).
- The clicks associated with TMS are loud and could potentially damage your hearing. To minimize this risk, you will be asked to wear earplugs throughout the testing session.

For electromyography of the arm muscle during transcranial magnetic stimulation:
You may get a skin rash under the sensors. A rash occurs in less than 5% of people. Rashes usually clear up within 2-3 hours of removing the sensors. No harm is expected to come of these pulses, or have been reported.

There may be other risks that have not yet been identified, and unexpected side effects that have not been previously observed may occur.

Arm Assessment Tests: There are no known risks associated with the assessment tests. However, you may become tired during these tests. In this case, please inform the researchers and you will be able to take a rest. You might also become anxious if you are having difficulty performing the tasks. If you wish, you can tell the researchers that you are uncomfortable at any time and they will stop the testing.

Kinarm Robot:
There are no known risks associated with performing tasks on the Kinarm robot. If at any point you feel uncomfortable you can tell the research team and they will stop the testing. Additionally, if you feel fatigued or require a break for any reason please tell the researchers and it will be provided.
Non-motor impairment tests: There are no known risks associated with the assessment tests. However, you may become tired during these tests. In this case you can ask the researchers and you will be able to take a rest. You might also become anxious if you are having difficulty performing the tasks. If you wish, you can tell the researchers that you are uncomfortable at any time and they will stop the testing.

There may be other risks that have not yet been identified, and unexpected side effects that have not been previously observed may occur.

In this research study we will be asking you questions about sensitive topics. As researchers we do not provide mental health services. However, if you talk about harming yourself or tell the research staff that you are thinking about self-harm or if you answer “Yes” to questions about having suicidal thoughts, we may give you contact information or resources for places you can call for help, or help you to call your doctor, a relative or therapist. The researcher may also help you to get to a medical facility for your safety.

What are the Benefits to You of Participating in the Study?
There is direct no benefit to you for participating in this study. It is hoped that additional information gained in this research study may be useful in the treatment of other patients with brain damage. You will be informed if any significant new findings develop during the course of the study that may affect your willingness to participate in this study.

Payments to Subjects
You will receive $35 for each clinic visit for giving your time and to offset your parking and/or travel expenses incurred to participate in this study.

In the Event of an Injury
In the event you experience a serious side effect during this study during normal business hours, you should report to an emergency room. If it is after 5:00 p.m., a holiday or weekend, you should report to an emergency room. By signing this form, you do not give up any of your legal rights and you do not release the researchers, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan. In case of a serious medical event resulting from this study, please report to an emergency room and inform them that you are participating in a research study and Lara Boyd (Principal Investigator) can be contacted for further information.

Confidentiality
Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of a designate of the investigator and by representatives of the UBC Clinical Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.
You will be assigned a unique study number as a subject in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc. Only this number will be used on any research-related information collected about you during the course of this study, so that your identity as a subject in this study. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your de-identified research data may be published or deposited into a publicly accessible location at the time of publication. This enhances the transparency of the research, but also allows others to access the data. This should not increase risks to you, but it does mean that other researchers may analyze the data for different reasons other than those described in this consent form. Once data is made publicly available, you will not be able to withdraw your data. The extent of the risk of you being identified through public data is unknown, but currently appears to be low.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected and also give you the right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

**Withdrawing from Study**

If you are not able to follow the requirements of the study or for any other reason, the study investigators may withdraw you from the study. On receiving new information about the study, the investigators might consider it to be in your best interests to withdraw you from the study without your consent if they judge that it would be better for your health.

You do not have to provide any reason for your withdrawal if you do not wish to do so. If you want to cancel permission to use your information, you should either verbally indicate your withdrawal or send a request to Dr. Boyd. If you cancel permission to use your information, you will be withdrawn from the study. The research team will stop collecting any additional information about you. The research team may use information that was gathered before they received your cancellation unless you specifically request withdrawal of your data, this request will be respected when possible.

**Questions**

You have read the information in this form. Dr. Boyd or their associates have answered your question(s) to your satisfaction. You know if you have any more questions after signing this you may contact Dr. Boyd or one of her associates.

If you have any concerns or complaints about your rights as a research subject, you may call the Research Participant Complaint Line at the University of British Columbia Office of Research Ethics at 604-822-8598 or toll free at 1-877-822-8598. Their email is RSIL@ors.ubc.ca. Please reference the study number (H22-01720) when calling so the Complaint Line staff can better assist you.
Future studies: You may be invited to take part in future studies. If Dr. Boyd thinks you might qualify for another study by her or her colleagues, she will contact you directly by mail or telephone and ask if you are interested. If you choose not to take part in future studies you should tell her. There will be no impact on you if you choose not to take part. You are not giving permission to do any future studies in this consent form.

Are you willing to be contacted in the future about participation in other studies?
_____ YES  _____ NO
PARTICIPANT CONSENT
Title: “Exploring I-waves: A Path to Personalized Stroke Recovery”

Dr. Boyd (or her associates) have given you information about this research study. They have explained what will be done and how long it will take. They explained any inconvenience, discomfort or risks that may be experienced during this study.

My signature on this consent form means:

- I have read and understood the subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the results will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me.

I freely and voluntarily consent to participate in this research study.

I will be given a signed and dated copy of the consent form to keep for my records.

____________________________________
Type/Print Subject's Name

____________________________________
Signature of Subject Date

____________________________________
Type/Print Name of Person Obtaining Consent

____________________________________
Signature of Person Obtaining Consent Date

Lara Boyd

____________________________________
Type/Print Name of Principal Investigator

____________________________________
Signature of Principal Investigator Date
APPENDIX B: TMS Screening Form

BRAIN BEHAVIOR LAB
TRANSCRANIAL MAGNETIC STIMULATION (TMS) SCREENING FORM

Below is a questionnaire used to exclude participants considered not suitable for transcranial magnetic stimulation (TMS). This information, as well as your identity, will be kept confidential.

PLEASE COMPLETE FORM BELOW:

Participant Code: __________________________

Please CIRCLE ONE:

<table>
<thead>
<tr>
<th>Condition / Device</th>
<th>YES</th>
<th>NO</th>
<th>Condition / Disease</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological or Psychiatric Disorder</td>
<td>YES</td>
<td>NO</td>
<td>Multiple Sclerosis</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Head Trauma</td>
<td>YES</td>
<td>NO</td>
<td>Depression</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Stroke</td>
<td>YES</td>
<td>NO</td>
<td>Clinical Depression</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Brain Surgery</td>
<td>YES</td>
<td>NO</td>
<td>Treatment with amitriptyline and haloperidol</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Metal in cranium</td>
<td>YES</td>
<td>NO</td>
<td>Implanted medication pump</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Brain Lesion</td>
<td>YES</td>
<td>NO</td>
<td>Intracranial Pathology</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Pacemaker</td>
<td>YES</td>
<td>NO</td>
<td>Abnormal</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>History of seizure</td>
<td>YES</td>
<td>NO</td>
<td>Intracranial anomaly</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Family history of epilepsy</td>
<td>YES</td>
<td>NO</td>
<td>Pregnant</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>History of epilepsy</td>
<td>YES</td>
<td>NO</td>
<td>Headaches or Hearing problems</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Intracranial electronic devices</td>
<td>YES</td>
<td>NO</td>
<td>Family History of Hearing Loss</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Intracardiac lines</td>
<td>YES</td>
<td>NO</td>
<td>Other medical conditions</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>

If you answered “yes” to any of the above questions, please provide details below.

_________________________________________________________________________