Neuromodulation Using Primed Paired Associative Stimulation

A DISSERTATION
SUBMITTED TO THE FACULTY OF
THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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October 2017

Acknowledgements

Success is built upon the shoulders of giants. I am indebted to the giants in my life.

Dr. James R. Carey, my doctoral advisor. You are a force. Your vision of humanity and your incredible intellect shine in everything you do and in every interaction that you have. I cannot express the ways in which you have impacted my own visions and actions. I am grateful to be your final doctoral student and will carry on your legacies of compassion and great science in every endeavor.

My Committee Chair, Dr. Teresa Kimberley. Your mentorship in all things science, career and life is greatly appreciated. I look up to your strength and sense of self.

My Committee: Dr. Colum MacKinnon, Dr. Bernadette Gillick, Dr. Kelvin Lim and Dr. James Hodges.

Dr. Jessica M. Cassidy, my academic sister. You taught me more than you'll ever know. To this day, I stream the Current and imagine our days of working on Hatch and Rob Peter Paul. You will go far and I am thankful to say "I knew you when."

Dr. Mo Chen, your humor, kindness and intelligence made all of my studies possible.

Fellow Rehabilitation Science Ph.D. students whose comradery will forever go unrivaled: Bekah Summers, Tonya Rich, Becky Lawrence, Justin Staker, Brittany Collins, Tara Mader, Joyce Trost, Dan Chantigian, Craig Kage, Leah Johnson, and Mia Larson.

Post-doctoral fellows who showed me that life goes on after the Ph.D.: Dr. Cecilia Prudente, Dr. Chao-Ying Chen, Dr. Samuel Nemanich, Dr. Jerel Mueller, Dr. Leo Ai

Dr. Nessa Johnson, Daniele De Patre, Maira Lixandrao, Dr. Naveen Elangoven, Iling Yeh

Faculty and staff within the Division of Rehabilitation Science and the Graduate Program in Neuroscience. Especially Dr. Dawn Lowe and Dr. Manda Keller-Ross.

My patient and loving family: Matt Frost, Bruce and Nancy Jahnke, Ross and Megan Jahnke, Scott Jahnke and Kylene Abbitt. The late Leon and Twilamae Johnson and Wilbur and Lucille Jahnke.

Ms. Frost's doctoral training was supported by the University of Minnesota Division of Rehabilitation Science, Division of Physical Therapy and Minnesota's Discovery, Research, and InnoVation Economy Graduate Fellowship in Neuromodulation.

Dedication

I dedicate this work to the number of women who have come before me and fought for respect as scientists, mathematicians and engineers.

"Do not undertake a scientific career in quest of fame or money. There are easier and better ways to reach them. Undertake it only if nothing else will satisfy you; for nothing else is probably what you will receive. Your reward will be the widening of the horizon as you climb. And if you achieve that reward you will ask no other."

-Cecilia Payne-Gaposchkin

I also dedicate this work to the inspiring men and women who find a second life in the wake of disease or disability.

"We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained."

-Marie Curie

Finally, I dedicate this work to my husband, Matt Frost, whose support has earned him rights to a significant portion of this Ph.D. May we learn, explore and love together for the rest of our days.

"Science makes people reach selflessly for truth and objectivity. It teaches people to accept reality, with wonder and admiration."

-Lisa Meitner

Abstract

Purpose: Neuroplasticity governs mechanisms of cortical reorganization, adaptation and recovery following neural injury. Paired associative stimulation (PAS) induces a long-lasting change in neuroplasticity by pairing a peripheral nerve stimulus with a cortical stimulus and inducing a spike-timing-dependent-like plasticity. However, preceding a principal bout of PAS that intends to induce neuroplastic change in one direction (e.g. facilitatory) with a priming PAS treatment that intends to weight synaptic plasticity in the opposite direction (e.g. suppressive) may deploy homeostatic synaptic mechanisms resulting in a larger and more consistent change in cortical excitability. Exploring principles of homeostatic synaptic plasticity in human motor cortex using all combinations of priming and principal suppressive PAS (PAS_{LTD}), facilitatory PAS (PAS_{LTP}) and sham PAS (PAS_{SHAM}), this study explores the efficacy of primed PAS as a method of neuromodulation and investigates a relationship between physiological characteristics and an individual's response to PAS.

Methods: Thirty-one healthy individuals were randomized into and completed either Experiment 1 (n=15, age 23.60 ± 2.33 years) or Experiment 2 (n=16, age 22.25 ± 2.28 years). Experiment 1 investigated priming of PAS_{LTD} using a cross-over of the following four interventions separated by at least one-week washout periods: 1. PAS_{SHAM}→PAS_{LTD}; 2. PAS_{LTP}→PAS_{LTD};3. PAS_{LTD}→PAS_{LTD};4. PAS_{SHAM}→PAS_{SHAM}. Experiment 2 investigated priming of PAS_{LTP} using a similar four-intervention cross-over of 1. PAS_{SHAM}→PAS_{LTP};2. PAS_{LTD}→PAS_{LTP};3. PAS_{LTP}→PAS_{LTP};4. PAS_{SHAM}→PAS_{SHAM}. The primary outcome measure for both experiments was an average of 20 peak-to-peak motor evoked potentials (MEPs) recorded from the preferred abductor pollicis brevis collected at baseline and 0, 10, 20, 30, 40, 50 and 60 minutes following intervention. Mixed linear models assessed within- and between- intervention change from baseline comparisons within each experiment. Secondary outcome measures assessing individual characteristics included presence of the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and the average latency of MEPs collected using an anterior-posterior current flow across the central sulcus.

Results: In Experiment 1, the PAS_{LTP}→PAS_{LTD} intervention produced a significant increase from baseline corticospinal excitability. Nonresponders had a significantly higher presence of the BDNF Val66Met polymorphism. In Experiment 2, no intervention produced a significant change from baseline excitability. Priming did not convert individual nonresponders to responders for any PAS intervention.

Discussion: Our results highlight the complexity of synaptic plasticity and the difficulty in harnessing mechanisms of plasticity to augment neuromodulation strategies. Individual characteristics may influence response to PAS_{LTD} and optimal protocols may need to be established for stratified groups.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ABP abductor pollicis brevis ***AMPAR*** α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptor ***BDNF*** brain-derived neurotrophic factor ***bAP*** back-propagating action potential ***CaMKII*** calcium/calmodulin-dependent protein kinase II ***cAMP*** cyclic adenosine monophosphate ***CIMT*** constraint-induced movement therapy ***CREB*** cyclic adenosine monophosphate response element-binding protein ***CSP*** cortical silent period ***DNA*** deoxyribose nucleic acid ***EEG*** electroencephalography ***EMG*** electromyography ***GABA*** gamma-Aminobutyric acid $\boldsymbol{GABA_A}$ gamma-Aminobutyric acid receptor A subtype $\boldsymbol{GABA_B}$ gamma-Aminobutyric acid receptor B subtype ***HIPAA*** health insurance portability and accountability act ***Hz*** hertz ***ICC*** intraclass correlation coefficient ***ICF*** intracortical facilitation ***IHI*** interhemispheric inhibition ***IPSP*** inhibitory post-synaptic potential ***ISI*** interstimulus interval ***LTD*** long-term depression ***LTP*** long-term potentiation ***M1*** primary motor cortex ***MAPK*** mitogen-activated protein kinases ***MEP*** motor-evoked potential ***ms*** millisecond ***MSO*** maximum stimulator output $\boldsymbol{\mu V}$ microvolt ***NIBS*** non-invasive brain stimulation ***NMDAR*** n-methyl-d-aspartic acid receptor ***PAS*** paired associative stimulation $\boldsymbol{PAS_{CONTROL}}$ control paired associative stimulation $\boldsymbol{PAS_{LTD}}$ suppressive paired associative stimulation $\boldsymbol{PAS_{LTP}}$ facilitatory paired associative stimulation $\boldsymbol{PAS_{SHAM}}$ sham paired associative stimulation ***PKA*** protein kinase a ***PKC*** protein kinase c ***PNS*** peripheral nerve stimulation ***PT*** perceptual threshold ***PPAS*** primed paired associative stimulation $\boldsymbol{PPAS_{LTD}}$ primed suppressive paired associative stimulation $\boldsymbol{PPAS_{LTP}}$ primed facilitatory paired associative stimulation ***REDCap*** research electronic data capture system ***RMT*** resting motor threshold ***rTMS*** repetitive transcranial magnetic stimulation ***SICI*** short intracortical inhibition ***SMC*** sensorimotor cortex ***STDP*** spike-timing-dependent plasticity ***TBS*** theta-burst stimulation ***tDCS*** transcranial direct current stimulation ***TES*** transcranial electrical stimulation ***TMS*** transcranial magnetic stimulation

1.0.0 Introduction

1.1.0 Identification of the Problem

This dissertation on healthy individuals is intended to lend value to the development of new scientific approaches that ultimately will improve rehabilitation strategies for people with stroke. A stroke results from ischemia or hemorrhage and causes irreversible neuronal damage and altered activity in surviving neurons, which impacts a variety of physiologic functions. In the United States, nearly 795,000 individuals suffer a first (76.7%) or recurrent (23.3%) stroke each year.[1] Stroke is currently the leading cause of long-term disability[2] with 80% of survivors experiencing some level of motor impairment.[3] Although conventional rehabilitation strategies aim to improve motor function following a stroke, less than 15% of those with a motor impairment achieve full motor recovery.[4] As acute medical care improves, the percentage of stroke survivors will continue to increase thus creating a need for improved rehabilitation strategies that parallel advancements in our understanding of neuroplasticity and the development of medical devices.

Non-invasively stimulating the brain provides new strategies for post-stroke motor recovery by recruiting dormant neurons in the penumbra following a stroke. The combination of non-invasive brain stimulation and conventional post-stroke therapy improves motor function.[5,6] However, efficacy reports are inconsistent and many studies are plagued by highly variable within- and between-participant responses.[7–11] Two meta-analyses reporting on the efficacy of a common form of brain stimulation, repetitive transcranial magnetic stimulation (rTMS), oppose each other's conclusions stating that rTMS is beneficial for motor recovery[12] and that there is not enough evidence to support the use of rTMS for motor recovery.[13] A more recent form of brain stimulation, paired associative stimulation (PAS), may improve excitability and motor function more than rTMS[14] but it still elicits highly variable responses between individuals. The current array of efficacy findings and inability to predict who will or will not benefit from brain

stimulation interventions presents a barrier that significantly hinders the progression and translation of brain stimulation from the lab to the clinic. It is thus important to pursue the potential for more effective approaches (e.g. PAS) and explore different characteristics that may influence an individual's response to brain stimulation.

1.2.0 Neuroplasticity

Neuroplasticity refers to intrinsic changes in the efficiency of communication (e.g. facilitation or suppression) along neural pathways. This property of the nervous system underlies fundamental functions including memory, learning and adaptation. In the context of neurorehabilitation, principles of neuroplasticity may drive the development of re-learning motor patterns through compensatory or restorative pathways. Mechanisms of neuroplasticity modify neural structures through changes in synaptic and non-synaptic properties, conferring changes in the strength of synaptic connections that underlie functions of learning and memory (see[15] for review). Following a stroke, the balance between facilitation and suppression of neural communication changes causing exaggerated suppression of excitability in the ipsilesional hemisphere.[16–18] However, understanding and implementing principles of neuroplasticity (e.g. experience-dependent plasticity, spike-timing-dependent plasticity and metaplasticity) enable strategic planning for more effective post-stroke motor recovery therapies that harness inherent mechanisms of motor-learning and facilitate communication from the ipsilesional primary motor cortex (M1) to the paretic limb.

1.2.1 Synaptic & Non-Synaptic Neuroplasticity

General mechanisms of neuroplasticity can be divided into two categories: *synaptic plasticity* and *non-synaptic plasticity*. Although both categories of neuroplasticity result in altered efficiency of communication between neurons, they differ in location and mechanism of action.[19] Synaptic plasticity refers to changes that occur at a synapse between two neurons. Acting pre and/or post-synaptically, these changes typically include altered neurotransmitter release and/or uptake resulting from changes in pathway

activation patterns.[20] Non-synaptic plasticity refers to changes that occur in areas remote from the synapse (e.g the soma, axon and dendrites). Unlike synaptic plasticity, these changes typically include altered resting or voltage-gated ion channel activity, influencing intrinsic neuronal excitability.[20] Although mechanisms of learning, memory and adaptation have historically been attributed to synaptic plasticity, recent evidence suggests that both synaptic and non-synaptic plasticity work in tandem to facilitate the functions of learning and memory.[19,22]

1.2.2 Experience-Dependent Plasticity

Experience modifies synaptic properties leading to facilitation or suppression of *specific neural pathways*. This experience-dependent plasticity contributes to the development of a nervous system as it responds to environmental stimuli and situations that are unique to an individual.[23] Dynamic processes of dendritic pruning and arborization[24,25] as well as synaptic strengthening[26] and weakening[27] through experience-dependent learning allow for variations in behavior to meet basic needs (e.g. sources of food and shelter), and social rules (e.g. familiar hierarchy) to be tailored into an individual's neural network. In the context of behavior modification, experience-dependent plasticity also explains why current behavior influences future behavior. Repetition of actions or thoughts reinforces specific neural pathways, lowering the threshold for those pathways to be used again in a similar situation.[26] Alternatively, pathways for actions that are not used weaken by raising the threshold for their future use.[27] The embedded principles of repetition, underscored by the "use it or lose it" mantra of forced use and learned non-use, are important concepts in understanding the origin, modification and restoration of motor patterns in people with stroke.[28]

1.2.3 Spike-Timing-Dependent Plasticity

Experience-dependent plasticity creates a relationship between behavior and neural pathway excitability. The cellular processes that support this relationship are described by *spike-timing-dependent plasticity (STDP)* and rely on the precise timing and order of

synaptic input to determine the direction of change in synaptic strength.[26,27,29–31] Facilitation of pathway excitability is characterized by the induction of long-term potentiation (LTP), defined as a long-lasting and reversible use-dependent increase in synaptic efficacy,[32] whereas suppression is characterized by the induction of long-term depression (LTD), defined as a long-lasting and reversible use-dependent decrease in synaptic efficacy.[33] Although mechanisms of LTD induction are less well understood, it is clear that the induction of either LTP or LTD depends on the speed and magnitude of the post-synaptic intracellular calcium influx.[34,35] In the case of LTP, pre-synaptic glutamate release activates post-synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) which depolarize the post-synaptic membrane. This leads to a release of magnesium and a conformational change within N-methyl-D-aspartate receptors (NMDARs) which opens cation channels allowing calcium to flow into the post-synaptic cell. The quick and transient rise in calcium leads to LTP through the insertion of additional post-synaptic AMPARs whereas a long and slow rise in calcium leads to LTD through the removal of post-synaptic AMPARs.

The induction of either LTP or LTD resulting from STDP also depends on the post-synaptic calcium influx. The timing and order of synaptic input influence post-synaptic calcium activity by creating a relationship between the initial AMPAR-mediated and secondary back-propagating action potential (bAP)-mediated calcium influx. Synchronous pre-synaptic spiking and post-synaptic depolarization create a single large influx in calcium due to a convergence of calcium from the AMPAR-mediated potential and bAP whereas asynchronous pre-synaptic spiking and post-synaptic depolarization create a weaker calcium influx due to overlap with a small afterdepolarization following the bAP as well as a calcium-mediated inactivation of NMDARs.[36] Therefore, the purpose of precise order and timing is to establish the proper intracellular calcium concentration in the proper amount of time to direct AMPAR insertion or resection. The cellular recognition of synchronous or asynchronous activation is impressive and

supports the theory that certain pathways can be strengthened or weakened in a use-dependent manner.

The facilitation and suppression of neural pathways via LTP and LTD, respectively, are both important for the promotion of restorative motor function in people with motor impairment following stroke. Experience-dependent plasticity enables motor practice to result in the facilitation of preferred pathways and suppression of accessory pathways to create more efficient motor patterns. Indeed, motor rehabilitation in people with stroke benefits from therapies that incorporate principles of experience-dependent plasticity.[28] Thus, the induction of LTP or LTD through STDP, the candidate mechanism that underlies experience-dependent plasticity, may expand the success of motor rehabilitation efforts when applied in conjunction with motor learning tasks that are rooted in principles of neuroplasticity. This dissertation describes an investigation into the use of PAS as a non-invasive method of inducing STDP and aims to contribute to the translation of neuroscientific principles into feasible rehabilitation strategies.

1.3.0 Metaplasticity & "Synaptic Wisdom"

Metaplasticity refers to the "plasticity of plasticity" and describes the influence of prior synaptic activity on thresholds for inducing further changes in synaptic weight (i.e. LTP or LTD).[37] The relationship between prior and future synaptic activity is described by the Bienenstock-Cooper-Munro theory whereby the time-average of prior post-synaptic activity alters thresholds for the induction of either LTP or LTD.[38] The ease and magnitude of LTP or LTD induction depends on the previous direction of synaptic weighting such that if prior activity weights plasticity in one direction (e.g. LTD), the subsequent weighting of plasticity in the opposite direction (e.g. LTP) will be easier to achieve but the subsequent weighting of plasticity in the same direction (e.g. LTD) will be more difficult.[38]

1.3.1 Homeostatic Metaplasticity

The relationship between prior and future synaptic activity is important for maintaining neural activity around a physiologically stable "set point." Excessive weighting of synaptic activity in a single direction, without restraint, renders a network vulnerable to an out-of-control positive-feedback loop. Such a positive-feedback loop comes from the possibility that continued use of a specific pathway could theoretically lead to limitless induction of LTP.[39] Without a complementary negative feedback system, damaging levels of LTP or LTD may be achieved within a system. This notion of impaired homeostatic metaplasticity is evidenced in individuals with writer's cramp, a form of task-specific focal dystonia. Kang et. al (2011) assessed homeostatic interactions using motor practice and PAS in 10 individuals with writer's cramp and 10 healthy individuals. Only those with writer's cramp lacked a homeostatic suppression of practice-dependent plasticity. Authors attribute this finding to a deficiency in homeostatic regulation of plasticity that may underlie the exaggerated plasticity theorized to lead to task-specific focal dysontias.[40] The Bienenstock-Cooper-Munro theory of sliding thresholds describes a homeostatic response that prevents an extreme and potentially damaging swing of synaptic weighting in a single direction.[38] This *homeostatic metaplasticity* allows neural adaptations to occur within a safe range by balancing synaptic modification and stabilization. Homeostatic synaptic scaling provides a solution to the need for balance, termed the "stability problem," by scaling current synaptic activity in response to previous activity thus maintaining activity around a certain set point.[41,42]

The reaction of a synapse to its own previous activity resembles a form of wisdom termed "synaptic wisdom.[43]" It alters future activity based on evidence of recent history and modifies response properties to ensure that activity remains within physiological limits. This inherent wisdom can be used to establish a known history of synaptic activity and then direct and magnify subsequent changes to achieve a desired neuroplastic effect.

1.4.0 Altered Neuroplasticity Following a Stroke

Mechanisms of metaplasticity create balance between suppression and facilitation of synaptic activity both within and between different regions of the brain. Following an acute stroke, a series of neurobiological events known as "the ischemic cascade" disrupts this balance and alters the excitability and viability of neurons affected by the infarct.[18,44] Consequently, during the post-infarct recovery period, physiological responsiveness and excitability levels change within the ipsilesional[45,46] and contralesional[46,47] hemispheres. The observed patterns of change are dynamic and complex making it difficult to pin a detailed timeline to the evolution of neuroplasticity following stroke.[18] However, the existence of a complicated evolution exemplifies the capacity for networks to continue to undergo plastic changes from seconds to years after an injury and creates an inherent target for motor rehabilitation therapies.

1.4.1 Diaschisis

Diaschisis occurs when cortical regions remote from the lesion site exhibit decreased activation even though they are not directly impacted by the injury.[16] These regions experience decreased blood flow and metabolic activity[48] resulting from vasogenic edema,[44] deafferentation,[49] exaggerated interhemispheric inhibition[17] and learned non-use.[50] The ensuing dysfunction and decreased excitability may contribute to decreased activity in response to motor and sensory stimuli.[18] A reduced response to stimuli hinders spontaneous recovery and functional reorganization, or remapping, following a stroke. However, these regions contain viable, albeit suppressed, neurons that are a target for potentiation and subsequent restoration of function.

In the early 1900's, Von Monakow postulated that three forms of diaschisis exist: *Diaschisis cortico-spinalis* (affecting corticospinal tract pathways), *Diaschisis associative* (affecting areas within the same hemisphere) and *Diaschisis commissuralis* (affecting homologous areas in the opposite hemisphere).[51] Each of these forms of diaschisis may

impact motor recovery by affecting spinal, ipsilesional and contralesional motor networks.

1.4.2 Interhemispheric Inhibition

Neurons within bilateral M1 areas communicate with each other through the corpus callosum.[52,53] Interhemispheric inhibition (IHI) is a transcallosal inhibitory drive that occurs during motor movement and is exerted from the primarily activated M1 toward the opposite, less activated M1.[54] In all individuals, IHI serves to prevent mirror movements.[55] Following a stroke, IHI becomes imbalanced resulting in an exaggerated inhibitory drive from the contralesional to ipsilesional M1 and a suppressed inhibitory drive from the ipsilesional to contralesional M1.[17] The excessive suppression of neural activity within ipsilesional M1 and diminished suppression of neural activity within contralesional M1 reduces the potential for use and functional reorganization of viable neurons in the ipsilesional M1. Thus, approaches that decrease excitability of the contralesional M1 may serve to decrease IHI exerted from the contralesional M1 to the ipsilesional M1 and effectively disinhibit the ipsilesional M1.

1.4.3 Quadruple Disablement

The direct damage and neuronal loss caused by a lesion is compounded by exaggerated ipsilesional inhibition resulting from imbalanced IHI. Together, these effects led to people with stroke being characterized as "doubly disabled."[56] However, IHI is a form of Von Monakow's *Diaschisis commissuralis* whereby homologous regions in the opposite hemisphere are impacted via transcollosal fibers. With this understanding, it makes sense to include the other forms of diaschisis, *Diaschisis cortico-spinalis* and *Diaschisis associative*, which describe the effects of mechanisms of diaschisis (e.g. deafferentation) on the corticospinal tract and ipsilesional cortical regions. Particularly for lesions affecting M1, the initial insult causes (1) permanent neuronal loss and mechanisms of diaschisis lead to (2) IHI, (3) altered corticospinal tract excitability and recruitment and (4) hypoactive regions of nearby sensorimotor cortices leading to a complex and global

reduction of corticomotor pathway activation. “Quadruple disablement” comprises the direct loss of neurons from the ischemic insult and the three indirect causes of decreased activity affecting the penumbra via Von Monakow’s three forms of diaschisis, and thus, more completely describes the neurological impact of stroke.

1.5.0 Neuromodulation

Neuromodulation refers to a stimulus-driven change in neural activity through pharmacological agents, implanted electrodes or external, non-invasive stimuli.[57] Transcranial magnetic stimulation (TMS) is a form of non-invasive neuromodulation that directs a changing magnetic field toward a targeted cortical region and induces current flow within resident neurons.[58] Single or paired pulses of TMS measure neuroplasticity through changes in intracortical, corticobulbar or corticospinal excitability[59–61] whereas patterned repeated TMS pulses modulate neuroplasticity through the induction of LTP or LTD.[62]

1.5.1 Neuromodulation using Paired Associative Stimulation

Paired associative stimulation (PAS) is a method of non-invasive brain stimulation that pairs a cortical stimulus with a peripheral nerve stimulus (PNS). Specific to measurement or modulation of corticospinal excitability, a single TMS pulse targeting a region in M1 is paired with an electrical stimulus targeting a nerve in the contralateral limb.[63] The frequency and number of pairs of stimuli range from 0.05 - 0.25 Hz and 50 - 270 pairs of pulses, respectively, and a review of evidence suggests that frequencies between 0.05 – 0.2 Hz are most effective.[64] PAS is unique in that it uses peripheral and central input to induce lasting changes in synaptic plasticity (i.e. LTP or LTD). Critically dependent on the timing and order of the arrival of each input to the sensorimotor cortex (SMC), the direction of change follows rules of STDP[65] whereby the arrival of TMS-induced followed by PNS-induced action potentials suppresses synaptic activity (PAS_{LTD}) and the arrival of PNS-induced followed by TMS-induced action potentials facilitates synaptic activity (PAS_{LTP}).[63,66] PAS measures the capacity for STDP in human SMC and it

modulates corticomotor excitability for up to 120 minutes following PAS_{LTD} and up to 90 minutes following PAS_{LTP}.[64] The unidirectional, lasting yet reversible nature of this neuromodulation make it ideal as an investigational technique and the induction of STDP-like plasticity which underlies experience-dependent motor learning makes it an ideal technique for improving motor recovery in people with stroke.

1.5.2 Priming with Paired Associative Stimulation

In neuromodulation, priming is both a concept and a method. It describes the concept of priming neural pathways with exercise or external stimulation to augment subsequent motor rehabilitation and promote long-term changes in the efficiency of neural pathway communication. Priming also describes the method of applying two bouts of stimulation (i.e. the first bout is termed 'priming' PAS followed by a second bout termed 'principal' PAS) within minutes of each other to capitalize on mechanisms of metaplasticity.[43,67] Specifically, priming utilizes homeostatic metaplasticity and the sliding threshold theory described by Bienenstock, Cooper and Munro[38] to either augment or suppress the aftereffects of the second, principal, bout of PAS. Priming PAS_{LTD} followed by principal PAS_{LTP} augments the facilitatory aftereffects of PAS_{LTP} whereas priming PAS_{LTP} followed by principal PAS_{LTP} suppresses facilitation which is theorized to result from a physiological ceiling-like effect.[68] Thus, combinations that weight synaptic plasticity in opposite directions appear to augment aftereffects of the principal bout and those that weight synaptic plasticity in the same direction appear to suppress aftereffects of the principal bout. Although this relationship has been described for principal PAS_{LTP}, evidence is sparse and the influence of priming on principal PAS_{LTD} is yet unpublished. The potential for priming to create a known history of synaptic activity and then utilize inherent mechanisms of metaplasticity to direct and magnify aftereffects of a subsequent, principal, bout of stimulation establishes a new method of neuromodulation that may improve the predictability of response. The combination of priming and PAS creates a potent approach based in principles of metaplasticity, experience-dependent plasticity and

STDP that may reduce variability and augment excitability changes leading toward greater improvements in post-stroke motor recovery.

1.6.0 Neuromodulation Using TMS for Post-Stroke Motor Recovery

Non-invasive neuromodulation augments stroke rehabilitation strategies by targeting the hypoactive, yet viable neurons within ipsilesional M1. Two approaches are consistently used to increase activity of hypoactive neurons in the penumbra: direct LTP-induction within the ipsilesional M1 or indirect disinhibition of ipsilesional M1 through LTD-induction within the contralesional M1. Either approach facilitates excitability within ipsilesional M1 and contributes to improved motor recovery.[56] Recent evidence suggests that people with different levels of impairment may benefit from different approaches. Specifically, LTD-induction within contralesionsal M1 improves function in individuals with mild impairment whereas a new approach of LTP-induction within the contralesional dorsal pre-motor cortex improves function in individuals with severe impairment.[69]

Neuromodulation that outlasts the stimulation period is important for post-stroke motor rehabilitation. Paretic limb exercises conducted following stimulation and during the period of altered excitability strengthen corticomotor pathways and improve motor recovery.[5,6] Thus, development of an appropriate and effective care plan combining neuromodulation and motor practice relies on knowledge of the time course of neuroplastic change following intervention.

1.6.1 Paired Associative Stimulation for Post-Stroke Motor Recovery

Paired associated stimulation improves motor function in people with stroke.[9,10,14,70,71] The induction of STDP-like plasticity mimics natural mechanisms of experience-dependent plasticity and the > 60 minutes of altered excitability provides time for post-stroke motor therapies. In people with dysphagic stroke, PAS outperformed rTMS in measures of corticobulbar excitability and swallowing function when compared to their

sham counterparts.[14] Previous studies show efficacy in neuromodulation of corticospinal pathways targeting the paretic upper limb when applying PAS_{LTP} to the ipsilesional M1[71] as well as corticospinal pathways targeting the paretic and non-paretic lower limbs when applying PAS_{LTD} to the contralesional M1.[70] Furthermore, motor recovery improvements in gait[10] and swallowing[14] demonstrate that PAS interventions successfully modulate pathway excitability and that this modulation contributes to improved motor function.

1.6.2 Variability in Response

Although studies of PAS in people with stroke demonstrate efficacy in neuromodulation and motor improvement, they also demonstrate high variability within- and between-individual responses.[9,10,70] Not only does the time course of change in corticospinal excitability differ between individuals[70] but the direction of change in excitability differs to the extent that only ~50% of individuals respond in the predicted manner following a given PAS intervention.[10] Considering that the point of using PAS in people with stroke is to alter corticomotor excitability and promote neural communication during subsequent motor training, the need to weight excitability in a desired direction is clear. The high response variability weakens efficacy interpretations and makes it difficult to determine which protocols to pursue. This variability is not unique to populations of people with stroke as it is also seen in healthy individuals where only 35-52% respond in a predicted manner.[8,72] Studies into variables that may explain an individual's response to PAS have identified the Val66Met polymorphism of brain-derived neurotrophic factor (BDNF), resting motor threshold, 1mV threshold, short intracortical inhibition (SICI) and age as potential factors.[8,72–74] However, the influences of these factors are currently ill-defined and do not create a detailed or whole characterization of those who will or will not benefit from intervention.

1.7.0 Healthy Participants

With the intended purpose to improve motor recovery following a stroke, the ability of PAS to predictably alter corticospinal excitability must first be characterized and

optimized. Although the recent push in neuromodulation research has been to define an effect of PAS in functional recovery, the high variability in response to neuromodulation plagues these studies with deceptively low effect sizes.[8,11] An inability to predict an individual's response also stifles the progression of PAS as a conventional clinical tool. Thus, the development of a more effective and predictive model of intervention is warranted. Primed PAS (PPAS) may augment changes in excitability thus capturing more individuals as responders to intervention. The following dissertation is the first study known to address all combinations of priming and principal PAS_{LTP} and PAS_{LTD}. Due to the increased number of factors that may contribute to variability in people with stroke (e.g. lesion size and location, time since stroke, severity of deficits) as well as the novelty of this investigation, it was conducted in people with no neurological disease or disorder and thus focuses on the influence of PPAS in healthy participants.

1.8.0 Statement of Purpose

The purpose of this dissertation was to assess the influence of $PPAS_{LTD}$ and $PPAS_{LTP}$ on corticospinal excitability and to investigate potential characteristics (e.g. the BDNF Val66Met polymorphism) that may indicate whether an individual will or will not benefit from brain stimulation.

1.9.0 Specific Aims

Specific Aim 1: Compare the effect of PAS_{LTP} priming followed by principal PAS_{LTD} (PAS_{LTP}→PAS_{LTD}) to sham-primed PAS_{LTD} (PAS_{SHAM}→PAS_{LTD}) on corticospinal excitability in healthy individuals.

Experimental hypothesis:

PAS_{LTP}→PAS_{LTD} will utilize homeostatic mechanisms of synaptic plasticity, resulting in a greater decrease in corticospinal excitability compared to PAS_{SHAM}→PAS_{LTD} as evidenced by a reduction in peak-to-peak amplitude of motor evoked potentials.

Specific Aim 2: Compare the effect of PAS_{LTD} priming followed by principal PAS_{LTP} (PAS_{LTD}→PAS_{LTP}) to sham-primed PAS_{LTP} (PAS_{SHAM}→PAS_{LTP}) on corticospinal excitability in healthy individuals.

Experimental hypothesis:

PAS_{LTD}→PAS_{LTP} will utilize homeostatic mechanisms of synaptic plasticity, resulting in a greater increase in corticospinal excitability compared to PAS_{SHAM}→PAS_{LTP} as evidenced by an increase in peak-to-peak amplitude of motor evoked potentials.

Specific Aim 3: Compare the ratio of responders to nonresponders between PAS_{LTP}→PAS_{LTD} and PAS_{SHAM}→PAS_{LTD} and between PAS_{LTD}→PAS_{LTP} and PAS_{SHAM}→PAS_{LTP}.

Experimental hypothesis:

Priming will weight synaptic plasticity in a known direction, making it easier to weight synaptic plasticity in the opposite direction. This will result in an increased ratio of responders to nonresponders following $PPAS_{LTD}$ and $PPAS_{LTP}$ interventions.

Specific Aim 4: Compare the presence of the BDNF Val66Met polymorphism and the latency of MEP onset between responders and nonresponders for both PAS_{SHAM}→PAS_{LTD} and PAS_{SHAM}→PAS_{LTP}.

Experimental hypothesis:

Presence of the Val66Met polymorphism will alter plasticity and thus be higher in individuals categorized as nonresponders. The MEP onset latency from MEPs collected with the induction of anterior-poster current flow will differ between individuals categorized as responders and those categorized as nonresponders.

2.0.0 Review of Literature

2.1.0 Neuroplasticity

2.1.1 A Cellular Basis for Learning, Memory & Adaptation

Neuroplasticity refers to the malleability of individual neurons, synapses and entire networks. This property of the nervous system underlies fundamental functions including memory, learning and adaptation. Understanding the mechanisms of neuroplasticity begins with development of the neuron doctrine. Set forward by Santiago Ramon y Cajal (1894), the neuron doctrine is the first to state that the neuron is the basic anatomical and physiological unit of the nervous system. Confirmed in the 1950s with the advent of electron microscopy and subsequent images of the synaptic cleft,[76] this doctrine laid the groundwork for investigating how a network of connected yet separate neurons could communicate to sustain functions of the nervous system. Although theories of neuroplasticity may be traced as far back as the psychologist William James (1890), a well-documented connection between cellular mechanisms, synaptic plasticity and behavior did not occur until the 1960s and 1970s when Eric Kandel and colleagues studied learning and memory in the simplified model of *Aplysia*.[15] By observing differences in behavior (the gill-withdrawal reflex), neural activity and synaptic strength following three types of learning (sensitization, habituation and classical conditioning), Kandel and colleagues demonstrated that pre-existing synapses within sensorimotor pathways are plastic and modifiable.[78–80] Further studies identified serotonin, PKA, cAMP, MAPK and CREB as key components influencing the observed presynaptic changes.[15] The findings that molecular substrates are influenced by previous activity, contribute to changes in synaptic weighting and subsequently change behavior established the foundation for neural correlates of learning and memory.

In a seminal paper by Bliss and Lomo (1973), a form of synaptic plasticity, now termed long-term potentiation (LTP), was discovered by stimulating the perforant pathway in the hippocampus of anesthetized rabbits. As an ideal model of long-term information storage,

LTP has been credited with functions underlying development and different forms of experience-dependent plasticity including learning and memory.[81] Because of its demonstrated importance, LTP has been extensively studied. Remarkable investigations into the mechanisms of LTP conducted by Morris (1986) and Malenka (1988) drew connections between post-synaptic NMDAR activity, post-synaptic calcium activity and the evolution of LTP within a synapse. Further studies by Morris (1986) demonstrate that blocking NMDAR activity not only altered LTP induction but also blocked memory storage. Thus, NMDAR activity and LTP could be directly linked to a functional consequence. It is now widely accepted that LTP induction relies on a quick post-synaptic calcium rise following strong or frequent stimuli. Briefly, the process of LTP induction begins with a presynaptic glutamate release and subsequent post-synaptic AMPAR activation enabling depolarization of the post-synaptic membrane. This depolarization results in the release of a magnesium blockade from NMDAR channel pores.[84] In the presence of glutamate, the release of this blockade and a glutamate-induced conformational change open the NMDAR cation channel allowing calcium to flow into the post-synaptic cell. Following high frequency or strong stimuli, the subsequent calcium influx will be fast, triggering a cascade involving CaMKII, PKC and protein synthesis. This results in the insertion of AMPARs within the post-synaptic membrane, increasing sensitivity to glutamate in future synaptic transmissions.[85] Although it is known that LTP induction, expression, and maintenance occur in three mechanistically distinct phases, the conversion to and maintenance of late-phase LTP remains under investigation.[86]

Long-term depression (LTD) complements LTP by weakening synaptic efficiency and maintaining bi-directional balance at a synapse. Discovered by Levy and Steward (1979), LTD, like LTP, was first observed in the hippocampus. Stimulation of entorhinal cortical pathways and recordings from the dentate gyrus of anesthetized rats demonstrated that when ipsilateral pathways were stimulated, crossed pathways exhibited a decrease in evoked response amplitudes. Authors concluded that potentiation could be reversed and

that a potentiated synapse could be depotentiated dependent on stimulation patterns.[87] Although the mechanism is not as well understood as that for LTP, LTD is thought to depend on a slow rise in post-synaptic intracellular calcium and activation of calcium-dependent phosphatases, resulting from low-frequency stimulation.[35] Subsequent AMPAR removal from the post-synaptic membrane provides the hallmark characteristic of LTD, a decrease in sensitivity to glutamate.[85]

As discussed above, glutamate is a neurotransmitter that governs activity (e.g. LTP and LTD) at excitatory synapses. Another neurotransmitter, γ-Aminobutyric acid (GABA), governs activity at inhibitory synapses. Although the discussion of learning and memory centers around the induction of LTP and LTD in excitatory networks, the role of GABA-mediated inhibitory circuitry in these functions is equally important. Until the mid-1960s, the role of GABA as a neurotransmitter was highly debated. Krnjevic and Schwartz (1967) showed that GABA may be the primary inhibitory neurotransmitter in the mammalian central nervous system by applying extracellular GABA to cortical neurons in anesthetized cats. By comparing neuron properties (e.g. membrane potential and resistance) following the application of GABA to those same properties during an inhibitory post-synaptic potential (IPSP), authors found that the actions of GABA, namely reversible hyperpolarization, mimic those that occur during an IPSP and thus concluded that GABA acts like a physiological inhibitory neurotransmitter.[88] Subsequent research discovered and characterized two distinct GABA receptor types, $GABA_A$ and $GABA_B$, that respectively mediate fast and slow hyperpolarization to accomplish different goals through inhibitory synaptic activity.[89] The balance between excitation and inhibition of cortical circuitry is critical. When this balance is disrupted, abnormal cortical activity may affect muscle or cognitive abilities. For this reason, several pharmacological agents have been developed to interfere with GABA signaling for management of conditions including spasticity and epilepsy.[90]

2.1.2 Applications to Post-Stroke Motor Recovery: Experience-Dependent Plasticity

Experience and activity alter network activity by "rewiring" the brain so that pathways that are frequently used become more easily used and those that are less frequently used become less easily used. This ensures efficient communication along neural networks to make tasks like grabbing a cup of coffee or recalling a piece of information a quick and less wasteful process. Mechanisms of learning and memory, i.e. LTP and LTD, are thus prime candidates for foundational mechanisms of this e*xperience-dependent plasticity.* Experience-dependent plasticity is operationally defined separately from experience-expectant plasticity whereby experience-expectant plasticity defines the neural response to and storage of information derived from ubiquitous experiences (e.g. visual processing of contrast) and experience-dependent plasticity defines the neural response to and storage of unique experiences (e.g. sources of food, shelter or social order).[23] Experience itself may be thought of as a collection of neural input that allows an individual to create an interpretation of its surroundings. Conceptually, experience-dependent plasticity encompasses the ability of the nervous system to remodel by strengthening and weakening specific neural connections in response to patterns of neural input.[23,28]

Early studies of experience-dependent plasticity focused on the use-dependency of cortical neurons in the visual cortex of cats. A series of classic studies by Hubel and Wiesel went from describing visual cortical neuron receptive fields[91,92] to demonstrating their capacity for use-dependent change.[93] When kittens underwent monocular deprivation from birth, they exhibited a pronounced lack of visual cortical neuron activity when the deprived eye was stimulated.[93] This finding was one of the first to support the idea that neural connections may be intact at birth and rendered defective by a lack of use. Another classic study being conducted around the same time by Held and Hein (1963) supported the importance of movement-produced sensory feedback in the development of visually-guided behavior in kittens. By comparing two kittens, one with its legs free to walk and the other in a basket so that it could not walk, authors created a scenario in which the two kittens, joined by a freely rotating bar, would experience the

same visual stimulation but one would be actively moving and the other would be passively moving. As demonstrated by various tasks (e.g. paw placement, visual cliff, blink response), the actively moving kittens outperformed the passively moving kittens in visually-guided behaviors and the passively moving kittens appeared functionally blind. The use-dependency of cortical circuitry highlights the need for specific and appropriate collections of neural input to effectively drive proper and efficient development of functional behaviors.

Physiological mechanisms of neural remodeling rely on changes in the number and strength of synaptic connections through dendritic pruning and arborization[24,25] as well as changes in protein expression[95] and neurotransmitter release or uptake. These changes provide structural evidence of functional changes observed in motor learning and memory. Importantly, the act of *learning* is critical to the structural changes observed in experience-dependent learning. Kleim et al. (1996) compared structural and protein measures in rats that were active in a complex environment (i.e. learned to run a complex obstacle course) to those that were active in a simple environment and those that were inactive. Layer II/III of the motor cortex of rats that were exposed to the challenge of a complex environment displayed increased synaptogenesis and Fos protein expression.[95] Furthermore, the synaptogenesis observed in rats that were active in a complex environment persisted without the need for continuous training.[96] Thus, the component of cognitive challenge that occurs during learning of complex motor tasks significantly impacts the structural changes that underlie experience-dependent plasticity and the complex tasks that are learned over the course of a training period may persist once training is completed.

The importance of experience-dependent remodeling following neural injury is clearly established.[97–99] Studies of cortical reorganization following stroke indicate that white matter tract integrity, functional connectivity, interhemispheric inhibition and sensorimotor representations shift following stroke.[17,97,100,101] In a series of studies

performed by Nudo et al. (1996a; 1996b; 1996c), cortical mapping with intracortical microstimulation demonstrated that functional remapping can occur in areas surrounding a lesion and that this remapping is associated with training as well as behavioral improvement. The foundational concepts derived from these studies are that (1) networks remain plastic following injury (2) the representation of a given cortical region can change and (3) mechanisms of experience-dependent plasticity can be used to improve functional recovery.

Enforcing the view that experience-dependent plasticity is a critical concept in neurorehabilitation for humans, Kleim and Jones (2008) highlight the theory that premier rehabilitation not only utilizes principles of experience-dependent plasticity but can be equated with the task of relearning. "Use-it or Lose-it" and "Use-it and Improve-it" are principles of experience-dependent plasticity that drive the widely-recognized strategy of forced-use through constraint-induced movement therapy (CIMT). This technique forces use of the more affected limb by effectively casting the less-affected limb for a variable length of time. As demonstrated by improved motor performance and increased neural excitability in the ipsilesional hemisphere of people with chronic stroke[104] as well as increased activation of the ipsilesional M1[105] and a better balance of bilateral sensorimotor network activity,[106] several studies support the efficacy of CIMT for post-stroke motor recovery. A recent review[107] questions this efficacy by highlighting evidence that other principles of experience-dependent plasticity such as the timing[108] and dosage[109] of a CIMT intervention may be as important as the foundational principles driving forced-use. Rehabilitation of motor function in people with stroke appears to mimic motor learning and benefits from incorporating multiple principles of experience-dependent learning and plasticity. Synaptogenesis, cortical remapping and improved paretic limb function are influenced by parameters that are easily adjusted and controlled (e.g. intensity, repetition and complexity of a behavior) by the individual assigning and the individual providing therapy. The next remodel of neurorehabilitation should pay special attention to the promotion and use of experience-dependent plasticity.

2.2.0 Hebb's Postulate

Donald Hebb (1949) postulated the theory that if a presynaptic cell, A, consistently and reliably fires and elicits a response (spiking) from a postsynaptic cell, B, the synaptic efficiency from A to B will be strengthened. Conversely, if presynaptic cell A consistently and reliably fires without eliciting a response from postsynaptic cell B, the strength of the synapse from A to B will be weakened.[27] This principle, now known as *Hebb's Postulate*, has defined the concepts of timing and order-dependent changes in synaptic plasticity, thus conferring a causal nature to synaptic weighting. Using whole cell voltage recordings from pyramidal neurons, Markram et al. (1997) confirmed Hebb's postulate, showing that the magnitude and sign of LTP and LTD changed depending upon stimulation (spike) timing and order.

2.2.1 Spike-Timing-Dependent Plasticity

Spike-timing-dependent plasticity (STDP) refers to the requirement of precise timing and order between pre and postsynaptic potentials resulting in a unidirectional modification of synaptic strength (either LTP or LTD) within a given time window.[29–31] Foundational studies by Markam et al. (1997) and Bi and Poo (1998) characterized the timing and order-dependency of STDP, contributing knowledge to the actual triggers for synaptic modifications that occur between neurons. Markram et al. (1997) demonstrated that potentiation occurred when presynaptic potentials led postsynaptic potentials by ~20ms and depression occurred when postsynaptic potentials led presynaptic potentials by 20-100ms. Using electrophysiological recordings in rat hippocampal pyramidal neurons, Bi and Poo (1998) demonstrated that the precise window for potentiation or depression was 5ms and noted that this window agreed with that observed in models of experience-dependent plasticity. Furthermore, these studies confirmed the roles of NMDARs and voltage-gated calcium channels in the potentiation and depression of synaptic weighting, indicating the recruitment of mechanisms of LTP and LTD. Functionally, these studies also support principles of experience-dependent plasticity by implying causality and sequence-effect in the relationship between neuronal use and the resulting strengthening

or weakening of neural pathways.[28] Thus, the use of Hebbian principles to induce LTP or LTD may strengthen strategies that capitalize on experience-dependent plasticity to promote motor learning and functional recovery.

Although initial studies have been in agreement with Hebb's postulate, defining Hebbian STDP, other forms of STDP exist and can act in an Anti-Hebbian manner, where pre-leading postsynaptic firing results in LTD, or in manners that unusually favor LTD (either Hebbian or Anti-Hebbian).[36] Understanding that the result of STDP is either LTP or LTD, it is logical that the mechanisms underlying the synaptic weighting following Hebbian STDP are the same as those previously discussed for NMDAR-depended LTP and LTD.[36] However, the importance of timing and order can be further defined mechanistically by understanding the influence of the back-propagating action potential (bAP) in the postsynaptic neuron. Because the magnitude of the intracellular calcium signal directs the induction of either LTP or LTD,[35] the temporal relationship between calcium influx from the initial, AMPAR-mediated postsynaptic potential and the secondary bAP is critical. As described by Feldman et al. (2013), the pre-leading post sequence of events results in a large influx of calcium due to a convergence of calcium influx from the AMPAR-mediated potential and bAP whereas the post-leading pre sequence results in a weaker calcium influx due to overlap with a small afterdepolarization following the bAP as well as a calcium-mediated inactivation of NMDARs. Therefore, the purpose of precise order and timing is to establish the proper intracellular calcium concentration in the proper amount of time to direct AMPAR insertion or resection.

Strategies that purposefully alter synaptic plasticity to promote post-stroke functional recovery may prefer induction using STDP over induction using high/low frequency driven changes in plasticity for several reasons. First, STDP more directly reflects physiologic mechanisms underlying changes in synaptic strength and may better mimic experience-dependent plasticity.[29,110] Second, the relationship between pre and

postsynaptic potentials may encode causality of external events, thus enforcing specific input/output sequences.[111] Finally, the short critical time window may enforce neural synchronization by forcing millisecond precision of input selection.[110]

2.3.0 Metaplasticity

2.3.1 Homeostatic Metaplasticity

Although Hebbian synaptic plasticity provides a well-accepted model of how information is stored in neural circuits, this model does not include checks or balances to prevent an extreme weighting of synaptic plasticity in a single direction. On its own, Hebb's Postulate states that repeated synchronous pre-synaptic spiking and post-synaptic depolarization will increase synaptic strength. Thus, there is no limit to the amount of strengthening that could occur which renders this model unstable. The capacity of neural circuitry to undergo experience-dependent modifications of synaptic activity requires a complementary balance between modification and stability.[112] One of the first studies to investigate changes in plasticity across an entire neuron and not just a single synapse demonstrated that all synapses associated with a neuron proportionally scale properties in response to neural activity.[42] This study of rat visual cortical pyramidal neurons found that average firing rates changed immediately following intervention and then showed a compensatory return toward baseline, reminiscent of a return to homeostasis.[42] The ability for neurons to alter intrinsic properties based on previous activity illustrates the capacity for neurons to use their own activity as a feedback signal. This "synaptic scaling" contributes to homeostatic control of neural properties to prevent limitless synaptic weighting. Although multiple feedback pathways and biological mechanisms have been suggested, the definitive roles of these pathways have not yet been elucidated. It is currently thought that intracellular calcium concentrations may direct receptor trafficking and that a calcium "sensor" may mediate synaptic scaling around a specific set point to maintain homeostasis (see [41,112] for reviews).

2.3.2 Bienenstock-Cooper-Munro Theory

The concept of homeostasis is not unique to synaptic plasticity, but *homeostatic metaplasticity* is supported by a unique theoretical mathematical framework that may explain changes in the ability to induce LTP or LTD. The term *metaplasticity* refers to the "changeability of change" and captures the property of changing neuroplasticity. Specifically, *homeostatic* metaplasticity governs the homeostatic balance between LTP and LTD induction at a given synapse. Introduced by Abraham and Bear (1996), the functional role of metaplasticity is to alter thresholds for LTP or LTD induction. A theoretical mathematical framework known as the Bienenstock-Cooper-Munro Theory provides a theory of sliding thresholds for the induction of LTP or LTD that is based upon the time-average of prior post-synaptic activity. This theory states that as a synapse undergoes LTP, the threshold for inducing LTD decreases (i.e. it becomes easier to induce LTD) and the threshold for inducing further LTP increases (i.e. it becomes more difficult to continue induction of LTP). Conversely, as a synapse undergoes LTD, the threshold for inducing LTP decreases (i.e. it becomes easier to induce LTP) and the threshold for inducing further LTD increases (i.e. it becomes more difficult to continue induction of LTD).[38] The seminal concept of a sliding threshold for induction is key to understanding the theoretical use of homeostatic metaplasticity to direct changes in neuroplasticity using tools of non-invasive neuromodulation.

2.3.3 Gating & Anti-Gating

Gating is a method of controlling changes in synaptic plasticity by decreasing intracortical inhibitory network activity or enhancing post-synaptic depolarization concurrently with motor training.[113] This concept differs from homeostatic metaplasticity because it does not rely on prior induction of LTP or LTD to alter the induction threshold for subsequent LTP or LTD. Instead, gating relies on the concurrent activity within intracortical inhibitory networks. An example of gating in people with stroke was accomplished through regional anesthesia which effectively led to deafferentation-deefferentation of the ipsilesional M1 while the participant was completing a thumb-

index finger pinch task.[114] Authors found that the regional anesthesia plus motor practice enhanced motor function of the paretic limb more than motor practice alone suggesting that the disinhibition resulting from regional anesthesia effectively gated, or augmented, the concurrent induction of LTP.[114] This method of gating allows LTP induction to occur at a lower threshold because of a lack of GABAergic-mediated network activity and thus, increasing the probability of NMDAR ion channel activity. Furthermore, the influence of intracortical inhibitory network activity provides a potential explanation for why a single protocol may result in an extensive variety of plasticity responses.

Gating has been discussed as its own concept,[113] as a form of homeostatic metaplasticity,[115] and as a complement to non-homeostatic plasticity.[116] Although the classification of gating is inconsistent, it is important to note that homeostatic plasticity, gating and non-homeostatic plasticity are three separate concepts. Recently, a discussion of non-homeostatic plasticity has led to the development of another concept called "Anti-Gating." A study of plasticity in human motor cortex assessed the effect of two consecutive interventions, a very-low frequency (0.1 Hz) rTMS intervention followed by a PAS intervention. The very-low frequency rTMS did not alter corticospinal excitability on its own but it did *increase* intracortical inhibition. Futhermore, the very-low frequency rTMS occluded both LTP and LTD after-effects of PAS_{LTP} and PAS_{LTD}, respectively.[115] An editorial opinion suggests that this finding resembles a type of "anti-gating" caused by the activation of intracortical inhibitory networks which may interfere with the subsequent induction of associative plasticity using PAS.[117] A potential mechanism of action, as demonstrated in animal models, suggests that increased GABA-ergic inhibition prevents the influx of calcium by hyperpolarizing the post-synaptic membrane, thus leaving the NMDAR magnesium blockade in place.[118] Thus, further efforts to induce LTP or LTD may be occluded secondary to a lack of calcium influx.

2.4.0 Neuromodulation using Non-Invasive Brain Stimulation

The concepts of neuroplasticity described above can be assessed in human motor cortex using non-invasive brain stimulation (NIBS). NIBS is composed of a variety of techniques that can both measure and modulate neuroplasticity. Unlike the surgically invasive nature of earlier brain stimulation techniques used to map the sensory and motor cortices,[119,120] NIBS provides methods of studying neuroplasticity in the awake human brain through a fully intact skull. Discoveries in the 1980s established the two primary categories of NIBS, transcranial electric stimulation (TES)[121] and transcranial magnetic stimulation (TMS).[58] Although methods of TES, namely transcranial direct current stimulation (tDCS), can modulate corticospinal excitability and improve functional recovery,[122] the focus of this dissertation is to investigate a particular approach using TMS. The primary reason for this is the desire to investigate mechanisms of synaptic plasticity induced by TMS, and not mechanisms of non-synaptic plasticity induced by tDCS. Thus, the following section will focus on the development and roles of TMS as they relate to measurement and modulation of synaptic plasticity.

2.4.1 Transcranial Magnetic Stimulation

Anthony Barker (1984) developed a transcranial magnetic method of brain stimulation known as transcranial magnetic stimulation (TMS). Using principles of Ampere's and Faraday's Laws, TMS acts by passing an electric current through a hand-held coil. This changing current induces a perpendicular, changing magnetic field (Ampere's Law) that can pass unimpeded through the skull and in turn, induces current flow (Faraday's Law) in the underlying cortex.[123] Each TMS pulse can be thought of as a brief yet powerful electric current lasting 100-200 μs, inducing a 1.5-2.0 T changing magnetic field.[62] When applied at a stimulus intensity that is suprathreshold, the result from the induced current flow within primary motor cortex is axonal depolarization leading to a direct corticospinal signal cascade (D-wave) followed by interneuron-mediated cascades (I-waves).[124] Early studies of motor cortical stimulation in cats and monkeys recorded the first traces and coined the terms for the resultant D- and I-waves. Patton and Amassian

(1953) found that stimulation of bulbar pyramidal cells in the ipsilateral motor cortex produced an initial stable trace, termed the D-wave, which was reliably present. The D-wave was followed by a series of subsequent low-frequency traces, termed I-waves, that depended on the integrity of nearby cortex.[125] It is now recognized that the D-wave occurs quickly and is theorized to represent a synchronous pyramidal volley resulting from direct pyramidal axon activation whereas the less understood I-waves may represent asynchronous delayed activity resulting from di- or monosynaptic activation of pyramidal neurons.[125,126]

2.4.2 TMS Coil Orientation

A relationship between coil orientation and motor pyramidal activation can be understood by re-visiting Ampere's and Faraday's laws with the addition of Lenz's law. The neurons that lie within a given cortical area may be represented by electrical wires that are oriented in a specific direction. When a changing magnetic field creates an electric field that surrounds these wires, the induced electrical current will flow in a direction that creates a magnetic field that will oppose the direction of the first-order induced magnetic field (Lenz's law). Thus, to produce an electrical current that ideally follows the anatomy of a series of connected neurons, it would make sense to orient the first-order induced magnetic field in a direction that opposes the magnetic field that will be created by the desired direction of current flow. Investigations confirm that the optimal coil orientation aligns with current flow through the underlying anatomy. Mills et al. (1992) studied the influence of coil orientation using a figure-of-eight coil by stimulating the hand area of the motor cortex and recording from the first dorsal interosseous muscle. Tests at each 45° increment through a circle of 360° demonstrated that that the largest MEPs elicited from the left M1 occurred at an angle of about 50° from the parasagittal line.[127] Another study conducted by Brasil-Neto et al. (1992) found 236.3° (corresponding to about 56.6° from the parasagittal line) to elicit the largest MEPs from the right M1. Recent modeling studies confirm that the coil orientation is an important factor that affects the depth and strength of penetration of the electrical field induced by a single TMS pulse.[129] An

extensive review of potential TMS mechanisms argues that the optimal coil orientation induces current flow in a posterior to anterior direction across the central sulcus and excites horizontal fibers preferentially at a site where the fibers bend down into the central sulcus.[130] Thus most TMS studies that target intrinsic hand muscles refer to holding the coil at a 45º angle to the parasagittal line with the handle pointing posterolaterally to induce a current flow in the posterior to anterior direction across the central sulcus.

2.4.3 TMS Measures of Corticospinal & Intracortical Excitability

The D- and I-waves elicited by TMS provide evidence that cortical pyramidal neurons can be directly or indirectly activated by an external stimulus. With contributions from extrapyramidal pathways as well as mono and poly-synaptic activation of alpha motoneurons in the spinal cord, the D-wave contributes to the creation of a motor-evoked potential (MEP). The MEP is a biphasic trace captured using electromyography (EMG) recordings from a muscle of interest. Resulting from suprathreshold single-pulse TMS, the MEP is an easily accessible measure of corticomotor pathway excitability. Alterations in the average peak-to-peak amplitude of a group of MEPs provide measures of facilitation (i.e. an increase in amplitude) or suppression (i.e. a decrease in amplitude).

The primary method of measuring neuroplasticity in the human motor cortex is through changes in neural pathway excitability as measured by paired- or single-pulse TMS.[110,131] Several questions can be answered by using either technique to assess the change in peak-to-peak MEP amplitude. Research questions of mechanism (e.g. what networks are modulated by PAS?) benefit from paired-pulse measures whereas questions of gross influence and change in corticospinal excitability benefit from single-pulse measures. Common paired-pulse techniques provide measures of intracortical excitability by testing glutamatergic or GABAergic network activity. Tests of intracortical facilitation (ICF) use a subthreshold TMS pulse followed 10-15 ms later by a suprathreshold TMS pulse to measure glutamatergic network activity.[59,60] Tests of short intracortical inhibition (SICI)

use the same unilateral paired-pulse set-up but the interval is typically 1-6 ms which primarily tests $GABA_A$ network activity.[59] Another test known as the contralateral or cortical silent period (CSP) measures $GABA_B$ network activity by assessing the length of interruption of corticospinal motor activity when a suprathreshold TMS pulse is given during voluntary muscle contraction.[132] Finally, interhemispheric inhibition (IHI) is tested using a bilateral paired-pulse technique where a coil is held over the primary motor area (M1) of each hemisphere. The most common test for IHI uses an interstimulus interval of 10 ms and assesses the transcallosal $GABA_B$-mediated inhibitory influence exerted by the first M1 onto the second M1.[54] Several variations of the aforementioned tests exist (see[133] for a review). However, this dissertation focuses on a question of gross change in corticospinal excitability and thus utilizes single-pulse TMS measures only.

2.4.4 Reliability of TMS Measures

Paired-pulse TMS measures provide information regarding the potential mechanisms of action that underlie neurotransmitter-mediated changes in neuroplasticity. However, the reliabilities of ICF, SICI and IHI are inconsistent. A study conducted by Du et al. (2013) tested a range of interstimulus intervals for SICI and ICF in twenty-three healthy individuals at two different time points. Both measures exhibited moderate-good test retest reliability indicating low within-individual variability but the optimal interstimulus interval to elicit peak SICI or ICF values differed considerably between individuals. Another study supports the reproducibility of within-individual SICI measures but not ICF.[135] Yet two other studies indicate poor test-retest reliability for SICI measures.[136,137] With the inconsistent reliability findings, measures of SICI and ICF are difficult to interpret. Cassidy et al. (2016) investigated the reliability of IHI in people with stroke and found that measures of IHI from contralesional M1 to ipsilesional M1 had moderate-strong reliability whereas measures from ipsilesional M1 to contralesional M1 had poor or invalid reliability. Unlike paired-pulse measures, single-pulse measures of MEP amplitude garner more agreement from reliability studies. Peak-to-peak MEP amplitudes show good test-retest reliability[139] and groups of 10, 15 and 20 MEPs collected at 120%

resting motor threshold in healthy individuals show a good reliability which is stronger than those collected at 1mV threshold.[140]

2.4.5 Neuronavigation and TMS

Neuronavigation is a method of guiding neurological procedures or measures with the help of imaging and/or 3D motion capture. Uses include pre- and intra-operative navigation (e.g. during the resection of brain tumors), navigated TMS testing, navigated TMS interventions and navigated TMS motor mapping.[141] The primary argument for using neuronavigation with TMS is to decrease variability of MEP measures. However, the evidence for reducing MEP variability with the use of neuronavigation is mixed. Julkunen et al. (2009) compared navigated and non-navigated motor thresholds and MEP amplitudes recorded from the right abductor pollicis brevis muscle in eight healthy individuals across two sessions. Although motor thresholds were similar regardless of navigation, MEPs had consistently higher amplitudes and shorter latencies with navigation.[142] Jung et al. (2010) conducted a similar study but took a stronger approach to address MEP variability by assessing reproducibility and coefficients of variance for MEP amplitudes. MEP amplitudes recorded from abductor pollicis brevis were assessed using navigated and non-navigated TMS at three different sessions in eight healthy individuals. There was no difference in MEP amplitudes or coefficients of variance between sessions or between navigated and non-navigated TMS use. Furthermore, MEP amplitudes were stable and reproducible with navigated and non-navigated TMS. Authors concluded that navigation does not reduce variability or improve reproducibility of MEP amplitudes.[143] Another study conducted by Gugino et al.(2010) assessed MEP amplitudes, areas and coefficients of variance following navigated and non-navigated TMS targeting the first dorsal interosseous muscle in five healthy individuals. Similar to Julkunen et al. (2009), Gugino et al. (2010) found that MEP amplitudes and areas were larger with navigated TMS but, importantly, the coefficients of variance did not differ between navigated and non-navigated TMS. Thus, the use of neuronavigation did not reduce the variability of MEP amplitudes.[144] Although the claim that neuronavigation can

reduce variability of TMS evoked MEPs is so far unsubstantiated, it is still the desired approach for the publication of results using TMS testing. Despite limitations of unconfirmed benefits, time-consuming procedures and extra equipment and training needed for neuronavigation, investigators and reviewers are drawn by the ~2mm coil placement accuracy and tend to feel more confident when neuronavigation is used for TMS procedures.

2.4.6 Using TMS to Modulate Excitability

In addition to measuring changes in corticospinal excitability, TMS can be used to induce changes in excitability by promoting LTP- or LTD-like plastic changes within neural networks. Two common methods of neuromodulation using TMS are rTMS and theta burst stimulation (TBS). These methods each use repeated cortical stimuli to drive changes in neuroplasticity but they differ in frequencies and patterns of stimulation. In the early 1990s, rTMS was developed as a means of investigating hemispheric language dominance and finding the epicenter of epileptogenic activity. An early study conducted by Pascual-Leone et al. (1994) described the influence of different frequencies and intensities of rTMS on motor activity in fourteen healthy individuals and laid the groundwork for protocol development and safety guidelines. Subsequent studies defined the suppressive influence of rTMS on corticospinal excitability following low frequency stimulation (e.g. 1 Hz)[146] as well as the facilitatory influence on excitability following high frequency stimulation (e.g. ≥1 Hz).[147] TBS is considered a form of patterned rTMS. Unlike the effects of rTMS which are primarily frequency-dependent, the effects of TBS are primarily pattern-dependent where continuous stimulation suppresses corticospinal excitability and intermittent patterns facilitate excitability.[148]

The neuromodulation induced by repeated TMS protocols appears to result from changes in LTP- and LTD-like neuroplasticity. A pharmacological study conducted by Huang et al. (2007) found that memantine, an NMDAR blocker, abolished the facilitatory and suppressive effects of TBS. The NMDAR-dependency of TBS after-effects suggests that

the underlying mechanism of action involves LTP- and LTD-like changes in neuroplasticity. A Cochrane Review of rTMS investigations supports the strength of evidence that TMS-induced changes in neuroplasticity are mediated by LTP- and LTD-like mechanisms.[13] Although rTMS and TBS both demonstrate an ability to alter corticospinal excitability and induce LTP- or LTD-like plasticity, their transition to the clinic has been fraught with challenges partially due to the lack of a clear understanding of how they work and how they may be influenced by unknown variables.[150] The development of a third method of TMS-induced neuromodulation called paired associative stimulation (PAS) provides another option for the induction of LTP- and LTD-like neuroplasticity that may be more effective and better harness natural mechanisms of experience-dependent plasticity.

2.4.7 Paired Associative Stimulation

In an effort to mimic protocols inducing associative LTP in animal models, Stefan et al. (2000) paired a peripheral nerve electrical stimulus with TMS and created a new method of NIBS, PAS. Similar to rTMS and TBS, PAS induces LTP- and LTD-like uni-directional changes in synaptic plasticity that are either facilitatory or suppressive. However, PAS differs in the mechanism of induction underlying plastic changes. Critically dependent on the timing between and order of peripheral and cortical stimuli, PAS is theorized to induce LTP-like and LTD-like changes through Hebbian STDP.[65] Thus, a TMS pulse applied to the motor cortex shortly *before* the arrival of a sensory action potential, resulting from peripheral nerve stimulation, tends to induce a suppressive aftereffect, whereas a TMS pulse shortly *after* the arrival of a sensory action potential tends to induce a facilitatory aftereffect.[66] Early studies demonstrated this hallmark characteristic of PAS-induced plasticity by testing the effect of different interstimulus intervals (ISIs) between peripheral and cortical stimuli on corticospinal excitability. The first study conducted by Stefan et al. (2000) tested ISIs ranging from 25 ms to 5000 ms and found that an ISI of 25 ms facilitated excitability and those above 100 ms had no effect. A subsequent study performed by Wolters et al. (2003) was the first to

demonstrate the ability of PAS to suppress corticospinal excitability using an ISI of 10 ms. The importance of timing between cortical and peripheral stimuli indicate that a Hebbian-like STDP may underlie the effects of PAS. Further evidence derived from pharmacological studies support the likelihood that PAS is STDP-mediated. Application of the NMDAR antagonist dextromethorphan blocks the facilitatory and suppressive effects of PAS, suggesting that the neuromodulatory effects are NMDA-dependent and thus LTP- and LTD-like.[66,151] The timing-dependent induction of LTP- and LTD-like plasticity strongly aligns with the nature of STDP and thus supports STDP as the leading candidate mechanism for PAS. The location of STDP is also theorized to be at the level of the cortex. Stefan et al. (2002) conducted a series of experiments that tested the F-wave, a measure of spinal motoneuron excitability, before and after facilitatory PAS. Following intervention, MEP amplitude increased but the F-wave (elicited using median nerve stimulation) was unchanged suggesting that cortical and not spinal input primarily drove the increase in MEP amplitude.[151] An extensive review of evidence[65] supports the argument that PAS induces STDP in the motor cortex. This finding is important for the potential applications of PAS because STDP underlies motor learning and thus motor recovery through principles of experience-dependent plasticity. The ability to measure or augment an individual's capacity for STDP may provide prognostic information or improve motor recovery strategies.

2.4.8 PAS for Neurorehabilitation

Since the inception of PAS, it has been used to measure or modulate neuroplasticity in people with stroke,[14,71,152] Parkinson's disease,[153] and focal hand dystonia.[40] In a recent study, the use of PAS in people with dysphagic stroke outperformed rTMS in both excitability and behavioral measures. Michou, et al. (2014) compared peripheral electrical stimulation only, PAS and rTMS in 18 dysphagic stroke patients. Results indicated that corticobulbar excitability and swallowing function increased to a greater extent following the two protocols providing afferent input (peripheral electrical stimulation only and PAS) compared to repeated cortical input only (rTMS).[14] Further

evidence comparing PAS to sensory input only (vibration) and motor practice (thumb movements) demonstrated that PAS enhances corticospinal excitability more than sensory input alone.[154] Thus, the combination of both cortical and peripheral stimuli in the form of PAS appears to be a more potent approach to neuromodulation compared to repeated cortical or peripheral stimuli alone.

In the context of neurorehabilitation, primary somatosensory cortical function is known to influence motor recovery. Recent reviews highlight neuroanatomical substrates, pathways and potential mechanisms of plasticity within the primary sensory cortex (S1) that may influence motor function.[155,156] Borich et al.(2015) and Fox et al. (2009) argue that further characterization of S1 and its role in motor control are needed to progress neurorehabilitation practices. Findings from studies investigating PAS corroborate these arguments by demonstrating improved motor function[14] and motor learning[157] following PAS intervention, but there is no clear indication of what sensorimotor pathways are being utilized. Although the importance of sensory input, in combination with a primary motor cortical stimulus, is evident, the mechanisms supporting this advantage are unclear. Carson and Kennedy (2013) highlight a number of candidate pathways including cortico-cortico, cerebello-thalamo-cortical and thalamo-cortical pathways that may mediate observed changes in primary motor cortex excitability induced by PAS, but no firm consensus has been reached.

Based on the arguments that PAS asserts its effect using Hebbian STDP and capitalizes on afferent input, its potential as a safe and effective NIBS approach to improve motor function and learning is clear. In addition to the evidence from individuals with dysphagic stroke, several groups have investigated the ability of PAS to alter excitability of corticomotor pathways targeting the paretic lower and upper limbs of people with stroke. Jayaram and Stinear (2008, 2009) conducted a series of studies assessing the influence of suppressive PAS targeting the contralesional tibialis anterior muscle in ten followed by nine individuals with chronic stroke. MEP recordings from the ipsilesional

and contralesional tibialis anterior confirmed that suppressive PAS decreased excitability of the ipsilesional muscle and increased excitability of the contralesional muscle.[70,152] Uy et al. (2003) investigated the alternative approach of applying facilitatory PAS to the ipsileional M1 and paretic tibialis anterior. Although group analyses showed no effect, individual analyses showed that five out of nine individuals exhibited an increase in corticospinal excitability targeting the paretic tibialis anterior. Furthermore, functional measures of cadence, stride length and time to heel strike improved by the fourth week of intervention.[10] No point is made regarding the correlation between individuals with significantly increased excitability and those with significantly increased functional measures but the improvement in both excitability and function is a promising trait for PAS as an adjunct to neurorehabilitation. Castel-Lacanal et al. (2007, 2009) studied the influence of facilitatory PAS on corticospinal projections to the upper limb of individuals with stroke. In two studies, authors found that corticospinal excitability increased following facilitatory PAS at 5 months and, to a lesser extent, 12 months following subcortical stroke.[71,159] Thus, time since stroke may impact the efficacy of PAS interventions. The ability for PAS to alter excitability and improve function in people with stroke is established. However, certain characteristics of intervention (e.g. the optimal protocol, timing of intervention, number of sessions) still need to be elucidated. The challenge of defining PAS for applications to neurorehabilitation begins with exploring different protocols that capitalize on natural mechanisms of neuroplasticity.

2.5.0 NIBS & Metaplasticity

The ability to capitalize on metaplasticity to improve efforts of neuromodulation and motor recovery is under investigation in human motor cortex using NIBS. Recent reviews illustrate the usefulness of priming motor cortex using rTMS, PAS and tDCS.[43,160] Developing a method of priming synaptic plasticity with rTMS began in healthy individuals. By providing a facilitatory (6 Hz) session of rTMS immediately followed by a depressive (1 Hz) session of rTMS, Iyer et al. (2003) demonstrated a method of priming that resulted in an increase of suppression of corticospinal excitability when compared to

suppressive (1 Hz) rTMS alone. This seminal study supports the argument that priming may be a better method of neuromodulation because of its enhanced effect on corticospinal excitability compared to unprimed rTMS. Subsequent studies used methods of priming to test homeostatic mechanisms of plasticity. Siebner et al. (2004) applied facilitatory (anodal) tDCS followed by suppressive (1 Hz) rTMS, finding excessive suppression of excitability. Authors also applied suppressive (cathodal) tDCS followed by suppressive (1 Hz) rTMS and found excessive facilitation.[161] These results indicate that homeostatic mechanisms do exist within primary motor cortex and that there is a limit to their physiological range, as demonstrated for suppression. Importantly, these studies also illustrate the influence that previous levels of synaptic activity may have on response to neuromodulation. Thus, priming to capitalize on metaplasticity may, in a sense, standardize the state of the brain, increasing the predictability of response to neuromodulation.

2.5.1 Priming with Paired Associative Stimulation

Purposeful priming of synaptic plasticity to induce a stronger change in synaptic weighting has been investigated using PAS. Although the earliest studies of priming and PAS used motor learning tasks as the method of priming and followed this with a bout of (principal) PAS, they demonstrate an ability of previous synaptic activity to influence response to neuromodulation.[162,163] Currently, two studies have investigated homeostatic metaplasticity through the use of PAS as the method to both prime and condition motor cortex. Muller et al. (2007) investigated primed versus unprimed facilitatory PAS by priming with (1) PAS_{LTD}, (2) PAS_{LTP} and (3) PAS_{SHAM}. The principal treatment was always PAS_{LTP}. From these studies, investigators found increased corticospinal excitability following PAS_{LTD} priming and PAS_{LTP} principal when compared to all other conditions. Similar to Siebner et al. (2004), this study also found a decrease in excitability following PAS_{LTP} priming and PAS_{LTP} principal, indicating a physiological limit of homeostatic plasticity, this time demonstrated in facilitation. Opie et al. (2017) investigated the effect of PAS priming and principal in young (20-27 years) and old (61-

79 years) healthy individuals. Again, the combinations began with a priming bout of PAS that was either (1) PAS_{LTP}, (2) PAS_{LTD} or (3) control and always ended with principal PAS_{LTP}. Interestingly, the PAS_{LTP} priming followed by PAS_{LTP} principal intervention resulted in the largest MEPs for the younger individuals and the PAS_{LTD} priming followed by PAS_{LTP} intervention was no different from the unprimed PAS_{LTP} (i.e. control) intervention. Older adults exhibited the opposite response to PAS_{LTP} priming followed by PAS_{LTP} principal in that MEPs were significantly smaller compared to the other conditions.[164] These results indicate that priming with PAS_{LTP} augmented MEP amplitudes in younger adults but not older adults. Thus, age may influence response to PAS. Furthermore, the increased amplitude in younger adults following PAS_{LTP} priming of PAS_{LTP} principal PAS is unexpected and opposes previous evidence that further facilitatory intervention results in a suppression of excitability.

Two other studies assessed priming and principal PAS but did not assess the opposing interactions of PAS_{LTD} followed by PAS_{LTP}. Each of these studies applied two sessions of PAS_{LTP}. One study varied the time between priming and principal (10, 30, 60 and 180 minutes) and found that after 30 minutes, a principal session of PAS_{LTP} will augment corticospinal excitability.[165] The other study compared two sessions of PAS_{LTP} (60 minutes apart) to (1) sham priming followed by PAS_{LTP} and (2) PAS_{LTP} followed by sham PAS. The results of this study indicate that two sessions of PAS_{LTP}, separated by 60 minutes, augmented corticobulbar plasticity in dysphagic stroke patients more so than a single bout of PAS_{LTP}. Of note, this study also found that more subjects responded (as defined by an increase in corticobulbar excitability) following the two sessions of PAS_{LTP} compared to a single session.[166] Although a secondary finding of the study, this underscores the argument that priming to standardize a brain-state may not only improve an individual's response to neuromodulation, but increase the likelihood of an individual responding in a predicable manner.

Although each study investigating priming of motor cortex significantly contributes to the understanding of homeostatic and non-homeostatic mechanisms that may underlie observed changes in neuroplasticity, a large gap in the literature exists. Only two studies have addressed the hypothesis that opposing directions of priming and principal PAS may capitalize on homeostatic mechanisms of metaplasticity and improve the magnitude of response to neuromodulation. Furthermore, no published study addresses the question of how priming with PAS_{LTP} may influence the after-effects of PAS_{LTD}.

2.6.0 Variables Influencing Response to NIBS

Although it is widely accepted that certain NIBS protocols induce facilitation or suppression of corticospinal excitability, it is also clear that variability in response reduces predictability and observed effect sizes. A recent study found that only 51.9% of healthy participants responded to PAS in the predicted manner (i.e. increased excitability following PAS_{LTP}) whereas the remaining 48.1% responded in the opposite manner.[167] A similar study investigating inter-individual variability in response to PAS, tDCS and TBS found that only 39%, 45% and 43% of people, respectively, responded in the predicted manner.[8] These studies identified resting motor threshold, 1mV threshold, SICI and age as influential variables in determining an individual's response. However, these variables alone do not provide a detailed enough measure of underlying variables that may influence measures of resting motor threshold and 1 mV threshold. In a letter to the editor of *Brain Stimulation*, Ulf Ziemann (2015) argues that the low to moderate effect sizes reported in NIBS literature are a result of high inter-subject or inter-session variability. The inability to correctly predict that an intervention will work (e.g. that PAS_{LTP} will increase excitability) diminishes confidence in published NIBS results and slows progression of NIBS as a clinical tool. To strengthen the argument for PAS and other forms of NIBS, variables informing individual profiles of likelihood of being a responder need to be defined.

Considering that TMS interventions act to modulate synaptic plasticity through LTP- and LTD-like mechanisms, it is logical to begin with an assessment of variables known to influence synaptic plasticity. Prior studies have established factors such as age,[164,167] prior synaptic activity,[168] time of day (i.e. cortisol levels),[169] menstrual cycle,[170] medication,[171] attention[172] and BDNF[73] as factors that may explain an individual's response to TMS. Of particular interest to the investigation of responders and nonresponders following PAS, the presence of a BDNF Val66Met single nucleotide polymorphism may explain why some individuals respond predictably to PAS whereas others do not. Cheeran et al., (2008) demonstrated that individuals without the Val66Met polymorphism exhibited significantly different changes from baseline MEP amplitude following PAS and those with the polymorphism did not exhibit any significant changes. Considering that the Val66Met polymorphism is fairly common in the Caucasian population (35%) and that many PAS studies to-date are conducted in Caucasian-dominant populations, it is possible that a portion of the reported high variability in response may be explained by genetic differences. Research conducted in cultured rat visual cortical cells provides foundational rationale for the role of BDNF in neuroplasticity. Rutherford et al. (1998) showed that the activity-dependent release of BDNF influences synaptic scaling and thus plays a role in the regulation of homeostatic plasticity. Further studies by Desai et al. (1999) show that BDNF mediates the activity-dependent regulation of intrinsic excitability of pyramidal neurons and interneurons possibly by altering the activity of sodium and potassium channels. A change in BDNF activity due to a genetic polymorphism may influence intrinsic excitability and thus establish a completely different pattern of response to neuromodulation efforts. The Val66Met polymorphism is demonstrated to influence responses to PAS[73] as well as MEP amplitudes and cortical reorganization following motor training.[175] Further characterization of variables like BDNF that are easily measured, reported and interpreted is a strong and viable route for beginning the creation of a portfolio of individual measures that will inform providers of who may or may not benefit from NIBS interventions.

Hamada et al. (2012) identified MEP latency as a factor that may describe an individual's response to neuromodulation. Authors defend the rationale that variability in response to TMS interventions may not be due to differences in synaptic plasticity. Instead, they may be a result of differences in populations of cortical neurons that are excited due to differences in the excitability of different neural populations. MEPs were collected with the typical coil orientation (inducing posterior-anterior current flow), the coil turned around 180° (inducing anterior-posterior current flow) and the coil at a 90° angle with the mid-sagittal line (inducing lateral-medial current flow). Two different interventions were applied: inhibitory TBS and facilitatory TBS. Although responses to each intervention were highly variable, 50% of this variability was explained by the difference between MEP onset latencies resulting from anterior-posterior stimulation and lateromedial stimulation. Because this difference in onset latencies depicts a difference in the recruitment of early I-waves vs. later I-waves, authors conclude that this difference is likely due to differences in the pools of interneurons that are recruited.[176]

2.7.0 Summary

Neuroplasticity is a driving force that supports learning of motor tasks. When individuals undergo rehabilitation to recover lost or impaired motor function, they are effectively re-learning motor patterns and motor tasks. Although the neural environment is altered following a cortical or sub-cortical injury, the brain remains plastic and events like cortical reorganization allow for continued compensation or restoration of re-learned motor patterns. However, current rehabilitation strategies are less than phenomenal, leaving a high percentage of individuals with impairments that impact activities of daily living. Progression of technologies that measure and modulate cortical activity as well as a better collective understanding of principles of experience-dependent plasticity led to the development of neuromodulation therapies as an adjunct to motor rehabilitation. PAS specifically stands out as a promising neurorehabilitative tool because it induces STDP which underlies experience-dependent plasticity and the addition of sensory input is shown to be more effective than cortical stimuli alone. Currently, high inter-individual

variability is reported following PAS in people with stroke as well as healthy individuals. This variability makes it difficult to interpret the efficacy of PAS. Furthermore, characteristics of who does and does not benefit from PAS have not been clearly defined. One proposed solution is to incorporate primed PAS (PPAS) to capitalize on mechanisms of homeostatic synaptic plasticity and augment response to PAS. Although primed facilitatory PAS ($PPAS_{LTP}$) has been thinly addressed, investigations into primed suppressive PAS ($PPAS_{LTD}$) are even less thorough. Thus, a complete investigation into the efficacy of all combinations of $PPAS_{LTP}$ and $PPAS_{LTD}$ and the exploration of characteristics that may influence an individual's response to each protocol is warranted.

3.0.0 Methods

3.1.0 Study Design

This study was composed of two Experiments. Experiment 1 assessed the effect of priming on suppressive PAS ($PPAS_{LTD}$) and Experiment 2 assessed the effect of priming on facilitatory PAS ($PPAS_{LTP}$). Enrolled participants were randomly assigned to a four-intervention cross-over within either Experiment 1 or Experiment 2. Each experiment was identical in design yet differed in the type of intervention that was given. Thus, two single-blinded, sham-controlled, two-factor repeated-measures cross-over designs were used to assess the $PPAS_{LTD}$ (Experiment 1) and $PPAS_{LTP}$ (Experiment 2) in healthy individuals. Participants came to the Noninvasive Neuromodulation Lab at the University of Minnesota – Twin Cities Delaware Clinical Research Unit on four separate days. Each day, the participant received baseline TMS testing, a randomly assigned intervention and one hour of TMS post-testing. A total of four interventions were applied to each individual with at least one week of washout between interventions (Figure 1). Regardless of assigned experiment, the sham-primed intervention was the first intervention for every participant due to the desire to ensure that adequate numbers of both responders and nonresponders to sham-primed PAS were enrolled. The remaining three interventions were randomized. A third-party individual randomized intervention orders and assigned labels (e.g. 1A, 1B, 1C and 1D) using Microsoft Excel. Table 1 lists the interventions for Experiment 1 and for Experiment 2. The participants and the individual completing TMS baseline testing and posttesting (i.e. the tester) were blinded to the assigned intervention. The same individual conducted all baseline tests and posttests. The individuals delivering the intervention were not blinded to the nature of the assigned intervention.

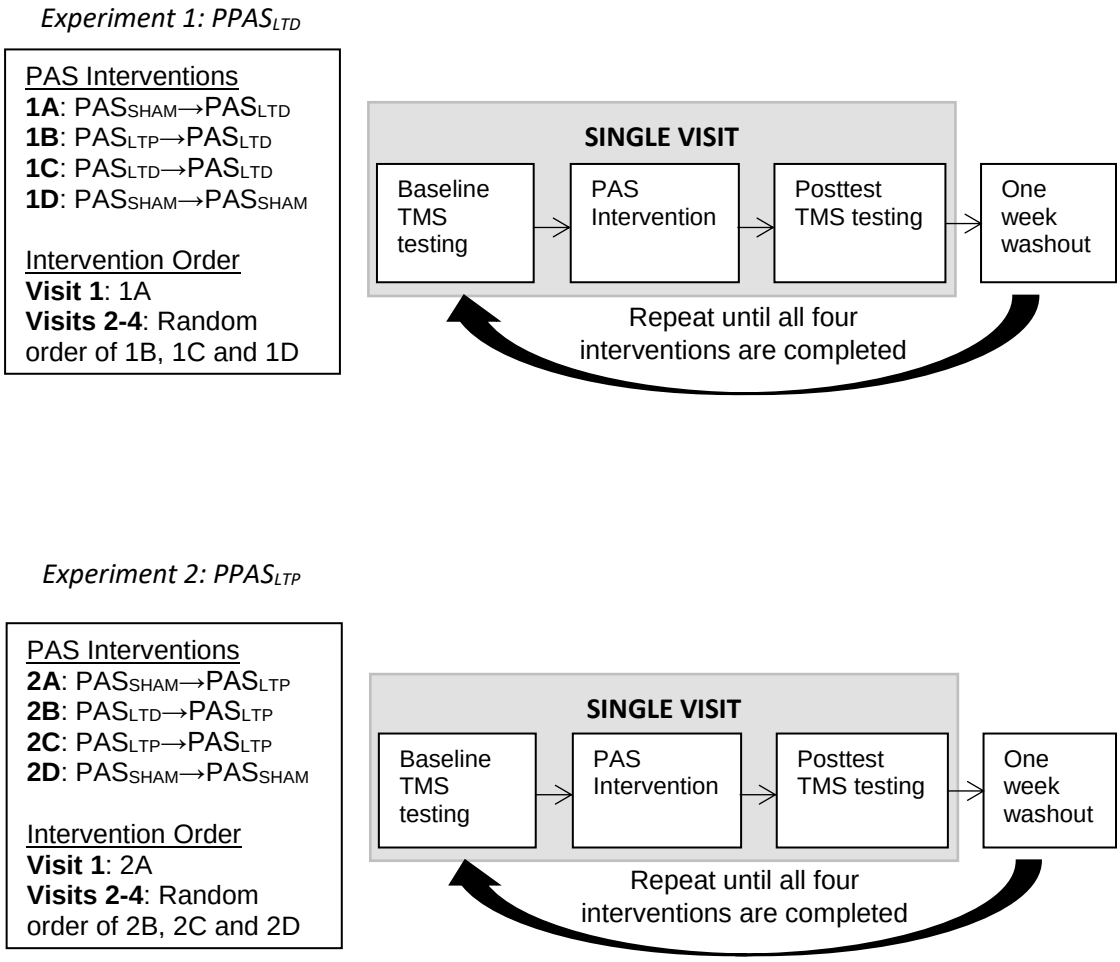

Figure 1. Study design for Experiment 1 (top) and Experiment 2 (bottom).

Table 1. List of Interventions

	Experiment 1	**Experiment 2**
Sham-primed Intervention	Sham priming of PAS_{LTD} (PAS_{SHAM}→PAS_{LTD})	Sham priming of PAS_{LTP} (PAS_{SHAM}→PAS_{LTP})
Primed Intervention 1	PAS_{LTP} priming of PAS_{LTD} (PAS_{LTP}→PAS_{LTD})	PAS_{LTD} priming of PAS_{LTP} (PAS_{LTD}→PAS_{LTP})
Primed Intervention 2	PAS_{LTD} priming of PAS_{LTD} (PAS_{LTD}→PAS_{LTD})	PAS_{LTP} priming of PAS_{LTP} (PAS_{LTP}→PAS_{LTP})
Sham Intervention	Sham priming of Sham PAS (PAS_{SHAM}→PAS_{SHAM})	Sham priming of Sham PAS (PAS_{SHAM}→PAS_{SHAM})

3.2.0 Prospective Power Analyses

This study was a novel exploratory investigation of the effect of priming on PAS. Considering the few published $PPAS_{LTP}$ and zero published $PPAS_{LTD}$ studies, determining a prospective sample size to achieve 80% power required a broad approach. Normative data regarding the percent change from baseline of MEP amplitude following unprimed PAS in healthy individuals have been established [64]. These data specify the effect size (cohen's *d*) for PAS_{LTP} and PAS_{LTD} at 0-5, 10, 15, 20, 30, 60, 90 and 120 minutes following intervention. However, they do not specify the effect size for a difference between primed and unprimed PAS. At the time of the prospective analyses, one previous study had investigated priming with PAS_{LTD} followed by principal PAS_{LTP}. This study found that PAS_{LTP} alone increased peak-to-peak MEP amplitude by 26% whereas priming with PAS_{LTD} followed by PAS_{LTP} principal resulted in a 43% increase. This is a difference of 17%, but no standard error or deviation of this difference were reported.[68]

Two power analyses were conducted using G*Power (Heinrich Heine Universitat, Dusseldorf, DE). The input for these power analyses were based on the previous study finding a difference of 17% for primed PAS_{LTP} and the normative data described by Wishcnewski et al. (2015). The standard deviation for each analysis was derived from normative data established for the unprimed intervention PAS_{LTD} for Experiment 1 and PAS_{LTP} for Experiment 2. Analysis using the repeated measures ANOVA was chosen based on the primary question of this study which is the effect of Primed Intervention 1 and Primed Intervention 2 compared to the Sham Intervention. G*Power input and output variables are available in Appendix A. Results from these analyses indicate that a sample size of 6 individuals per group (i.e. per intervention and thus per cross-over experiment) are needed for PAS_{LTP} and a sample size of 6 per group are needed for PAS_{LTD}. Due to the exploratory nature of this study as well as the studies from which these power analyses are based, the sample size was increased to at least 15 people per experiment in an effort to find a potentially smaller effect size and to account for a roughly 50-50 mix of responders and nonresponders in each experiment.

3.3.0 Participants

3.3.1 Recruitment & Eligibility Screening

Recruitment efforts included flyers posted in buildings at the University of Minnesota – Twin Cities campus and in-class presentations given through the University of Minnesota Division of Physical Therapy. Interested individuals were encouraged to provide an e-mail address or to send an e-mail to the Student Principal-Investigator. To screen individuals for eligibility, the investigator e-mailed an eligibility survey through the University of Minnesota's Research Electronic Data Capture (REDCap) system. REDCap is a secure system that was used to track the number of interested individuals, the number of surveys sent, the number of surveys completed and the nature of survey responses. The survey itself was composed of questions to screen each potential participant for inclusion/exclusion criteria and general TMS safety guidelines [177,178] (Appendix B).

3.3.2 Inclusion & Exclusion Criteria

Inclusion criteria:

- ≥ 18 and ≤ 30 years of age
- Elicitable motor evoked potential from the motor cortex contralateral to the preferred hand
- Elicitable N20 sensory evoked potential

Exclusion criteria:

- History of neurological disease
- Seizure within the past 2 years
- Currently taking epileptogenic medication
- Peripheral neuropathy
- Cognitive impairment and/or major psychiatric disorder
- Metal in the head (dental permitted)
- Pacemaker or other indwelling devices
- Pregnancy

3.3.4 Consent Process

Eligible individuals were invited to the University of Minnesota's Noninvasive Neuromodulation Lab for an in-person consent process. At this visit, the investigator described the purpose of the study and the procedures that would be required. To ensure that the potential participant was adequately informed, the investigator and the participant talked through each section of the consent form (Appendix C). If the participant chose to enroll in the study, the participant and the investigator signed and dated the consent form. Once enrolled, the participant was given a copy of the consent form for his/her record.

3.3.5 On-site Surveys & Authorization Forms

Following the consent process, surveys and authorization forms were discussed and completed. The Edinburgh Handedness Survey [179] (Appendix D) assessed for the preferred hand of each participant. The Health Insurance Portability and Accountability Act authorization form (Appendix E) ensured that the participant understood that the information we were collecting could be considered protected health information however we did not obtain medical records for any individual. The Photograph Release Form (Appendix F) was signed only if an individual consented to the taking of photos and the potential use of photos taken of that individual for scientific purposes. Individuals could participate in this study without signing this form.

3.4.0 Responder & Nonresponder Characteristic Testing

3.4.1 Responder & Nonresponder Classifications

Individuals were categorized as responders or nonresponders to the sham-primed intervention. For Experiment 1 ($PPAS_{LTD}$), responders were classified as having a ratio of grand average post-intervention to average baseline MEP amplitude of ≤ 0.9. All other individuals were classified as nonresponders. For Experiment 2 ($PPAS_{LTP}$), responders had a ratio of ≥ 1.1.[165] Unless otherwise noted, responder and nonresponder classifications for analyses were based on the response to the sham-primed intervention for each experiment.

3.4.1 MEP Onset Latency

The MEP onset latency may relate to an individual's response to NIBS because of a difference in the pools of neurons that are stimulated. Following the TMS pretest of the first visit only, 10 MEPs were collected using the same TMS testing and EMG set-up described in section 3.5 but the TMS coil was turned 180° so that the tail pointed anteromedially and the induced current flow across the central sulcus was anterior-posterior. The onset latency was defined as the amount of time (ms) from the TMS stimulus artifact to the beginning of the take-off of the MEP using the EMG recording.

3.4.2 Saliva Sample for Genotyping

During the consent process, participants agreed to provide a saliva sample that would be tested for the presence of the BDNF Val66Met single-nucleotide polymorphism. At the end of the first visit, each participant provided a ~2 mL saliva sample using Oragene Discover 500 Saliva Tubes and Sample Kits (DNA Genotek, subsidiary of OraSure Technologies, Inc., PA,USA). At the end of the study, all samples were sent to the University of Minnesota Genomics Center where DNA was extracted and a subsequent Taqman Assay assessed presence of the Val66Met polymorphism (see Appendices G and H for DNA extraction quality control and Taqman results reports, respectively).

3.5.0 Corticospinal Excitability Testing

The primary outcome measure for both Experiment 1 and Experiment 2 is change from baseline corticospinal excitability. Change from baseline corticospinal excitability was assessed using the average peak-to-peak amplitude of 20 MEPs collected from the abductor pollicis brevis (APB) of the preferred hand. Single-pulse TMS elicited MEPs at baseline and 0, 10, 20, 30, 40, 50 and 60 minutes following intervention (Figure 2).

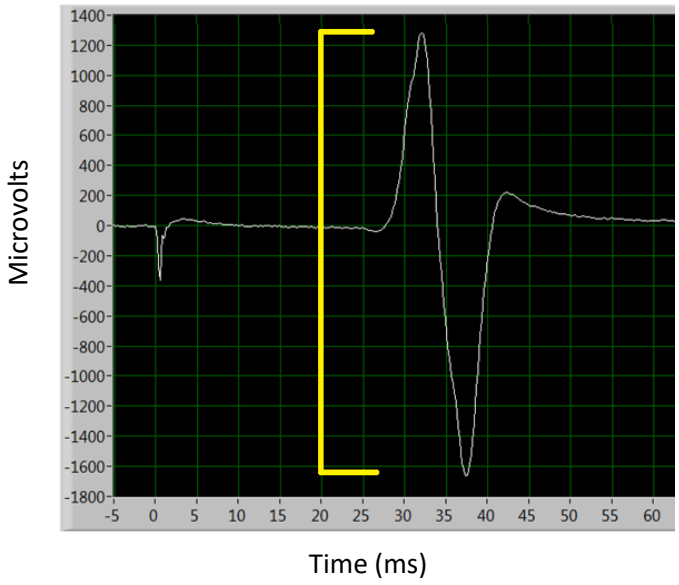

Figure 2. Example EMG recording of a motor-evoked potential recorded from abductor pollicis brevis following a single transcranial magnetic stimulus. The yellow brackets illustrate the peak-to-peak amplitude (2,970 µV)

3.5.1 Single-pulse TMS Instrumentation & Methodology

During TMS testing and PAS intervention, the participant sat in a chair in a semi-reclined position. The participant was offered ear plugs to reduce the level of noise experienced from the TMS coil. TMS was applied with a D70^2 TMS coil connected to a Magstim 200^2 stimulator with a Bistim^2 connecting module (Magstim Co., Whitland, UK). The cortical hotspot for the APB was defined as the location where single-pulse TMS produced at least 5 out of 10 MEPs with a peak-to-peak amplitude of at least 50μV at the lowest possible stimulator output. The corresponding stimulator output defines the resting motor threshold (RMT) in percent maximum stimulator output (%MSO). All testing and interventions were completed with a TMS intensity of 130% RMT. To collect 20 MEPs at each baseline and posttest time point, single-pulse TMS (0.1 Hz, 130% RMT) was delivered to the hotspot for APB corresponding to the preferred hand.[180] MEPs that were compromised by neuromuscular pre-innervation were discarded.

3.5.2 Neuronavigation

All TMS testing and interventions were completed with the guidance of neuronavigation. Because we did not have access to individual anatomical magnetic resonance images, BrainSight neuronavigation was used in conjnction with a template brain (Rogue, Toronto, CA). At the beginning of each session, a coil-tracker was placed on the coil and calibrated using the BrainSight calibration block (Figure 3A). In anticipation of coil-overheating and the need for the use of a second coil, a second coil-tracker was placed on a back-up coil and calibrated. A subject-tracker was also placed on the forehead of the participant. With the subject-tracker in place, the dimensions of the participant's head were registered with the template brain. The TMS coil locations, in reference to the subject-tracker, were saved for each hotspot and testing location (Figure 3B).

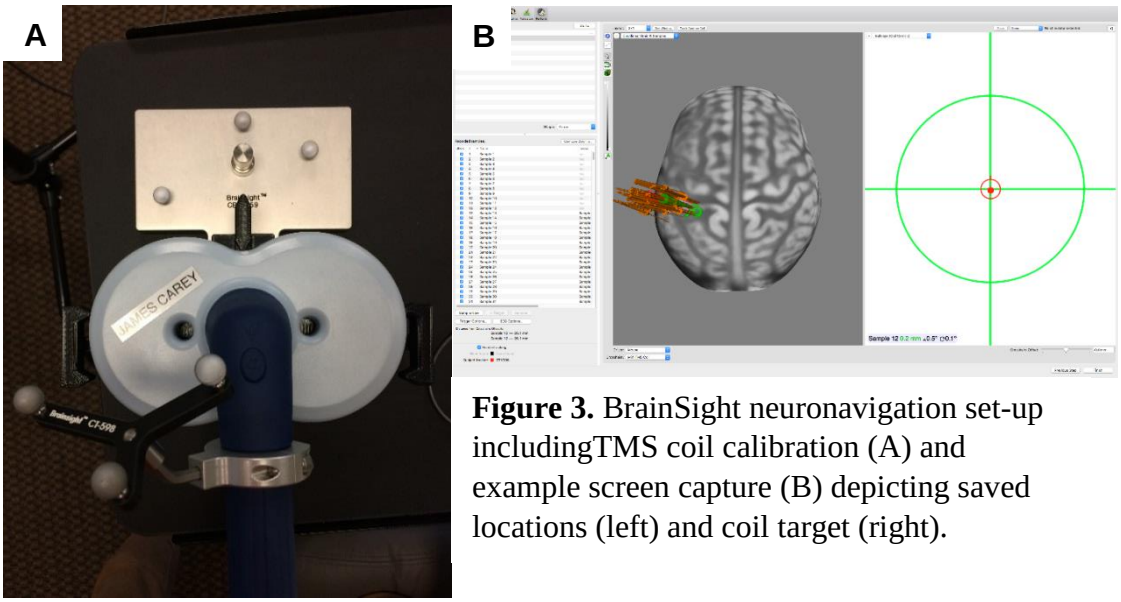

Figure 3. BrainSight neuronavigation set-up includingTMS coil calibration (A) and example screen capture (B) depicting saved locations (left) and coil target (right).

3.5.3 EMG Set-up

The skin over the preferred APB was abraded and cleansed with an alcohol pad. Two stainless steel disc electrodes were placed in a belly-tendon montage on the skin overlying APB (Figure 4). EMG signals were amplified using a bipolar EMG amplifier (Y03-2, Motion Lab Systems, Inc., Baton Rouge, Louisiana, USA) (Gain: 300, band-pass filter: 20-2000Hz), then digitized by a NI 9234 analog-to-digital convertor (National Instruments, Austin, Texas, USA) with a 24-bit resolution at a sampling rate of 6.4kHz. EMG data were displayed in real time to monitor muscle activity during each session. All data were stored by a custom LabVIEW program (v2012, National Instruments, Austin, Texas, USA) in a laptop computer.

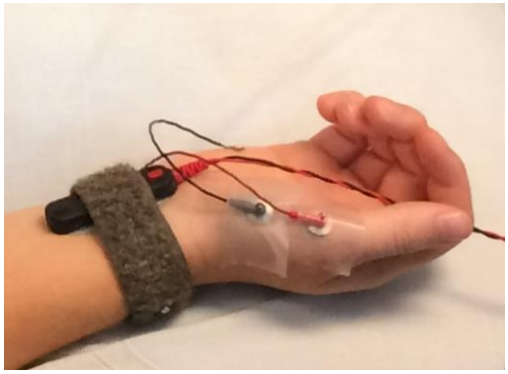

Figure 4. EMG set-up with the peripheral stimulating bar electrode shown secured under a ground strap at the wrist.

3.6.0 PAS Intervention Instrumentation and Methodology

PAS pairs a central stimulus with a peripheral nerve stimulus. For this study, the central stimulus was cortical stimulation applied using single-pulse TMS and the peripheral nerve stimulus was applied using an electrical stimulator (Grass S88, SIU5 and CCU1, Natus Neurology Inc., Warwick, Rhode Island, USA) and a stimulating bar electrode (cathode proximal) over the median nerve at the wrist of the preferred hand[68] (Figure 5). Two different ISIs were used: N20+2ms (PAS_{LTP}) and N20-5ms (PAS_{LTD}). Table 1 lists the priming and principal combinations for each experiment. Each bout of active PAS consisted of 180 pairs of peripheral (3x perceptual threshold, 200 μs duration) and cortical (130% RMT) stimulation delivered at a rate of 0.2 Hz.[181] PPAS interventions consisted of two sequential bouts of PAS within the same session separated by ~3-5 minutes.

Figure 5. Active PAS set-up. Transcranial magnetic stimulation is paired with contralateral median nerve stimulation.

3.6.1 Sham PAS Intervention

To ensure a full sham intervention, no cortical or pheripheral stimulation were given. A novel approach for sham PAS was applied whereby a sham air-film rTMS coil connected to Magstim Rapid^2 device (Magstim Co., Whitland, UK) replaced the active D70^2 coil and single-pulse TMS (Figure 6). The rate of stimulation was set at 0.2 Hz and the instensity was 75% MSO, high enough to mimic the level of sound and tactile sensation of single-pulse TMS. The sham air-film coil mimics the sound of the active coil yet does not produce a magnetic pulse and therefore does not provide any stimulation. Furthermore, the peripheral stimulator was turned off so that no peripheral stimulation was given. To maintain blindednes of the participant and the tester, all other procedures (e.g. hotspot finding, pretesting, neuronavigation, N20) were performed at each session. Further efforts were made during the description of the study whereby participants were told that the effect of different machines (i.e. the Magstim 200^2 and the Magstim Rapid^2) would be tested and thus they were to expect the use of different machines, coils and intensities.

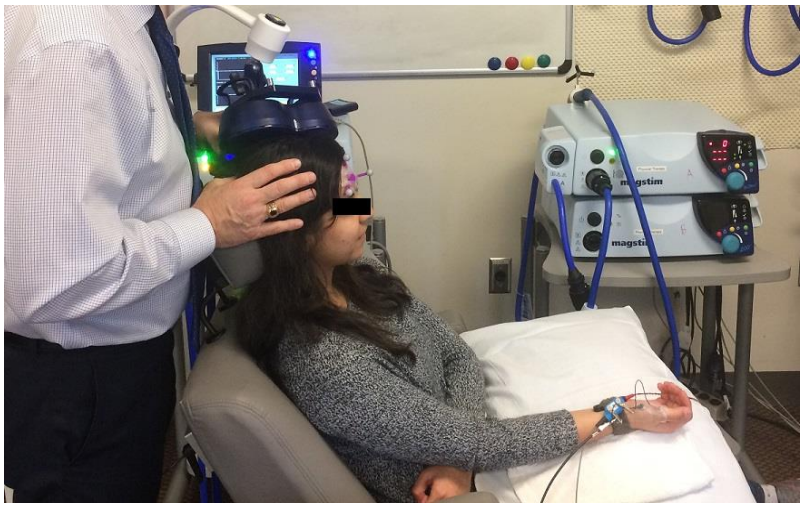

Figure 6. Sham PAS set-up. A sham air-film coil mimics the tactile and auditory traits of active TMS. The bar electrode is placed at the wrist but provides no stimulation during intervention.

3.6.2 N20 Measurement

At the start of each session, the participant's N20 latency, defined as the latency of arrival of a sensory evoked potential resulting from median nerve stimulation at the wrist, was measured using electroencephalogry (EEG). To acquire the EEG signal, a Precision EEG Cap (Brain Vision LLC., Morrisville, North Carolina, USA) and gelled EEG electrodes were placed on the scalp through holes provided in the cap at Cp3/Cp4 with Cz as the reference.[182] An electrode impedance checker (El-Check, BIOPAC Systems, Inc., Goleta, California, USA) was used to confirm that the impedance for each electrode was <10 kOhms (Figure 7). A stimulating (bar) electrode was placed over the median nerve (cathode proximal) of the preferred hand at the wrist. To determine the N20 latency, three hundred trials of suprathreshold (3x perceptual threshold, 200 μs duration) constant current electrical stimuli were applied to the median nerve at 2.0 Hz using an electrical stimulator (Grass S88, SIU5 and CCU1, Natus Neurology Inc., Warwick, Rhode Island, USA). The latency was defined as the amont of time (ms) between the peripheral nerve stimulus artifact and the highest positive peak (Figure 8).

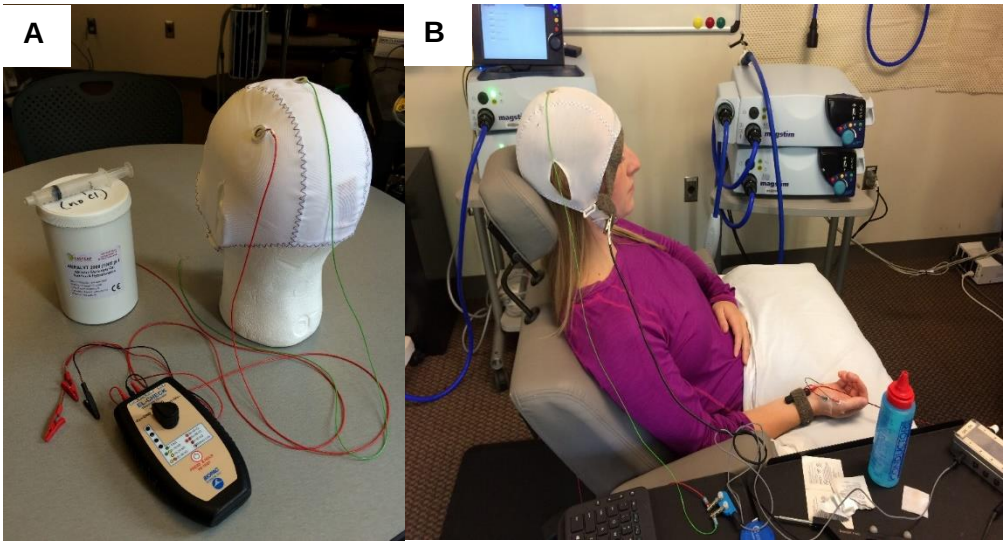

Figure 7. EEG Set-up. (A) EEG equipment includes an EEG cap, abrasive gel, electrodes and an impedence checker. (B) N20 measurement set-up with median nerve stimulation at the preferred wrist and EEG recording from the contralateral primary sensory cortex.

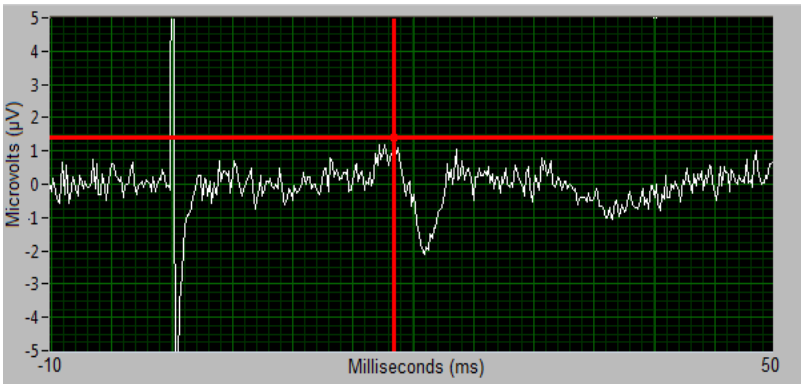

Figure 8. Example EEG recording of the N20 SEP. The red crosshairs mark the measurement of the N20 latency (18.4ms).

3.7.0 Participant Report of Symptoms

To ensure that single-pulse TMS or PAS intervention did not result in any level of adverse event, investigators used a questionnaire to survey for possible symptoms and adverse events that have been reported in previous NIBS literature.[183] This questionnaire was completed at the beginning, middle and end of each visit (Appendix I).

3.8.0 Data Analysis

Each participant received four separate interventions. At each intervention, 20 peak-to-peak MEP amplitudes were collected at baseline and 7 post-intervention time points (0, 10, 20, 30, 40, 50 and 60 minutes following intervention). The peak-to-peak amplitude served as the primary outcome measure. Amplitudes were assessed using the grand average change from baseline (i.e. the average change from baseline MEP amplitude over all 7 post-intervention time points) and the linear trend across each individual post-intervention time point.

The original peak-to-peak MEP amplitudes collected from each individual at each time point for each intervention were highly skewed right for both Experiment 1 and

Experiment 2 (Appendix J: A & B). Values were log-transformed using $\log_{10}$ which resulted in normal distributions for Experiment 1 as well as Experiment 2 (Appendix J: C & D).

All analyses used the log-transformed data. The log-transformed MEP amplitudes were averaged at each time point for each intervention for each individual. Two statistical models were created using these averages. One assessed Whole Group effects for each experiment and the other assessed Responder, Nonresponder effects for each experiment.

All analyses were done using JMP (v. 13 Pro, SAS Institute, Cary, NC).

3.8.1 Whole Group Model

A Mixed Linear Model (Standard Least Squares Regression) assessed main effects of three fixed within-subject factors: Time, Session ID (i.e. intervention) and the Time*Session ID interaction. Three random effects: Participant, the Participant*Session ID interaction and the Participant*Time interaction and the residual error (three-way interaction between Participant*Session ID*Time) were included in the model (Appendix K).

3.8.2 Responder, Nonresponder Model

This model only differs from the Whole Group Model because of the addition of a fourth variable, Responder (Y/N). A Mixed Linear Model (Standard Least Squares Regression) assessed main effects of seven fixed within-subject factors: Time, Session ID, Responder, the Time*Session ID interaction, the Session ID*Responder interaction, the Time*Responder interaction and the Time*Session ID*Responder interaction. Three random effects: Participant, the Participant*Session ID interaction and the Participant*Time interaction and the residual error (three-way interaction between Participant*Session ID*Time) were included in the model (Appendix L).

3.8.3 Contrasts

Within each model, the grand average change from baseline and linear trend analyses were carried out using contrasts in the Session ID*Time interaction (Whole Group Model) or the Session ID*Time*Responder interaction (Responder, Nonresponder Model). Example contrasts are presented in Appendix M. Because this is an exploratory study, p-values were not adjusted for multiple comparisons.[184,185]

Results are presented as percent change from baseline which was derived from the back-transformed ratio using the following steps:

1) $Log_{10}(posttest) – log_{10}(baseline) = log_{10}(posttest/baseline)$
2) $10^{(log_{10}(posttest/baseline))} = posttest/baseline$
3) $((Posttest/baseline) – 1)*100$ = percent change from baseline

3.8.4 Secondary Data Analyses

MEP Onset Latency

The difference in average MEP onset latency between responders and nonresponders was analyzed separately for Experiment 1 and Experiment 2. Due to unequal sample sizes between the two independent samples, the Mann-Whitney U test was used to assign ranks and assess a difference in onset latency between responders and nonresponders (Appendix N).

BDNF Val66Met

The proportions of those with the Val66Met polymorphism were compared between responders and nonresponder for both Experiment 1 and Experiment 2. The z-test for a difference between proportions assessed for a significant difference in this proportion between the two groups within each experiment.

3.8.5 Sub-Analyses

MEP Reliability

Test-retest reliability of baseline peak-to-peak MEP measures was assessed using the Measurement Systems Analysis feature of JMP statistical software. Intraclass correlation coefficients (ICCs) were set-up to determine the proportion of variability described by the Session ID within an individual. Thus, the baseline measures for each of the four interventions were compared within each individual and a single ICC value is reported for each experiment. The JMP input is available as Appendix O.

Carry-over

Potential carry-over of neuromdoulatory effects may influence baseline values and intervention effects in subsequent sessions. Thus, the possibility of carry-over was determined by creating contrasts (see Section 3.8.3) to statistically compare baseline MEP measures between the four interventions within each individual. Contrasts were created using the Participant ID*Session ID*Time Least Squares Means table within the Whole Group Model.

4.0.0 Results

4.1.0 Participants

4.1.1 Recruitment & Enrollment

Recruitment took place through flyers posted on the University of Minnesota – Minneapolis campus and through in-class presentations given to students in the Division of Physical Therapy. Forty-two people completed eligibility surveys. Thirty-two people subsequently enrolled in this study. One enrolled individual dropped out due to scheduling complications. Thirty-one people successfully completed this study (including all four data collection sessions and provision of a saliva sample). The consort diagram illustrates recruitment and drop-outs prior to and after enrollment (Figure 9).

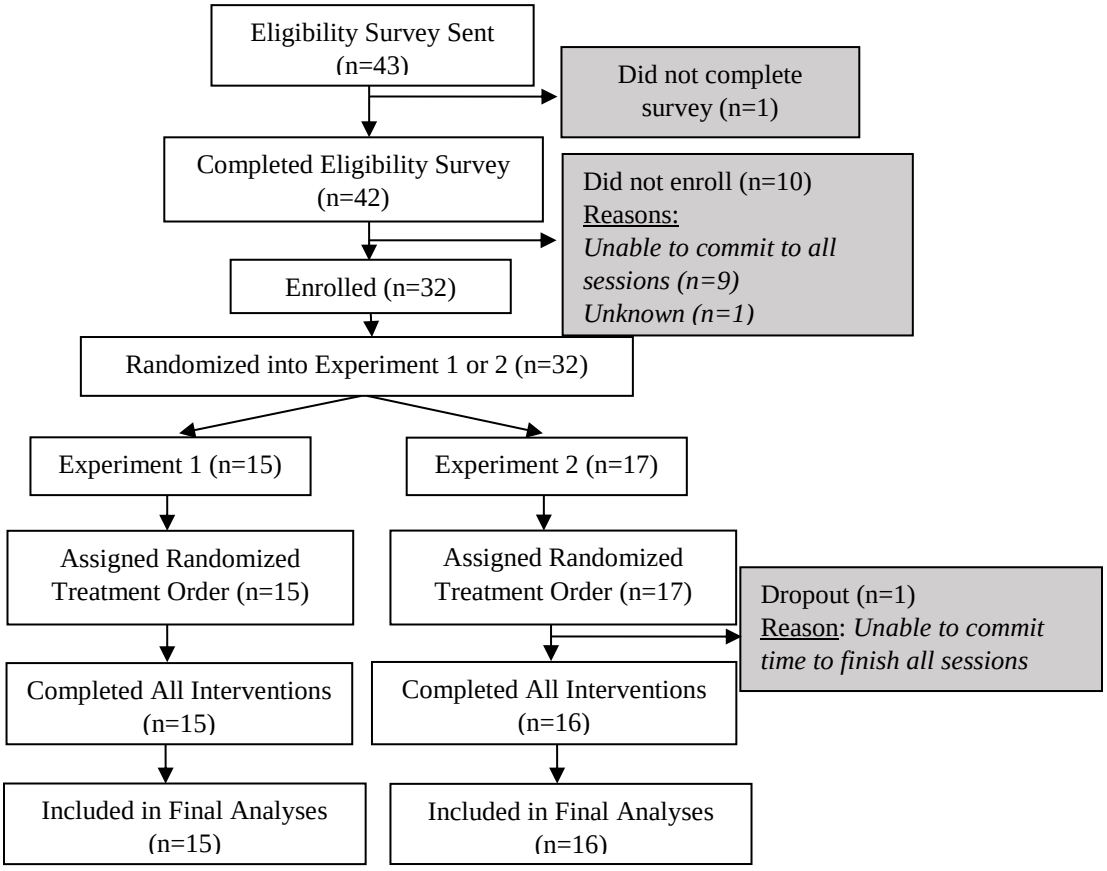

Figure 9. Consolidated Standards of Reporting Trials (CONSORT) Diagram

4.1.2 Individual Characteristics & Demographics

Fifteen individuals (6 males, 9 females; average age 23.6 ± 2.3 years) completed Experiment 1. Sixteen individuals (7 males, 9 females; average age 22.3 ± 2.3 years) completed Experiment 2. Due to the substantial amount of demographics and neurophysiological data collected for each individual, tables of this information are available as Appendix P (Experiment 1) and Appendix Q (Experiment 2).

4.2.0 Experiment 1 ($PPAS_{LTD}$)

The primary aim for Experiment 1 was to compare the effect of PAS_{LTP} priming followed by principal PAS_{LTD} (PAS_{LTP}→PAS_{LTD}) to sham-primed PAS_{LTD} (PAS_{SHAM}→PAS_{LTD}) on corticospinal excitability in healthy individuals. The results that follow are broken into two sections, Whole Group Analyses and Responder & Nonresponder Analyses. Results from the Grand Average Change from Baseline and Linear Trend analyses will be described separately within each section.

4.2.1 Experiment 1 ($PPAS_{LTD}$): Whole Group Analyses

Grand Average Change from Baseline

The PAS_{LTP}→PAS_{LTD} intervention resulted in a significant increase from baseline peak-to-peak MEP amplitude (p=0.0149) (Figure 10 & Table 2). Notably, the *increase* from baseline opposes the hypothesized direction of change. This increase from baseline excitability significantly differs from the non-significant change produced by the sham-primed intervention, PAS_{SHAM}→PAS_{LTD} (p=0.0067) (Figure 10).

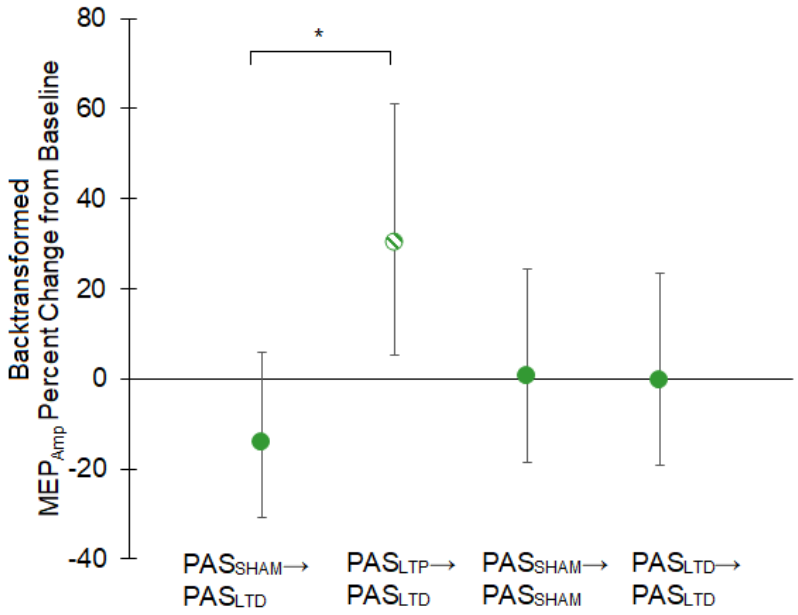

Figure 10. Grand average (average of all posttests) percent change from baseline excitability for the whole group (n=15). MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Striped circle indicates significant change from baseline ($p<0.05$). Asterisk (*) indicates significant difference between interventions ($p<0.05$). Error bars are 95% CIs.

Table 2. Whole Group Grand Average Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention (Experiment 1: $PPAS_{LTD}$)

	PAS_{SHAM}→PAS_{LTD}	**PAS_{LTP}→PAS_{LTD}**	**PAS_{SHAM}→PAS_{SHAM}**	**PAS_{LTD}→PAS_{LTD}**
Grand Average	-14.30	30.35*	0.60	-0.23
95% CI	(-30.71%, 6.00%)	(5.39%, 61.22%)	(-18.66%, 24.43%)	(-19.34%, 23.40%)
p-value	0.158	0.0149	0.9558	0.982

* Significant change from baseline $p<0.05$

Linear Trend

No intervention resulted in a significant linear trend in change from baseline corticospinal excitability over the 60-minute post-intervention period. Between-intervention comparisons show that the trends in change from baseline excitability produced by PAS_{LTP}→PAS_{LTD} and PAS_{SHAM}→PAS_{SHAM} significantly differ from each other ($p=0.0424$) with the decreasing slope produced by PAS_{SHAM}→PAS_{SHAM} trending toward significance at $p=0.067$ (Figure 11).

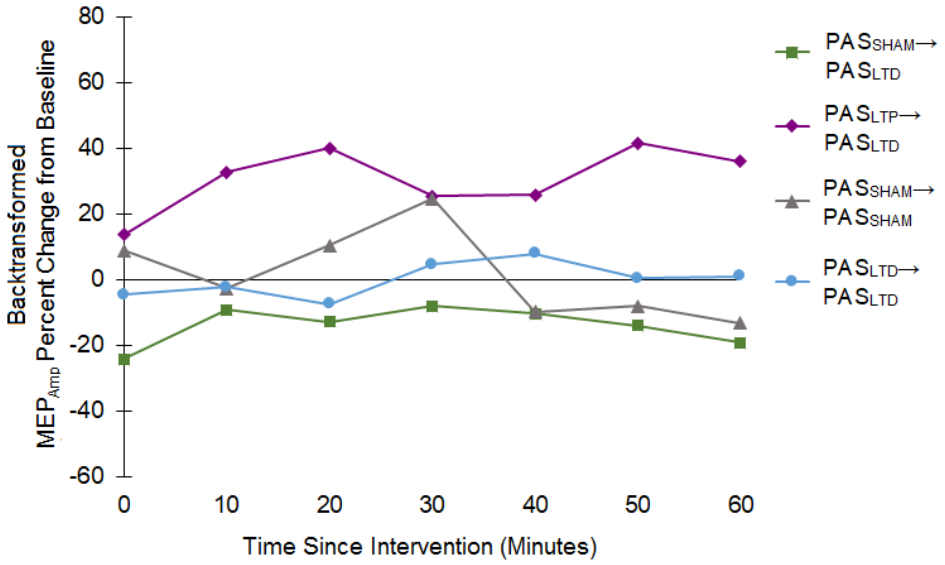

Figure 11. Whole group (n=15) time course of percent change from baseline excitability at each posttest. MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Linear trend analyses do not reveal significance at $p<0.05$ but the decreasing trend of PAS_{SHAM}→PAS_{SHAM} reaches $p=0.067$.

The average percent changes from baseline corticospinal excitability at each post-intervention time point for each intervention are listed below in Table 3. These describe the average increase or decrease in corticospinal excitability measured from all 15 individuals in Experiment 1. The PAS_{LTP}→PAS_{LTD} intervention significantly increased excitability at 10, 20, 50 and 60 minutes following intervention. These increases range from 32.6% to 41.5% and oppose the hypothesized direction of change. PAS_{LTP}→PAS_{LTD} significantly increased excitability compared to the sham-primed

intervention, PAS_{SHAM}→PAS_{LTD}, at 0, 20, 50 and 60 minutes, the PAS_{SHAM}→PAS_{SHAM} intervention at 50 and 60 minutes and the PAS_{LTD}→PAS_{LTD} intervention at 20 minutes.

Table 3. Whole Group Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention at each Time Point (Experiment 1: $PPAS_{LTD}$)

Time since intervention (minutes)	PAS_{SHAM}→PAS_{LTD}	PAS_{LTP}→PAS_{LTD}	PAS_{SHAM}→ PAS_{SHAM}	PAS_{LTD}→PAS_{LTD}
0	-24.32[b]	13.71[a]	8.72%	-4.72%
10	-9.22	32.62[*]	-2.73%	-2.28%
20	-12.90[b]	39.96[ad]	10.26%	-7.53%[b]
30	-8.17	25.34	24.65%	4.54%
40	-10.46	25.81	-9.84%	7.80%
50	-14.10[b]	41.45[ac]	-8.17%[b]	0.42%
60	-19.28[b]	35.89[*ac]	-13.31%[b]	0.86%

Values are estimates of the average

[*] Significant change from baseline $p<0.05$

[a] Significant change from PAS_{SHAM}→PAS_{LTD} $p<0.05$

[b] Significant change from PAS_{LTP}→PAS_{LTD} $p<0.05$

[c] Significant change from PAS_{SHAM}→PAS_{SHAM} $p<0.05$

[d] Significant change from PAS_{LTD}→PAS_{LTD} $p<0.05$

4.2.2 Experiment 1 ($PPAS_{LTD}$): Responder & Nonresponder Analyses

Responder to Nonresponder Ratios

The third, exploratory aim of this dissertation was to assess the ratio of responders to nonresponders for each intervention. The ratio of responders to nonresponders changed for each intervention in Experiment 1 (Table 4). The sham-primed intervention resulted in the highest number of responders with 8/15 (53.33%) individuals that exhibited a suppression of grand average excitability by ≥10%. The PAS_{LTP}→PAS_{LTD} intervention produced the fewest number of responders to a suppressive intervention. This finding contradicts the hypothesis that priming may augment changes in excitability resulting in a higher number of individuals who respond to intervention. Interestingly, the

PAS_{SHAM}→PAS_{SHAM} intervention with no stimulation still resulted in a suppressive response in 33.33% of individuals. When the responder or nonresponder categorization for each individual was determined following each intervention, no pattern of response emerged (e.g. those that were nonresponders to sham-primed PAS did not convert to responders following a PPAS intervention).

Table 4. Responder to Nonresponder Ratios for each Intervention (Experiment 1: $PPAS_{LTD}$)

	PAS_{SHAM}→PAS_{LTD}	**PAS_{LTP}→PAS_{LTD}**	**PAS_{SHAM}→PAS_{SHAM}**	**PAS_{LTD}→PAS_{LTD}**
R:NR	8:7	3:12	5:10	4:11

Responder (R); Nonresponder (NR)

Responders: Grand Average Change from Baseline

The PAS_{SHAM}→PAS_{LTD} intervention resulted in a significant decrease from baseline excitability ($p=0.0013$) (Figure 12 & Table 5). This decrease is expected because responders were categorized based on a suppressive response to PAS_{SHAM}→PAS_{LTD}. No other intervention produced a significant change from baseline for those that were categorized as responders to PAS_{SHAM}→PAS_{LTD}. For this group, the response to PAS_{LTP}→PAS_{LTD} significantly differed from PAS_{SHAM}→PAS_{LTD} ($p=0.0022$) but those that responded significantly to PAS_{SHAM}→PAS_{LTD} did not appear to respond significantly to any other intervention.

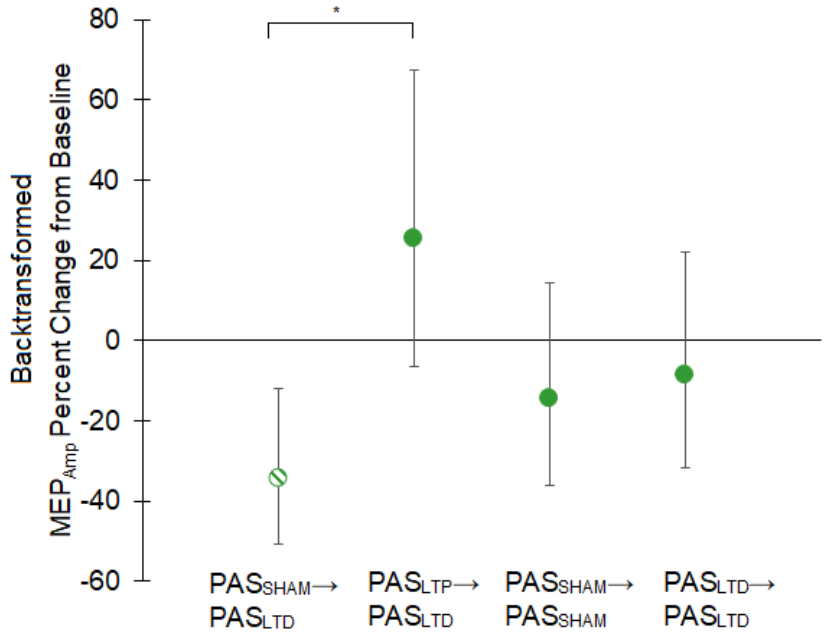

Figure 12. Grand average (average of all posttests) percent change from baseline excitability for responders only (n=8). MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Striped circle indicates significant change from baseline ($p<0.05$). Asterisk (*) indicates significant difference from another intervention ($p<0.05$). Error bars are 95% CIs.

Table 5. Responders Only Grand Average Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention (Experiment 1: $PPAS_{LTD}$)

	PAS_{SHAM}→PAS_{LTD}	**PAS_{LTP}→PAS_{LTD}**	**PAS_{SHAM}→PAS_{SHAM}**	**PAS_{LTD}→PAS_{LTD}**
Grand Average	-34.23%*	25.23%	-14.49%	-8.59%
95% CI	(-50.80%, -12.09%)	(-6.31%, 67.39%)	(-36.03%, 14.30%)	(-31.61%, 22.19%)
p-value	0.0048	0.1294	0.2901	0.5495

* Significant change from baseline $p<0.05$

Responders: Linear Trend

When separated from the whole group, responders still did not exhibit a significant linear trend in change from baseline excitability following any intervention. However, PAS_{SHAM}→PAS_{LTD} produced a consistently suppressed change from baseline excitability during the entire 60-minute post-test period (Figure 13).

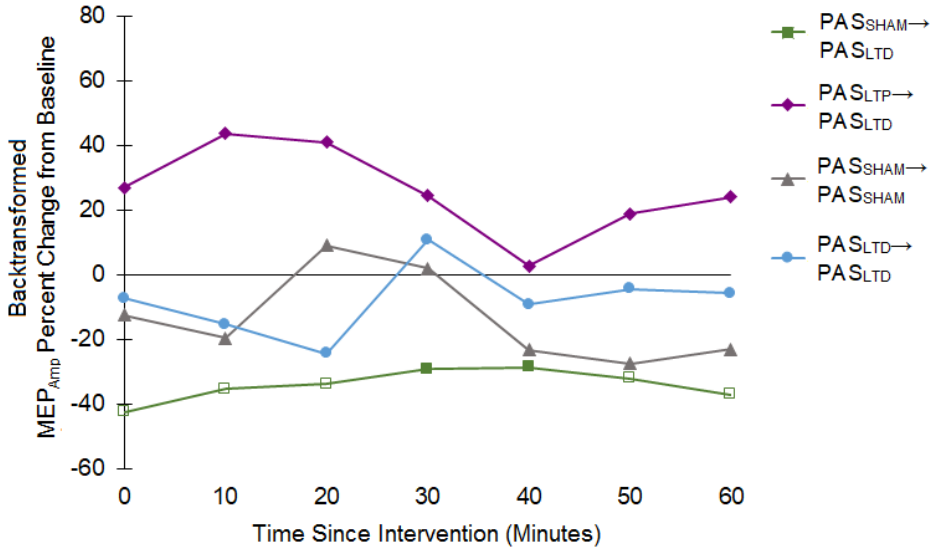

Figure 13. Responders only (n=8) time course of percent change from baseline excitability at each posttest. MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Linear trend analyses do not reveal significance but the PAS_{SHAM}→PAS_{LTD} intervention remains steadily suppressed. Open markers indicate significant difference from baseline ($p<0.05$).

The average percent change from baseline in peak-to-peak MEP amplitude for responders to the sham-primed intervention (n=8) are shown in Table 6. The sham-primed intervention, PAS_{SHAM}→PAS_{LTD} is the only intervention to produce significant changes from baseline in this sub-group. These occur at 0, 10, 20, 50 and 60 minutes following intervention and range from -42.5% to -32.2%. The PAS_{LTP}→PAS_{LTD} intervention creates a 43.5% and a 45.0% increase from baseline excitability at 10 and 20 minutes, respectively but these do not reach significance ($p=0.06$, $p=0.09$, respectively). The PAS_{SHAM}→PAS_{LTD} intervention significantly decreased excitability compared to PAS_{LTP}→PAS_{LTD} at all time points except 40 minutes following intervention.

PAS_{LTP}→PAS_{LTD} significantly increased excitability compared to PAS_{SHAM}→PAS_{SHAM} at 10 minutes and PAS_{LTD}→PAS_{LTD} at 20 minutes (Table 6).

Table 6. Responders Only Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention at each Time Point (Experiment 1: $PPAS_{LTD}$)

Time since intervention (minutes)	PAS_{SHAM}→PAS_{LTD}	PAS_{LTP}→PAS_{LTD}	PAS_{SHAM}→ PAS_{SHAM}	PAS_{LTD}→PAS_{LTD}
0	-42.46%[*b]	26.89%[a]	-12.50%	-7.32%
10	-35.43%[*b]	43.52%[ac]	-19.65%[b]	-15.47%
20	-33.93%[*b]	40.99%[ad]	8.97%	-24.50%[b]
30	-29.21%[b]	24.34%[a]	1.88%	10.71%
40	-28.71%	2.68%	-23.26%	-9.22%
50	-32.08%[*b]	18.71%[a]	-27.56%	-4.50%
60	-37.19%[*b]	24.08%[a]	-23.09%	-5.81%

Values are estimates of the average

[*] Significant change from baseline $p<0.05$

[a] Significant change from PAS_{SHAM}→PAS_{LTD} $p<0.05$

[b] Significant change from PAS_{LTP}→PAS_{LTD} $p<0.05$

[c] Significant change from PAS_{SHAM}→PAS_{SHAM} $p<0.05$

[d] Significant change from PAS_{LTD}→PAS_{LTD} $p<0.05$

Nonresponders: Grand Average Change from Baseline

Those categorized as nonresponders to PAS_{SHAM}→PAS_{LTD} exhibited no significant grand average change in baseline excitability following any intervention (Figure 14 & Table 7). However, the increase in excitability following PAS_{LTP}→PAS_{LTD} came close to significance at $p=0.0501$.

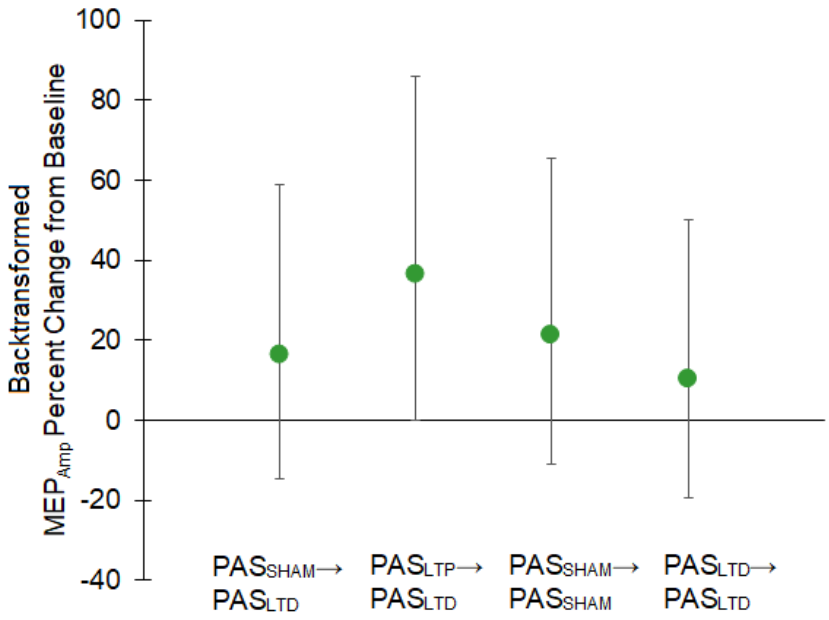

Figure 14. Grand average (average of all posttests) percent change from baseline excitability for nonresponders only (n=7). MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Error bars are 95% CIs.

Table 7. Nonresponders Only Grand Average Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention (Experiment 1: $PPAS_{LTD}$)

	PAS_{SHAM}→PAS_{LTD}	**PAS_{LTP}→PAS_{LTD}**	**PAS_{SHAM}→PAS_{SHAM}**	**PAS_{LTD}→PAS_{LTD}**
Grand Average	16.44%	36.49%	21.34%	10.15%
95% CI	(-14.64%, 58.84%)	(0.10%, 86.10%)	(-11.05%, 65.52%)	(-19.21%, 50.19%)
p-value	0.3377	0.0501	0.2211	0.544

Nonresponders: Linear Trend

The PAS_{LTP}→PAS_{LTD} intervention produced a significantly increasing trend in corticospinal excitability over the 60-minute post-intervention period (p=00087) (Figure 15). The increasing trend in excitability produced by PAS_{LTP}→PAS_{LTD} significantly

differs from those produced by the PAS_{SHAM}→PAS_{LTD} (p=0.0367) and PAS_{SHAM}→PAS_{SHAM} (p=0.005) interventions.

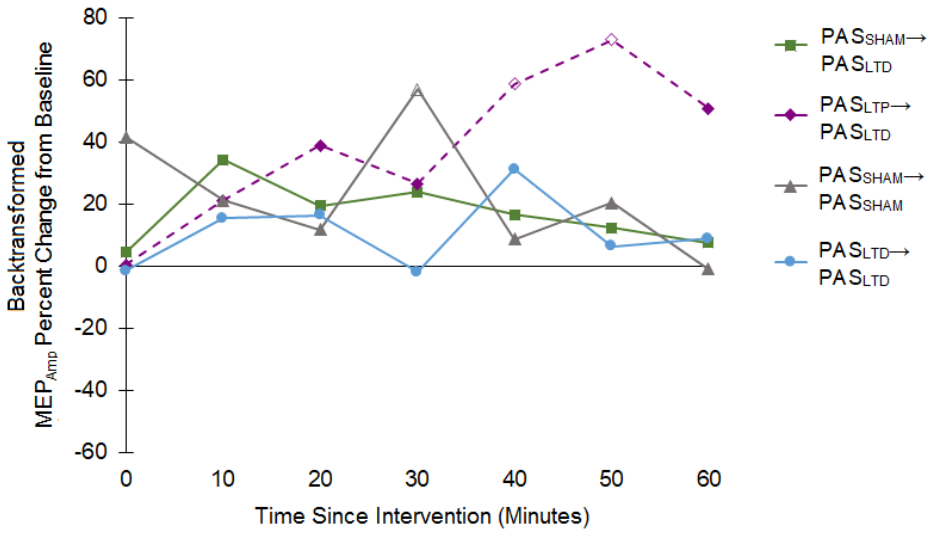

Figure 15. Nonresponders only (n=7) time course of percent change from baseline excitability at each posttest. MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Dotted line indicates significant (p<0.05) trend. Open markers indicate significant (p<0.05) difference from baseline.

The average percent change from baseline corticospinal excitability for each timepoint is given in Table 8. PAS_{LTP}→PAS_{LTD} significantly increased corticospinal excitability at 40 and 50 minutes. Interestingly, the PAS_{SHAM}→PAS_{SHAM} intervention significantly increased excitability (57%) at 30 minutes following intervention. Change from baseline excitability did not differ between interventions at the same time point.

Table 8. Nonresponders Only Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention at each Time Point (Experiment 1: $PPAS_{LTD}$)

Time since intervention (minutes)	PAS_{SHAM}→PAS_{LTD}	PAS_{LTP}→PAS_{LTD}	PAS_{SHAM}→ PAS_{SHAM}	PAS_{LTD}→PAS_{LTD}
0	4.30%	0.30%	41.48%	-1.60%
10	34.09%	21.20%	21.17%	15.37%
20	19.32%	38.77%	11.74%	16.28%
30	23.72%	26.47%	56.96%*	-2.05%
40	16.44%	58.67%*	8.59%	31.04%
50	12.36%	72.78%*	20.28%	6.22%
60	7.37%	50.76%	-0.92%	8.89%

Values are estimates of the average

* Significant change from baseline $p<0.05$

4.3.0 Experiment 2 ($PPAS_{LTP}$)

The primary aim for Experiment 2 was to compare the effect of PAS_{LTD} priming followed by principle PAS_{LTP} (PAS_{LTD}→PAS_{LTP}) to sham-primed PAS_{LTP} (PAS_{SHAM}→PAS_{LTP}) on corticospinal excitability in healthy individuals. Like the previous section, the results that follow are broken into two sections, Whole Group Analyses and Responder & Nonresponder Analyses. Results from the Grand Average Change from Baseline and Linear Trend analyses will be described separately within each section.

4.3.1 Experiment 2 ($PPAS_{LTP}$): Whole Group Analyses

Grand Average Change from Baseline

No intervention resulted in a significant change from baseline excitability (Figure 16 & Table 9).

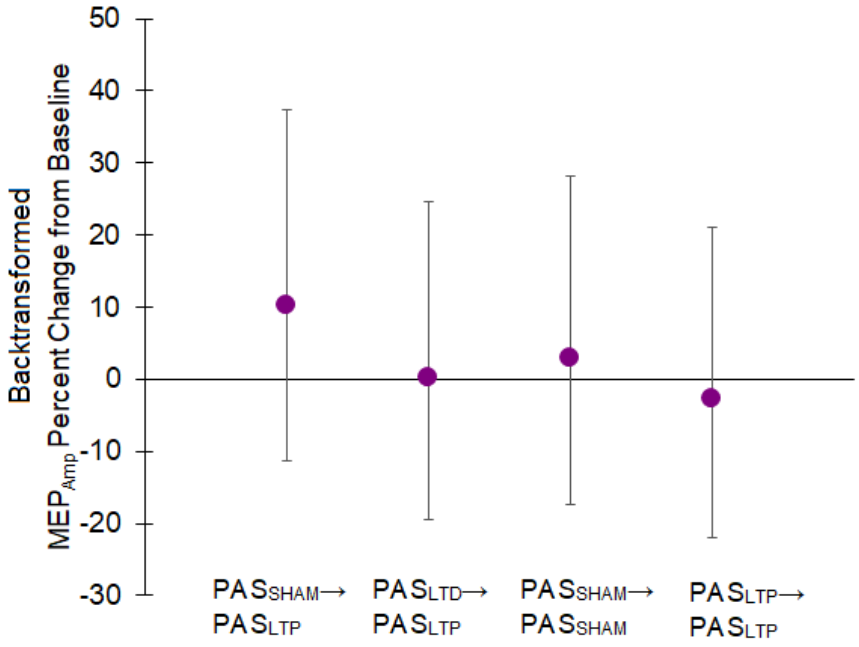

Figure 16. Grand average (average of all posttests) percent change from baseline excitability for the whole group (n=16). MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Error bars are 95% CIs.

Table 9. Whole Group Grand Average Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention (Experiment 2 :$PPAS_{LTP}$)

	PAS_{SHAM}→PAS_{LTP}	**PAS_{LTD}→PAS_{LTP}**	**PAS_{SHAM}→PAS_{SHAM}**	**PAS_{LTP}→PAS_{LTP}**
Grand Average	10.31%	0.16%	2.97%	-2.73%
95% CI	(-11.42%, 37.36%)	(-19.57%, 24.73%)	(-17.31%, 28.22%)	(-21.88%, 21.13%)
p-value	0.381	0.9878	0.7938	0.7254

Linear Trend Analyses

No intervention resulted in a significant linear trend in change from baseline corticospinal excitability over the 60-minute post-intervention period. There were no significant differences between trends in change in excitability following any intervention (Figure 17).

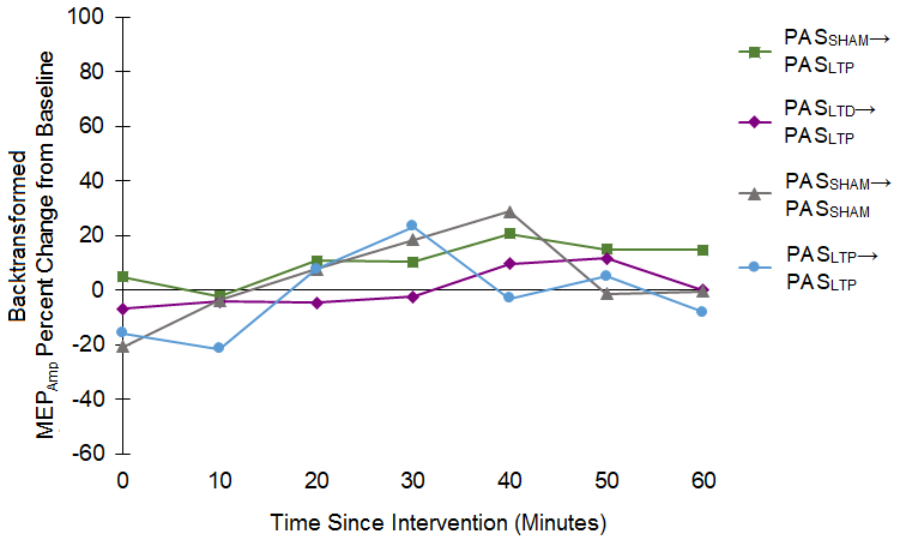

Figure 17. Whole group (n=16) time course of percent change from baseline excitability at each posttest. MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Linear trend analyses do not reveal significance at $p<0.05$.

The average percent change from baseline excitability at each time point for each intervention are listed in Table 10. No intervention elicited a significant change from baseline at any time point during the 60-minute post-test period. Furthermore, no intervention produced a change in excitability that significantly differed from another intervention at the same time point (Table 10).

Table 10. Whole Group Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention at each Time Point (Experiment 2: $PPAS_{LTP}$)

Time since intervention (minutes)	PAS_{SHAM}→PAS_{LTP}	PAS_{LTD}→PAS_{LTP}	PAS_{SHAM}→ PAS_{SHAM}	PAS_{LTP}→PAS_{LTP}
0	4.83%	-6.89%	-20.75%	-15.86%
10	-2.28%	-4.28%	-3.83%	-21.48%
20	10.64%	-4.72%	7.62%	7.67%
30	10.28%	-2.50%	18.33%	23.23%
40	20.64%	9.62%	28.65%	-3.17%
50	14.82%	11.63%	-1.37%	5.12%
60	14.74%	0.003%	-0.46%	-8.17%

Values are estimates of the average

4.3.2 Experiment 2 ($PPAS_{LTP}$): Responder & Nonresponder Analyses

Responder to Nonresponder Ratios

The ratio of responders to nonresponders varied from 7:9 to 10:6 for the four different interventions (Table 11). Similar to Experiment 1, the sham-primed intervention resulted in the highest number of responders with 10 out of 16 (62.5%) individuals that exhibited a facilitation of grand average excitability by ≥10%. The PAS_{LTD}→PAS_{LTP} intervention produced 50% responders and the PAS_{LTP}→PAS_{LTP} intervention produced 56.25% responders. Although the PAS_{SHAM}→PAS_{SHAM} intervention produced the fewest number of responders, it still elicited a ≥10% increase from baseline excitability in 7 out of 16 individuals. Again, there was no pattern in individual response to each intervention.

Table 11. Responder to Nonresponder Ratios for each Intervention (Experiment 2: $PPAS_{LTP}$)

	PAS_{SHAM}→PAS_{LTP}	PAS_{LTD}→PAS_{LTP}	PAS_{SHAM}→PAS_{SHAM}	PAS_{LTP}→PAS_{LTP}
R:NR	10:6	8:8	7:9	9:7

Responder (R); Nonresponder (NR)

Responders: Grand Average Change from Baseline

The PAS_{SHAM}→PAS_{LTP} intervention resulted in a significant increase from baseline excitability (p=0.0009) (Figure 18 & Table 12). This increase in excitability is expected because responders were categorized based on a facilitatory response to PAS_{SHAM}→PAS_{LTP}. No other intervention produced a significant change from baseline. The increase from baseline excitability following PAS_{SHAM}→PAS_{LTP} significantly differed from PAS_{SHAM}→PAS_{SHAM} (p=0.0226).

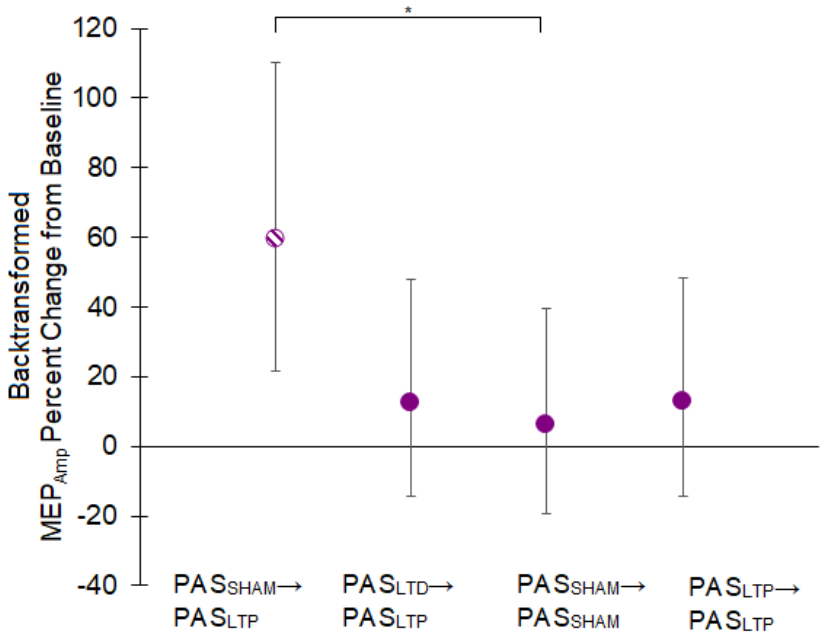

Figure 18. Grand average (average of all posttests) percent change from baseline excitability for responders only (n=10). MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Striped circle indicates significant change from baseline (p<0.05). Asterisk (*) indicates significant difference between interventions (p<0.05). Error bars are 95% CIs.

Table 12. Responders Only Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention (Experiment 2: $PPAS_{LTP}$)

	PAS_{SHAM}→PAS_{LTP}	PAS_{LTD}→PAS_{LTP}	PAS_{SHAM}→PAS_{SHAM}	PAS_{LTP}→PAS_{LTP}
Grand Average	59.81%*	12.62%	6.07%	12.80%
95% CI	(21.57%, 110.08%)	(-14.37%, 48.11%)	(-19.31%, 39.44%)	(-14.23%, 38.35%)
p-value	0.0009	0.3959	0.6973	0.4732

* Significant change from baseline $p<0.05$

Responders: Linear Trend Analyses

PAS_{LTP}→PAS_{LTP} and PAS_{SHAM}→PAS_{SHAM} produced significant linear trends in change from baseline excitability over time ($p=0.0271$ and $p=0.0067$, respectively) (Figure 19). No other significant trends or differences between trends were revealed.

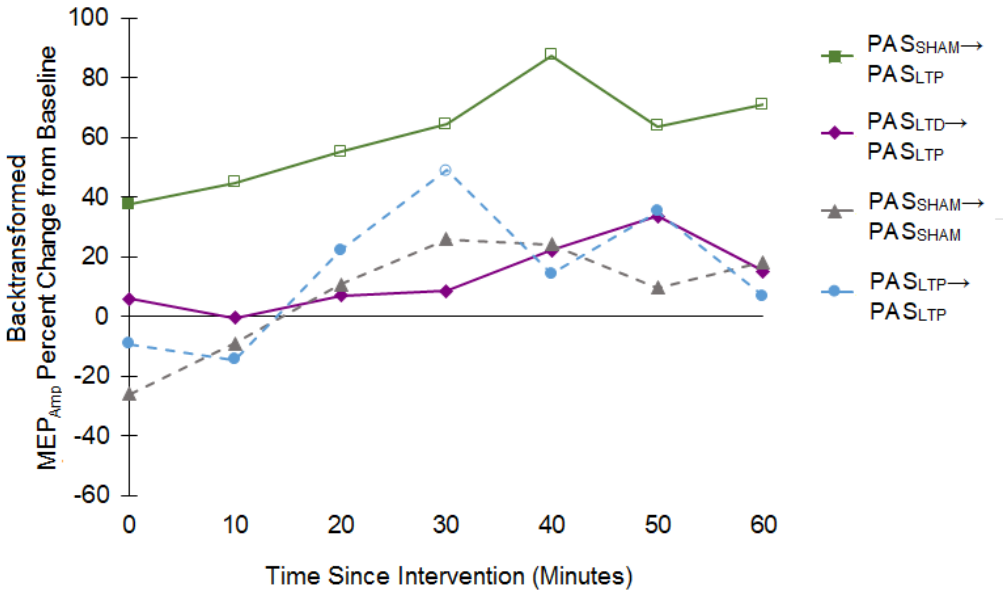

Figure 19. Responders only (n=10) time course of percent change from baseline excitability at each posttest. MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Dotted line represents significant linear trend ($p<0.05$). Open markers indicate significant difference from baseline ($p<0.05$).

The average percent change from baseline in peak-to-peak MEP amplitude for responders (n=10) are shown in Table 13. PAS_{SHAM}→PAS_{LTP} significantly increased corticospinal excitability at 10, 20, 30, 40, 50 and 60 minutes following intervention. Contrary to the hypothesis, PAS_{LTP}→PAS_{LTP} also significantly increased excitability at 30 minutes following intervention. PAS_{SHAM}→PAS_{LTP} more effectively increased excitability at 10 and 40 minutes compared to PAS_{LTP}→PAS_{LTP} but only increased excitability at 10 minutes compared to the PAS_{SHAM}→PAS_{SHAM} intervention.

Table 13. Responders Only Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention at each Time Point (Experiment 2: $PPAS_{LTP}$)

Time since intervention (minutes)	PAS_{SHAM}→PAS_{LTP}	PAS_{LTD}→PAS_{LTP}	PAS_{SHAM}→ PAS_{SHAM}	PAS_{LTP}→PAS_{LTP}
0	37.56%[c]	5.85%	-25.87%	-9.22%
10	44.74%[*cd]	-0.46%	-9.01%[a]	-14.49%[a]
20	55.20%[*]	6.91%	10.71%	22.07%
30	64.32%[*]	8.47%	25.95%	48.90%[*]
40	87.37%[*d]	22.07%	24.02%	14.29%[a]
50	63.68%[*]	33.60%	9.62%	35.08%
60	71.00%[*]	15.21%	18.11%	6.78%

Values are estimates of the average

[*] Significant change from baseline $p<0.05$

[a] Significant change from PAS_{SHAM}→PAS_{LTD} $p<0.05$

[c] Significant change from PAS_{SHAM}→PAS_{SHAM} $p<0.05$

[d] Significant change from PAS_{LTD}→PAS_{LTD} $p<0.05$

Nonresponders: Grand Average Change from Baseline

PAS_{SHAM}→PAS_{LTP} significantly decreased excitability in the nonresponder group ($p=0.0042$). This change from baseline excitability significantly differs from that produced by PAS_{SHAM}→PAS_{SHAM} ($p=0.0315$) (Figure 20 & Table 14).

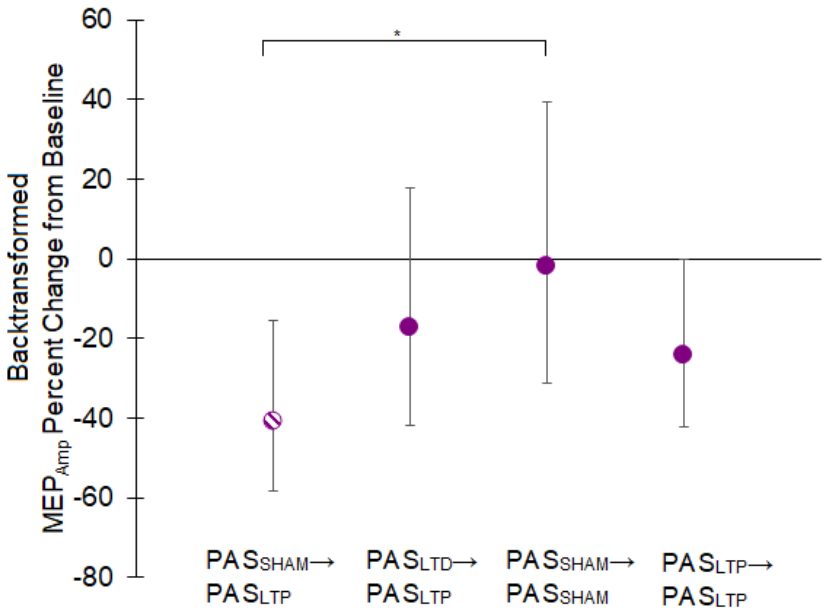

Figure 20. Grand average (average of all posttests) percent change from baseline excitability for nonresponders only (n=6). MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Striped circle indicates significant change from baseline ($p<0.05$). Asterisk (*) indicates significant difference between interventions ($p<0.05$). Error bars are 95% CIs.

Table 14. Nonresponders Only Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention (Experiment 2: $PPAS_{LTP}$)

	PAS_{SHAM}→PAS_{LTP}	**PAS_{LTD}→PAS_{LTP}**	**PAS_{SHAM}→PAS_{SHAM}**	**PAS_{LTP}→PAS_{LTP}**
Grand Average	-40.57%*	-17.40%	-2.05%	-24.14%
95% CI	(-58.26%, -15.38%)	(-41.99%, 17.62%)	(-31.21%, 39.47%)	(-42.32%, -0.24%)
p-value	0.0042	0.2874	0.9116	0.1249

* Significant change from baseline $p<0.05$

Nonresponders: Linear Trend Analyses

No significant linear trends were observed following any intervention (Figure 21).

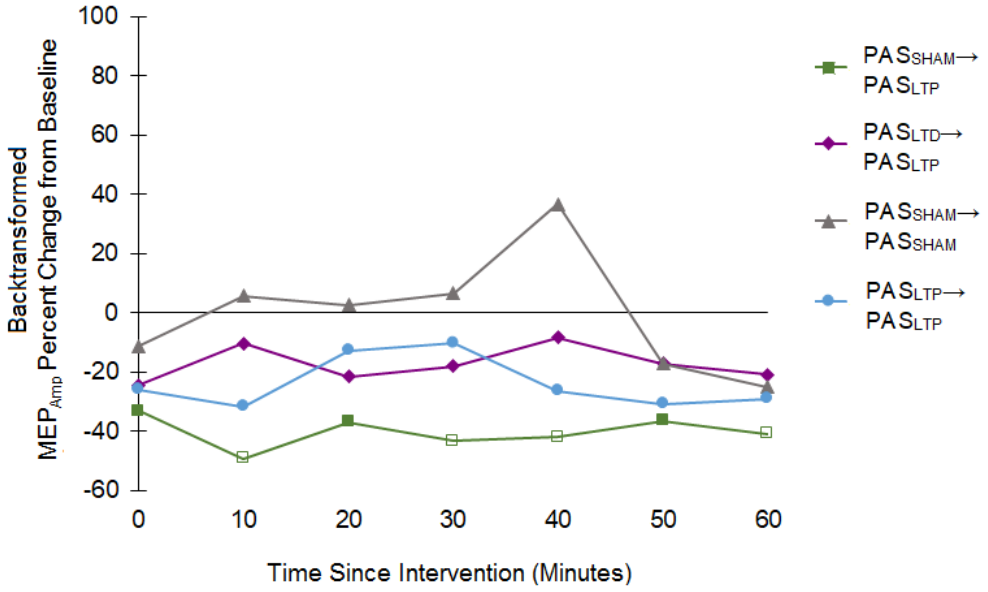

Figure 21. Nonresponders only (n=6) time course of percent change from baseline excitability at each posttest. MEP_{Amp} refers to peak-to-peak motor-evoked potential amplitude. Linear trend analyses do not reveal significance at $p<0.05$. Open markers indicate significant difference from baseline.

The average change from baseline excitability for each intervention at each time point is listed in Table 15. PAS_{SHAM}→PAS_{LTP} significantly decreased excitability in the nonresponder group at 10, 30, 40 and 60 minutes following intervention. At 10, 30 and 40 minutes, this decrease in excitability significantly differs from the average change produced by PAS_{SHAM}→PAS_{SHAM}.

Table 15. Nonresponders Only Percent Change from Baseline Peak-to-Peak MEP Amplitude for each Intervention at each Time Point (Experiment 2: $PPAS_{LTP}$)

Time since intervention (minutes)	PAS_{SHAM}→PAS_{LTP}	PAS_{LTD}→PAS_{LTP}	PAS_{SHAM}→ PAS_{SHAM}	PAS_{LTP}→PAS_{LTP}
0	-33.32%	-24.32%	-11.28%	-25.87%
10	-49.18%*c	-10.46%	5.71%a	-31.77%
20	-37.05%	-21.48%	2.64%	-12.70%
30	-43.25%*c	-18.15%	6.64%a	-10.05%
40	-42.06%*c	-8.38%	36.71%a	-26.38%
50	-36.47%	-17.21%	-17.21%	-30.82%
60	-40.98%*	-20.93%	-25.18%d	-29.04%c

Values are estimates of the average

* Significant change from baseline $p<0.05$

a Significant change from PAS_{SHAM}→PAS_{LTD} $p<0.05$

c Significant change from PAS_{SHAM}→PAS_{SHAM} $p<0.05$

4.4.0 Responder & Nonresponder Characteristics

The fourth, exploratory, aim of this study investigated the possibility that there exists a relationship between individual characteristics (MEP onset latency and presence of the BDNF Val66Met polymorphism) and an individual's categorization as a responder or nonresponder to sham-primed PAS.

4.4.1 Experiment 1 ($PPAS_{LTD}$)

MEP Onset Latency

The induction of posterior-anterior current flow across the central sulcus resulted in elicitable MEPs from five responders and six nonresponders. The average MEP onset latencies were 25.07 ± 0.24 ms and 25.65 ± 0.51 ms for responders and nonresponders, respectively. This difference was not significant ($p=0.7482$) (Figure 22).

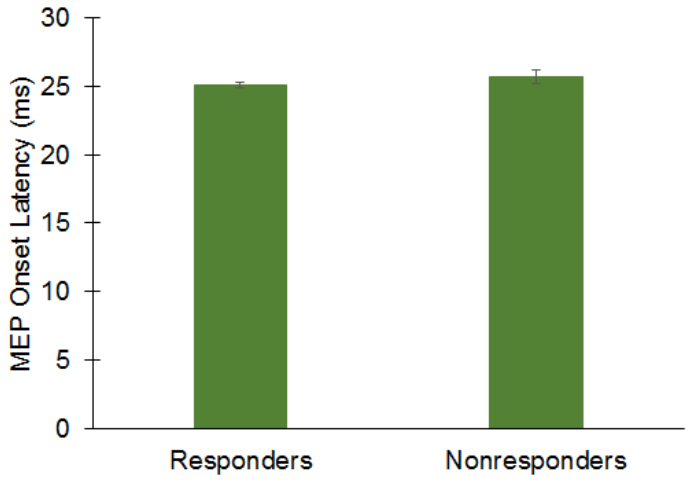

Figure 22. Average motor-evoked potential (MEP) onset latencies for responders and nonresponders. Error bars are SEM

Val66Met polymorphism

Five out of 15 individuals (33.33%) had the Val66Met BDNF polymorphism. Presence of the polymorphism was significantly higher among nonresponders (4 out of 7) compared to responders (1 out of 8) ($p<0.0001$) (Figure 23).

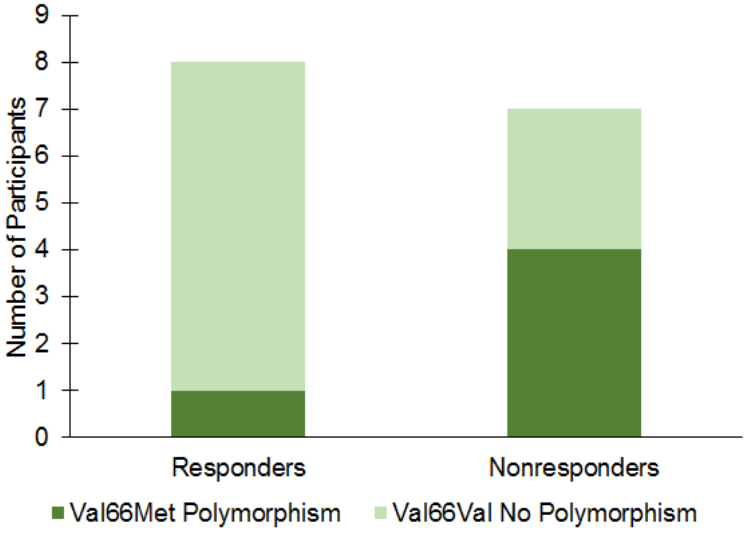

Figure 23. Frequency of individuals with (dark) and without (light) the Val66Met polymorphism for both responders and nonresponders.

4.4.2 Experiment 2 (PPAS$_{LTP}$)

MEP Onset Latency

The posterior-anterior current flow elicited MEPs from nine responders and four nonresponders. The average latencies were 25.62 ± 1.12 ms and 24.36 ± 0.96 ms for responders and nonresponders, respectively. This difference was not significant (p=0.4875) (Figure 24).

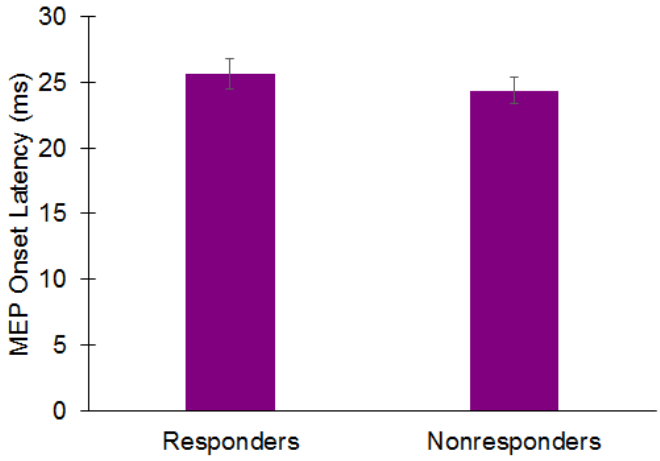

Figure 24. Average motor-evoked potential (MEP) onset latencies for responders and nonresponders. Error bars are SEM

Val66Met Polymorphism

Six out of 16 individuals (37.5%) had the Val66Met BDNF polymorphism. Unlike Experiment1, presence of the polymorphism appears slightly higher among responders (4 out of 10) compared to nonresponders (2 out of 6) but this difference is not significant (p=0.790) (Figure 25).

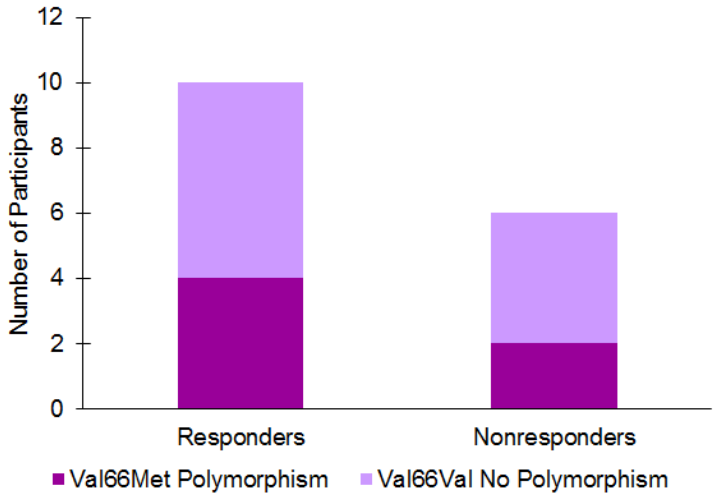

Figure 25. Frequency of individuals with (dark) and without (light) the Val66Met polymorphism for both responders and nonresponders.

4.5.0 MEP Test-Retest Reliability

The ICCs revealed low between-session test-retest reliability across all four baseline sessions within individuals for Experiment 1 (0.07) and Experiment 2 (0.005). Between-session variability is expected and was accounted for in the study design by re-establishing the motor hotspot and RMT each session and by using a percentage of RMT (i.e. 130% RMT) for testing and interventional intensities.

4.6.0 Carry-over

Although significant differences were found between average baseline measures (within an individual), these differences did not differ systematically across individuals. Thus, we conclude that there was no carry-over effect between sessions and that the one-week washout period was sufficient.

4.7.0 Alternative Exploratory Analyses

Several alternative analyses were completed in an exploratory attempt to view the data from multiple perspectives. Although the detailed methodology and results of these analyses will not be described in this dissertation, it is important to continue a multi-faceted approach to neuromodulation data analysis because investigators do not yet know which approach is favorable and it appears that the landscape of analyses is shifting toward recognition of individual analyses and the important roles that these may play in designing individualized treatment plans.

4.7.1 Individual Visual Analyses

Bar graphs and linear trend plots were created to depict the grand average change from baseline excitability for each individual following each intervention. A visual assessment was used to look for systematic variations in individual responses that correlated with gender, age, MEP onset latency or presence of the Val66met polymorphism. No systematic variations were found.

4.7.2 Responders vs. Nonresponders

Contrasts were created to compare the average change from baseline following each intervention between responders and nonresponders. This type of analysis may provide an estimate for how much more or less an intervention influences responders compared to nonresponders. Because there were no significant differences from baseline excitability (except for the expected difference following sham-primed intervention) for responders or nonresponders, finding a difference in response between responders and nonresponders is statistically irrelevant. However, nonresponders may be considered a type of control group (i.e. no predictable response to neuromodulation) thus a statistical difference in response may describe the influence that an intervention has on those who would benefit from intervention compared to those who do not. For Experiment 1, responders and nonresponders significantly differed ($p<0.05$) at 0, 10 and 20 minutes following $PAS_{SHAM} \rightarrow PAS_{LTD}$. No other differences were found. For Experiment 2, the

response of responders and nonresponders significantly differed ($p<0.05$) at each post-test time point following PAS_{SHAM}→PAS_{LTP}. No other differences were found.

4.8.3 Sham-Removal

Corticospinal excitability is inherently variable.[11,186] Theoretically, measures taken following PAS_{SHAM}→PAS_{SHAM} represent normal variations in excitability. Because of this, the values of corticospinal excitability at each time point following PAS_{SHAM}→PAS_{SHAM} may be subtracted from the values of excitability at each time point following each other intervention. This provides a value that seemingly accounts for inherent variability and represents the true difference in excitability caused by the intervention. However, the statistical estimates for excitability following PAS_{SHAM}→PAS_{SHAM} are actually an unknown combination of true value and error. When these estimates are subtracted from estimates of another intervention, the uncertainty of the amount of error increases. Thus, it provides an estimate with more uncertainty and effectively a lower "signal-to-noise" ratio. Despite this limitation, analyses were completed to see if sham-removal affected previously established results. The removal of sham estimates did not change the significance of any findings in grand average change from baseline for the Whole Group, Responders and Nonresponders for both experiments.

4.8.4 Raw MEP Values

Although the raw MEP values were highly skewed left and thus $\log_{10}$ transformed for the primary analyses, some argue that transforming data may not reduce variability and that results of statistical tests performed on transformed data do not translate to the raw data.[187] Furthermore, most statistical tests are more robust to non-normal distributions than what is commonly believed. Thus, the same statistical analyses that were performed on the transformed data were also performed with the raw data. When compared to results from the transformed analyses, the raw analyses did not produce any differences in statistical significance findings.

4.7.5 BDNF Val66Val and Val66Met

Presence of the Val66Met polymorphism of BDNF may influence response to PAS.[73] Each participant in this study provided a saliva sample from which DNA was extracted and assessed for either the Val66Val (no polymorphism) or the Val66Met (polymorphism) sequence. Within-intervention grand average change from baseline analyses for each group (Val66Val and Val66Met) in each experiment may explain differences in response to PAS intervention better than responder and nonresponder categorizations. Thus, a model similar to the Responder, Nonresponder model was developed and contrasts were created to assess within-intervention grand average change from baseline for Val66Val and Val66Met individuals. No difference from baseline excitability was found for either group in Experiment 1. A significant increase from baseline excitability was found in individuals with no polymorphism (Val66Val) following $PAS_{LTP} \rightarrow PAS_{LTD}$ in Experiment 2 ($p=0.018$). This was the only significant finding. Thus, those without the Val66Met BDNF polymorphism did not exhibit a significant change in excitability following $PPAS_{LTP}$ interventions but they did exhibit an *increase* in excitability following $PAS_{LTP} \rightarrow PAS_{LTD}$. As expected, those with the Val66Met polymorphism did not exhibit a significant change from baseline excitability following any intervention. Due to the exploratory nature of this analytical approach as well as the lack of statistically significant change from baseline excitability findings, between-intervention and between-group analyses were not pursued.

5.0.0 Discussion

The purpose of this study was to assess the influence of $PPAS_{LTD}$ and $PPAS_{LTP}$ on corticospinal excitability and to investigate potential characteristics that may indicate whether an individual will or will not benefit from brain stimulation. Thirty-two healthy participants were randomized into and completed one of two experiments. The first experiment assessed changes in corticospinal excitability following $PPAS_{LTD}$ interventions: PAS_{SHAM}→PAS_{LTD}, PAS_{LTP}→PAS_{LTD}, PAS_{LTD}→PAS_{LTD} and PAS_{SHAM}→PAS_{SHAM}. The second experiment assessed changes in corticospinal excitability following $PPAS_{LTP}$ interventions: PAS_{SHAM}→PAS_{LTP}, PAS_{LTD}→PAS_{LTP}, PAS_{LTP}→PAS_{LTP} and PAS_{SHAM}→PAS_{SHAM}. The interest in using PAS to alter excitability stems from evidence that the addition of peripheral input augments motor function and changes in corticomotor pathway excitability in people with stroke.[14] Furthermore, the mechanism of action appears to be the induction of STDP which primarily drives experience-dependent motor learning.[65] Therefore, the clinical potential of PAS lies in its ability to mimic natural mechanisms of motor learning and enhance the use of previously dormant motor pathways. Although PAS has a demonstrated ability to alter excitability, it is not yet possible to foresee who will respond predictably and who will not. Priming is hypothesized to improve the predictability of response by inducing a larger change from baseline corticospinal excitability through the use of sliding thresholds. To date, this study is the first to assess the effects of all combinations of $PPAS_{LTP}$ and $PPAS_{LTD}$ on corticospinal excitability. A summary of major findings along with a discussion of considerations, limitations, and future implications for each experiment follow.

5.1.0 Summary of Findings: Specific Aims

Across both experiments, the primary finding is that PAS_{LTP}→PAS_{LTD} significantly increased excitability in healthy young adults. Furthermore, presence of the BDNF Val66Met polymorphism was significantly higher in individuals that did not respond predictably to PAS_{SHAM}→PAS_{LTD} compared to those who did respond predictably.

Although categorization of responders and nonresponders may help to identify individuals who will benefit from unprimed or sham-primed PAS interventions, this categorization does not seem to influence response to PPAS interventions. The primary findings for each specific aim are listed below:

5.1.1 Specific Aim #1 (Experiment 1: $PPAS_{LTD}$)

Compare the effect of PAS_{LTP} priming followed by principal PAS_{LTD} (PAS_{LTP}→PAS_{LTD}) to sham-primed PAS_{LTD} (PAS_{SHAM}→PAS_{LTD}) on corticospinal excitability in healthy individuals.

Experimental hypothesis:

PAS_{LTP}→PAS_{LTD} will utilize homeostatic mechanisms of synaptic plasticity, resulting in a greater decrease in corticospinal excitability as evidenced by a reduction in peak-to-peak amplitude of motor evoked potentials.

Findings:

PAS_{LTP}→PAS_{LTD} significantly increased excitability over a 60-minute post-intervention period in healthy young adults and the excitability following PAS_{LTP}→PAS_{LTD} was significantly higher than that resulting from PAS_{SHAM}→PAS_{LTD}. Within the 60-minute period following PAS_{LTP}→PAS_{LTD}, excitability was greatest at 10, 20, 50 and 60 minutes. At 50 and 60 minutes, change from baseline excitability was significantly greater than that resulting from the control and sham interventions, PAS_{SHAM}→PAS_{LTD} and PAS_{SHAM}→PAS_{SHAM}. These findings oppose our hypothesis stating that the PAS_{LTP} priming would decrease the threshold for suppression thus leading to a greater amount of suppression following PAS_{LTD} principal.

5.1.2 Specific Aim #2 (Experiment 2: $PPAS_{LTP}$)

Compare the effect of PAS_{LTD} priming followed by principal PAS_{LTP} (PAS_{LTD}→PAS_{LTP}) to sham-primed PAS_{LTP} (PAS_{SHAM}→PAS_{LTP}) on corticospinal excitability in healthy individuals.

Experimental hypothesis:

PAS_{LTD}→PAS_{LTP} will utilize homeostatic mechanisms of synaptic plasticity, resulting in a greater increase in corticospinal excitability as evidenced by an increase in peak-to-peak amplitude of motor evoked potentials.

Finding:

No significant within- or between- intervention differences in corticospinal excitability were observed. This does not support our hypothesis that PAS_{LTD} priming will decrease the threshold for inducing facilitation thus increasing facilitatory effects of PAS_{LTP} principal.

5.1.3 Specific Aim #3

Compare the ratio of responders to nonresponders between PAS_{LTP}→PAS_{LTD} and PAS_{SHAM}→PAS_{LTD} and between PAS_{LTD}→PAS_{LTP} and PAS_{SHAM}→PAS_{LTP}.

Experimental hypothesis:

Priming will weight synaptic plasticity in a known direction, making it easier to weight synaptic plasticity in the opposite direction. This will result in an increased ratio of responders to nonresponders following $PPAS_{LTD}$ and $PPAS_{LTP}$ interventions.

Findings:

PAS_{LTP}→PAS_{LTD} produced fewer responders and thus a smaller ratio (3:12) compared to PAS_{SHAM}→PAS_{LTD} (8:7). PAS_{LTD}→PAS_{LTP} resulted in a slightly

smaller ratio of responders to nonresponders (8:8) compared to PAS_{SHAM}→PAS_{LTP} (10:6). Both of these findings oppose our hypothesis that priming will increase the number of responders by increasing the aftereffects of PAS.

5.1.4 Specific Aim #4

Compare the presence of the BDNF Val66Met polymorphism and the latency of MEP onset between responders and nonresponders for both PAS_{SHAM}→PAS_{LTD} and PAS_{SHAM}→PAS_{LTP}.

Experimental hypothesis:

Presence of the Val66Met polymorphism will alter plasticity and thus be higher in individuals categorized as nonresponders. The MEP onset latency from MEPs collected with the induction of anterior-poster current flow will differ between individuals categorized as responders and those categorized as nonresponders.

Findings:

MEP Onset Latency: Results indicate that the MEP onset latency did not differ between responders and nonresponders for PAS_{LTP} or PAS_{LTD}.

BDNF Val66Met Polymorphism: Over 1/3 of the individuals in this study had the Val66Met polymorphism. Nonresponders following PAS_{LTD} had a significantly higher proportion of individuals with the Val66Met polymorphism compared to those that were categorized as responders. Interestingly, there was no difference in the presence of the Val66Met polymorphism between nonresponders and responders following PAS_{LTP}.

5.2.0 Considerations & Limitations

The two experiments within this study are technically identical except for the nature of the intervention (i.e. the ISI between the peripheral and cortical stimuli). Thus, common decisions regarding the protocol may influence the interpretation of findings from either experiment.

Study Design & Primary Outcome Measure

The cross-over design of this study allows for individual responses to be compared following each intervention. This design enables a smaller sample size and strengthens the interpretation of between-intervention variability by assigning less variability to differences between participants. Although carry-over of effects is a concern when using a cross-over design, our analysis found no evidence of carry-over suggesting that the one-week washout period was sufficient. For this study, a cross-over design is especially important for the interpretation of responder and nonresponder ratios following each intervention. Because each individual received each intervention, investigators could track who was categorized as a nonresponder to sham-primed PAS and see if this assignment changed following active-primed interventions. There was no pattern of change in those who converted from responders to nonresponders or in those who converted from nonresponders to responders following PPAS for either experiment. This finding provides evidence that priming does not convert nonresponders to responders and, in conjunction with our corticospinal excitability, supports the conclusion that PPAS does not improve response to PAS by augmenting excitability changes and capturing more individuals as responders.

This study only had one primary outcome measure which was the average of 20 peak-to-peak MEP amplitudes. Other measures of intracortical excitability including SICI and ICF would have provided a stronger interpretation of which networks may have been modulated by each PPAS intervention. These measures could have also provided evidence of neuromodulation in networks that may not be obvious in gross measures like

MEP amplitude. However, a recent review of evidence suggests that the influence of PAS on SICI is highly inconsistent and that PAS exerts no influence on ICF.[158] The use of MEP amplitude is more common and the influence of PAS is more consistent which makes this an outcome measure that is more easily compared between studies. Furthermore, the reliability of SICI and ICF is less consistent than that of MEP amplitude. Although our ICC values exhibit low test-retest reliability, several other studies[139,140] support the reliability of MEP amplitude. Due to the novelty of this study, the apparent lack of a consistent influence on intracortical measures and the higher reliability of MEP amplitude measures, MEP amplitude was chosen as the sole primary outcome measure.

Attention

Stefan et al (2004) showed that attention may influence an individual's response to PAS_{LTP}. By attending to the peripheral nerve stimulus at the wrist, participants focused attention on a specific task which resulted in a larger change in peak-to-peak MEP amplitudes. Although this is a viable approach to account for attentional differences and potentially reduce variability in response, it has only been tested for PAS_{LTP}. It is possible that attending to a task serves to increase excitability, thus contributing to the increase in peak-to-peak MEP amplitudes. If this is true, it would counteract the attempt to suppress excitability using PAS_{LTD}. The purpose of this study was not to address the effect of attention on response to PAS so participants were not directed to attend to a specific task. Because this study was separated into two experiments, it would have been possible to ask participants within Experiment 2 ($PPAS_{LTP}$) to attend to a task without concern about how attention would influence interventions with principal PAS_{LTD}. However, the tester was blinded to the assignment of each participant and having only one group of participants attend to a task would unblind the tester.

Other Variables

Variables other than attention may influence an individual's response thus contributing to the observed variability and large SEM values. Cortisol levels are known to fluctuate throughout the day according to a circadian rhythm. These fluctuations may influence corticospinal excitability.[169] Investigators addressed this concern by scheduling participants at the same time of day and the same day of the week each week. Although some sessions strayed from this pattern due to scheduling conflicts, most adhered to the same weekly time and day thus reducing variability from circadian cortisol levels. The influence of caffeine appears to be minimal or non-existent[188] so caffeine intake was not monitored and participants were only asked to keep caffeine intake consistent prior to each session. A woman's menstrual cycle is known to influence measures of corticospinal excitability.[189] This is relevant to our findings because over half (58%) of participants were women of child-bearing age and each individual returned for a session on one day a week for four weeks. Thus, the duration of an individual's participation in this study spans the average 28-day cycle of menstruation and captures a woman at each week within this cycle. Further evidence is needed to determine what impact sex hormones have on corticospinal excitability and if stage of menstrual cycle should be controlled for in future neuromodulation studies.

Number of Pulses

This study was designed with comparisons between active-primed, sham-primed and sham-sham interventions. Sham priming allows participants to remain blinded to the nature of the intervention (primed or unprimed) and allows for the same amount of time to pass between pretest and posttest measures regardless of the intervention so the length of time between pretest and posttest did not differ between interventions. However, the nature of our sham intervention caused the number of cortical and peripheral pulses to differ between interventions. Earlier studies have constructed sham interventions to utilize an interstimulus interval of N20+100 ms[157] or an alternation between different intervals.[167] It is argued that this provides the same number of stimuli without inducing

STDP-like changes in neuroplasticity. However, it is possible that peripheral nerve or cortical stimulation alone still alter excitability. To avoid the possibility that an active-sham intervention would influence excitability, we used a sham air-film coil which provides no cortical stimulation and did not apply peripheral stimuli. This provides a true period of time without externally-influenced neuromodulation. A limitation of this approach is that a different number of stimuli are delivered between interventions. Although this would not have an impact on the interpretation of within-intervention analyses, it may impact the interpretation of comparisons between the sham-primed and active-primed interventions because the active-primed interventions provide twice the number of stimuli. Increasing the number of TMS pulses is known to impact corticospinal excitability by increasing MEP amplitudes.[190] The influence of increasing pairs of transcranial magnetic and peripheral nerve stimuli is unknown. If the increased number of TMS pulses in active-primed PAS increases excitability, all active-primed PAS interventions (facilitatory and suppressive) should increase excitability more than their sham-primed counterparts. This study did not find that relationship. Thus the effect of different numbers of pairs of pulses during PAS may be more complicated. Yet it is interesting to consider the impact that multiple TMS pulses may have on outcome measures during the 60-minute post-intervention period. Applying 20 pulses at a rate of 0.1 Hz every 10 minutes may influence excitability through mechanisms like anti-gating. Our study did not find significant positive or negative trends in whole group analyses however a visual analysis of linear trends shows that most are either near zero for $PPAS_{LTD}$ interventions or slightly positive for $PPAS_{LTP}$ interventions. It remains possible that the repeated TMS pulses given during post-intervention testing may suppress suppressive effects and/or augment facilitatory effects.

Length of Time between Priming and Principal PAS

The existence of multiple PAS and PPAS protocols allows a variety of parameters to be defined but it reduces the number of reproduced PAS studies thus challenging the interpretation of general efficacy. The priming protocol for this study mimics previous

studies whereby the principal bout of PAS occurs 3-5 minutes after the priming bout of PAS. Our findings do not support this protocol as an effective method of priming. Recent evidence suggests that the length of time between priming and principal may be a significantly influential parameter. A 10 minute break between bouts increases the duration of $PPAS_{LTP}$ effects whereas a break of 30 minutes actually increases both the magnitude and duration of $PPAS_{LTP}$ effects.[165] The shorter break (less than 5 minutes) used in this study may not have given sufficient time for the aftereffects of priming PAS to be captured during the application of principal PAS.

Unintended Interruption of Priming or Principal PAS

This study used the Magstim 200^2 stimulator and a $D70^2$ coil for TMS delivery. During PPAS, we experienced technical errors that included coil overheating and machine error codes E86, E80 and E74 indicating grounding faults, capacitor errors and stimulator overheating. These errors caused the machine to automatically shut down requiring 5-30 minutes of machine cooling, coil switching or investigator problem solving to resolve the issue. This occurred in ~7.5% of sessions. Because this introduced a variable length of time between priming and principal PAS for some individuals and caused an unintended interruption of priming or principal PAS, investigators conducted an outlier analysis by marking these sessions as outliers and comparing analyses that included these outliers to those that excluded potential outliers. This comparison found no differences in results that included the potential outlier sessions compared to those without the outlier sessions. Thus, the sessions with unintended interruptions were not treated as outliers.

5.2.1 Considerations for Experiment 1: $PPAS_{LTD}$

This is the first known investigation of $PPAS_{LTD}$. We tested the influence of two different $PPAS_{LTD}$ interventions: PAS_{LTP}→PAS_{LTD} and PAS_{LTD}→PAS_{LTD} on corticospinal excitability. The observed increase in corticospinal excitability following PAS_{LTP}→PAS_{LTD} was unexpected. According to the Bienenstock-Cooper-Munro theory of sliding thresholds, the application of priming PAS_{LTP} should have decreased the

threshold of induction of LTD which makes it easier to induce LTD and suppress excitability. Our findings indicate that principal PAS_{LTD} did not suppress excitability more when primed with PAS_{LTP} compared to when it is sham-primed (i.e. unprimed). Instead, principal PAS_{LTD} significantly increased excitability when primed with PAS_{LTP}. When responders and nonresponders to sham-primed PAS_{LTD} were analyzed separately, the trend in change from baseline excitability following PAS_{LTP}→PAS_{LTD} was significantly positive for nonresponders only. Thus, it appears that the unexpected facilitatory influence of PAS_{LTP}→PAS_{LTD} primarily occurs in those who do not respond predictably to PAS_{LTD}. Considering the short window of efficacy for PAS_{LTD} (<4.5 ms),[191] it is possible that the N20-5ms interstimulus interval does not optimally suppress excitability for all individuals. Investigators controlled for individual differences in afferent pathways by measuring the N20 latency for each individual on each day. However, other differences in cortical anatomy (e.g. motor pathways) or activation of different groups of neurons[176] by TMS may contribute to delays and altered signaling patterns that cause peripheral and central potentials to reach the sensorimotor cortex outside of the window of efficacy. Furthermore, the use of N20-5ms may not be ideal. Others have successfully suppressed excitability using intervals as short as 10 ms.[66,191] Thus, the problem may lie in the protocol and the solution may be to either stratify individuals into groups that respond best to certain protocols or to individualize each protocol for each participant.

Nonresponders to sham-primed PAS_{LTD} also had a higher proportion of individuals with the BDNF Val66Met polymorphism which may explain the unexpected increase in excitability following PAS_{LTP}→PAS_{LTD}. A previous study found a relationship between presence of the Val66Met polymorphism and response to PAS whereby those that had the polymorphism did not exhibit any significant changes in excitability following PAS_{LTP}.[73] This may result from an alteration of mechanisms that regulate homeostatic plasticity[173] leading to an abnormal response to neuromodulation. Because the theory behind priming relies on homeostatic plasticity, it is logical to theorize that the Val66Met polymorphism

impacts an individual's response to PPAS and that the next step would be to understand how. Aside from an exploratory analysis, our study did not compare data between those with and those without the polymorphism because the design focused on whole group effects and responder, nonresponder effects. With our finding that 35% of participants have this polymorphism, studies can now be designed to focus on the influence of this polymorphism without hesitation regarding the need to enroll an excessive number of individuals.

Although the influence of two consecutive identical bouts of PAS (PAS_{LTD}→PAS_{LTD}) was not a primary aim of this study, it is of interest because the double-dose of suppression may capitalize on homeostatic plasticity by reaching the bottom of the physiologic range of LTP and LTD. This has been demonstrated using two bouts of anodal (facilitatory) tDCS where a separation of 3 minutes led to a significant decrease in corticospinal excitability. Authors hypothesized that the top of the physiologic range for LTP was reached which led to the activation of homeostatic mechanisms and thus a significant suppression of excitability.[192] In our attempt to reach the bottom of this range, we applied two bouts of PAS_{LTD} separated by 3-5 minutes. In each analysis model (Whole Group and Responder, Nonresponder), we found no significant within or between intervention differences. We did not assess excitability during either bout (priming or principal) of PAS so we cannot say whether or not excitability was suppressed after priming, then reached a low point and began to climb, thus appearing like no change from baseline excitability by the time post-intervention tests took place. Future studies of homeostatic plasticity using PPAS would benefit from assessing excitability during each bout of PAS (e.g. by measurement of peak-to-peak MEP amplitudes elicited by the TMS pulse during PAS) to better define the timeline of the induction of homeostatic metaplasticity.

5.2.2 Considerations for Experiment 2: $PPAS_{LTP}$

Our findings indicate no significant effect of priming with either PAS_{LTD} or PAS_{LTP} on a principal bout of PAS_{LTP}. One previous study supports the hypotheses that PAS_{LTD}→PAS_{LTP} significantly increases excitability and that PAS_{LTP}→PAS_{LTP} significantly decreases excitability when compared to PAS_{LTP} alone.[68] The primary difference between this study and our own is the length of time between principal and priming bouts. Muller et al. (2007) allowed 30 minutes between bouts whereas our protocol only allowed 3-5 minutes. As discussed earlier, a 30-minute break between priming and principal may provide sufficient time for neuroplastic mechanisms to take place following PAS and shorter, 3-5 minute breaks may not be long enough. Opie et al. (2017) allowed 10 minutes between priming and principal bouts and also found no differences between PAS_{LTD}→PAS_{LTP} and $PAS_{CONTROL}$→PAS_{LTP} for groups stratified into younger and older age brackets. However, authors did find an age-dependent difference in response to PAS_{LTP}→PAS_{LTP} whereby younger individuals exhibited a significant increase in excitability following PAS_{LTP}→PAS_{LTP} and older individuals exhibited a significant decrease.[164] Our study only assessed effects in younger (18-30 yrs) adults and found a similar significant increase in linear trend in change from baseline excitability for PAS_{LTP}→PAS_{LTP} in those who were categorized as responders to PAS_{SHAM}→PAS_{LTP}. The interpretation of this finding is complicated by a significant linear trend in the same group of individuals following PAS_{SHAM}→PAS_{SHAM} indicating that those who exhibit a ≥10% increase in excitability following PAS_{SHAM}→PAS_{LTP} may have a variable range of excitability that skews more toward facilitation than suppression. Our method of PAS_{SHAM} utilized a sham air-film coil and no peripheral nerve stimulation to ensure that no external modulation of excitability would occur. The increase in excitability can only be attributed to internal factors which may be influenced by attention or other inherent internal processes that are not yet elucidated. The explanation for this variability and the significant response to PAS_{SHAM}→PAS_{SHAM} is unclear which makes it unwise to draw strong conclusions regarding the positive trend in excitability following PAS_{LTP}→PAS_{LTP} in this experiment.

Presence of the Val66Met polymorphism did not have a clear influence on the response to $PPAS_{LTP}$. Nearly an equal percentage of responders and nonresponders (40% and 33.34%, respectively) have the polymorphism thus it does not appear to influence response to PAS_{SHAM}→PAS_{LTP}. This contradicts findings by Cheeran et al. (2008) where individuals with the Val66Met polymorphism did not exhibit a significant increase in excitability following PAS_{LTP}. We may have seen significant increases in excitability in individuals with the polymorphism that were confounded by variability as noted in the significant increase of excitability following PAS_{SHAM}→PAS_{SHAM}. Another possibility is that we did not restrict the time frame of PAS delivery. Cheeran et al. (2008) only applied PAS to individuals within a 4 hour window each day which was an attempt to reduce inter-individual variability by controlling for the time of day and thus circadian variations in neuromodulators (e.g. cortisol). Although we restricted each individual to the same time of day and same day of the week (e.g. 8:00am-11:00am on Mondays), different individuals received PAS at different times of the day. For some individuals, the difference between appointment times was as large as 12 hours. We addressed this difference by using post-MEP amplitude to pre-MEP amplitude ratios for data analysis however it remains possible that a difference in the level of neuromodulators leads to a difference in the magnitude of change from baseline excitability and thus contributes to the large amount of variability in response between individuals.

5.2.3 Sham-primed PAS

The whole group analyses for each experiment found no difference between sham-primed PAS and PAS_{SHAM}→PAS_{SHAM}. Across both experiments, PAS_{LTP}→PAS_{LTD} was the only intervention to elicit a change in excitability that differed from PAS_{SHAM}→PAS_{SHAM} (an increase in excitability at 50 and 60 minutes following intervention). This suggests that PAS_{LTP}→PAS_{LTD} was the only intervention to modulate excitability and that this modulation did not occur until 50 and 60 minutes following intervention. Although this may be true, the sources of variability and considerations previously discussed likely obscured some neuromodulatory effects. It is also possible that the low-frequency (0.1

Hz) TMS testing that occurred prior to PPAS activated inhibitory networks and blocked the effects of PAS in a manner similar to that described by Delvendahl et al. (2010) for anti-gating. This finding spotlights major concerns regarding the unknowns of neuromodulation. These include the unknown influence that TMS pre-testing may exert on GABA-ergic or glutamatergic network activity, the unknown contribution to variability in response by factors like attention and hormone levels as well as the unknown sources of variability that lead to significant findings following full sham interventions. The future of PAS research is bright with directions toward refining ideal protocols, defining influential variables and translating this knowledge into clinical tools for altering motor pathway excitability.

5.3.0 Future Directions

The recent push toward individualized medicine will expand from chemotherapy treatments to rehabilitation techniques. Not every body is physiologically identical and thus it is illogical to assume that a single treatment will not only work for everyone but that it will also affect everyone in the same way. Neuromodulation therapies need to adopt this mindset and shift focus to understand the sources of within- and between-individual differences in response to the same intervention. If investigators can begin to identify influential factors, they can define efficacy of different protocols and recommend different protocols for specific individuals or stratified groups of individuals.

From this study, we have learned that the protocol for PPAS will benefit from further investigation into the length of time between priming and principal PAS, the influence of attention on PAS_{LTD} and the influence of circadian variations of neuromodulators. We have also learned that non-modifiable individual characteristics like the BDNF Val66Met polymorphism may be an easy way to begin a portfolio of measures that aid clinicians in the identification of who will and will not benefit from certain PAS interventions. Further investigation into the influence of modifiable lifestyle factors (e.g. exercise, psychological stress, sleep) may help to clarify unknown sources of variability and

provide clinicians with evidence supporting recommendations for lifestyle changes that can augment neurorehabilitation strategies.

Although this study was conducted in healthy individuals, it is meant to provide evidence and contribute to the development of neuromodulation therapies that can improve motor recovery in people with stroke. There were no adverse events reported in this study which supports the safety of PPAS and encourages a more quick and easy transition into feasibility studies in people with stroke. The lack of significant effects observed in healthy individuals may result from an attempt to alter excitability in an uninjured brain. Repeating this study in people with stroke allows for an exact comparison of protocols between healthy individuals and those with stroke which provides insight into how PPAS differs, if at all, between the two groups. Furthermore, the addition of motor function testing and the completion of a motor task during the 60-minute post-intervention period would provide evidence regarding the influence of PPAS on motor function in people with motor impairment. Thus, the translation of PPAS to people with stroke is warranted and our findings suggest that if the exact protocol is not repeated for the sake of a direct comparison, some protocol parameters (e.g. length of time between priming and principal PAS) should be reconsidered.

5.4.0 Conclusions

The purpose of this study was to assess the influence of priming on PAS_{LTD} ($PPAS_{LTD}$) and PAS_{LTP} ($PPAS_{LTP}$) on corticospinal excitability in healthy individuals and to investigate potential characteristics that may indicate whether an individual will or will not benefit from brain stimulation. We found no significant decrease in excitability in the whole group analyses for $PPAS_{LTD.}$ However, PAS_{LTP}→PAS_{LTD} caused an unexpected significant increase in excitability. We found no significant differences in whole group analyses for $PPAS_{LTP}$. Responders exhibited a significant increase in linear trend following PAS_{LTP}→PAS_{LTP} but the interpretation of this finding is confounded by a significant increase in linear trend for PAS_{SHAM}→PAS_{SHAM} in the same group of

individuals. Further characterization of non-modifiable factors (e.g. presence of the BDNF Val66Met polymorphism) and modifiable factors (e.g. attention, protocol parameters) will improve future neuromodulation efforts by unveiling variables that influence response to neuromodulation, identifying those who will benefit from neuromodulation therapies and defining the optimal method of applying PAS so that a range of individuals will benefit from its safe approach to neuromodulation.

6.0.0 References

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7.0.0 Appendices

Appendix A. Prospective Power Analyses: G*Power Input and Output

Experiment 1 ($PPAS_{LTD}$):

F tests – ANOVA: Repeated measures, within factors

Analysis:	A priori: Compute required sample size	
Input:	Effect size f	= 1.77
	α err prob	= 0.05
	Power (1−β err prob)	= 0.80
	Number of groups	= 3
	Number of measurements	= 7
	Corr among rep measures	= 0.5
	Nonsphericity correction ε	= 1
Output:	Noncentrality parameter λ	= 263.1636
	Critical F	= 2.6613045
	Numerator df	= 6.0000000
	Denominator df	= 18.0000000
	Total sample size	= 6
	Actual power	= 1.0000000

Experiment 2 ($PPAS_{LTP}$):

F tests – ANOVA: Repeated measures, within factors

Analysis:	A priori: Compute required sample size	
Input:	Effect size f	= 0.77
	α err prob	= 0.05
	Power (1−β err prob)	= 0.80
	Number of groups	= 3
	Number of measurements	= 7
	Corr among rep measures	= 0.5
	Nonsphericity correction ε	= 1
Output:	Noncentrality parameter λ	= 49.8036000
	Critical F	= 2.6613045
	Numerator df	= 6.0000000
	Denominator df	= 18.0000000
	Total sample size	= 6
	Actual power	= 0.9982097

Appendix B. REDCap Survey

Confidential

Study Participation Survey

Welcome to the Study Participation Survey for the study investigating effects of non-invasive peripheral nerve and brain stimulation. This survey will help to determine if you are eligible to participate. It is important to answer honestly and completely. A study investigator will let you know the results of your eligibility via e-mail.

Thank you for your time.

Gender
- ◯ Male
- ◯ Female
- ◯ Other

Are you pregnant?
- ◯ Yes
- ◯ No

Are you willing to take a pregnancy test?
- ◯ Yes
- ◯ No

Birth date _____

Medical History. Please answer the following questions honestly. Some answers may prompt further questioning by a study investigator.

	Yes	No
Are you taking or have you recently (within the last month) stopped using any medications?	◯	◯
Are you taking or have you recently (within the last month) stopped using any illicit or recreational drugs?	◯	◯
Do you have epilepsy or have you ever had a convulsion or seizure?	◯	◯
Have you ever had severe (ie. followed by loss of consciousness) head trauma?	◯	◯
Have you ever had a stroke?	◯	◯
Have you ever had a fainting spell or syncope?	◯	◯
Do you have diabetes?	◯	◯
Do you have any peripheral neuropathy or carpal tunnel syndrome?	◯	◯

Do you have any metal in your head?	○	○
Do you have any intracardiac lines?	○	○
Do you have any aneurysm clips or vascular clamps?	○	○
Do you have metal slivers or fragments that have not been removed?	○	○
Do you have any in-dwelling or implanted devices (e.g. neurostimulator, pacemaker, catheter, insulin pump, medication infusion device, other)	○	○
Have you ever been diagnosed with a mental or psychiatric disorder?	○	○
Do you have any hearing problems or ringing in your ears?	○	○
Do you have cochlear implants?	○	○
Did you ever have surgical procedures to your spinal cord?	○	○
Did you ever undergo transcranial magnetic stimulation in the past?	○	○

Are you taking any tricyclic anti-depressants (e.g. imipramine, clomipramine, doxepin, nortryptaline, other)

○ Yes
○ No
○ I don't know

Please list the name(s) of any medication(s) that you are currently using or recently (within the last month) stopped using.

_____

Please list the illicit or recreational drug(s) that you are currently using or recently (within the last month) stopped using.

_____

Study Preferences

What day(s) of the week would you prefer?

☐ Monday
☐ Tuesday
☐ Wednesday
☐ Thursday
☐ Friday

What time of day would you prefer?

☐ Morning
☐ Afternoon
☐ Evening

How do you prefer to be contacted?

☐ E-mail
☐ Phone

What is your phone number? _____

Appendix C. Consent Form

Consent to Participate in a Research Study

Primed vs. Unprimed Facilitatory and Depressive Paired Associative Stimulation

You are invited to participate in a research study exploring different methods of brain stimulation and how they may influence brain excitability. You were selected as a possible participant because you responded to a flyer or a presentation and you completed the online REDCap survey. We ask that you read this form carefully and ask any questions you may have before agreeing to be in this study.

This study is being conducted by Kate Frost, Ph.D. Candidate and James Carey, Ph.D., P.T. (faculty advisor) from the Rehabilitation Science Graduate Program of the University of Minnesota as well as Dr. LeAnn Snow, M.D. from the Department of Physical Medicine and Rehabilitation at the University of Minnesota. This study is funded internally by Physical Therapy Program funds.

Study Purpose

New developments in technology allow researchers to measure and influence brain activity without needing participants to undergo invasive procedures. These non-invasive methods of brain stimulation are driving the development of new rehabilitation approaches that might help people recover motor function following a brain injury. The purpose of this study is to explore the most effective method of applying paired associative stimulation to influence the excitability of the brain. Paired associative stimulation is a method of non-invasively influencing brain activity by applying an electrical stimulus to a nerve at the wrist followed soon thereafter by a transcranial magnetic stimulation (TMS) stimulus to the brain. It is known that this combination of two stimuli can change the brain's excitability, which can be helpful to promote functional recovery following brain injury. Because this study is investigating a new method of applying this stimulation, we want to see if it is effective in healthy individuals before moving on to populations of people with brain injury.

TMS is a way to stimulate the brain without surgery. We will use a special magnetic coil that we will hold on your head in a specific spot. The use of the Magstim 200^2 device in this study is an "investigational use." This means it is being tested and the device is not cleared for sale for this use in the United States by the U.S. Food and Drug Administration (FDA).

How it works is that the study device creates a magnetic effect, which goes through bone and creates an electrical current inside the head. This current may change brain activity, but we do not know the most effective way to change brain activity and that is why we are doing this study. As described below, all people will receive four different non-invasive brain stimulation interventions.

If we learn any new information that might change your mind about continuing in the study, we will tell you about it.

Participation Criteria

We are not allowed to enroll pregnant females or females currently breast-feeding in this study, so we need to give a pregnancy test to each female. We need to do this because pregnant females and unborn children need extra protection for their safety. If you have a neurological disorder, cardiac pacemaker, or metal in your head (such as cochlear implants, aneurysm clips, etc.) you cannot be in the study. If you've had a seizure or take medications that make you more likely to have a seizure, you cannot participate in this study. Furthermore, if we are unable to elicit responses from stimuli applied to a nerve at your wrist or from stimuli applied to your head using TMS, you cannot participate in this study.

If you are eligible to participate and would like to participate, all testing and non-invasive brain stimulation sessions will occur at the Clinical Translational Science Institute at the University of Minnesota.

Study Duration

You will be enrolled in this study for four weeks. Visits will take place one day a week for four weeks (you will come for four visits total). Each visit will last approximately two to three hours (total of 8-12 hours). We would like to see you at the same time of day on the same day of the week for each visit.

Study Procedures

If you agree to participate in this study, we would ask you to do the following.

First Visit:

- Investigators will measure your blood pressure, heart rate and your weight.

- Investigators will ask you a set of questions to see how you have been feeling over the last week and you will fill out a form that asks questions to determine which hand is your preferred hand.
- Investigators will collect a saliva sample from you by having you spit into a tube. This saliva will be used to analyze your genetic material. Studies have indicated that people without a certain gene are less likely to show changes in brain excitability during TMS than people with this gene. Whether you have this gene or not will not influence the procedures you get in this study or any other medical treatments you get in the future. The genetic test will only be used to help us learn the possible factors that might influence how people respond to TMS. This procedure will be done only once. You will read more about genetic testing later in this form
- Investigators will check the responsiveness of the hemisphere of your brain that is responsible for moving your preferred hand. You will be seated in a reclining chair and we will use an alcohol swab to clean an area of you preferred hand. Electromyography (EMG) electrodes will be attached to a muscle in your preferred hand to check whether a response can be detected in that muscle when a TMS pulse is applied to the corresponding hemisphere. Earplugs will be inserted into your ears to diminish the loudness of the TMS coil's clicking noise. Marks will be made on your head and a device called Brainsight will be positioned on your head to aid in reliable placement of the TMS coil. The investigator will position the coil over the desired hemisphere and apply a single pulse of stimulation. You will hear a click and you may feel a tap on your head. If no response is observed, a stimulus of higher intensity will be applied. The stimulation intensity and coil position will be continually adjusted to try to produce a response on the EMG screen with a pulse given once every 10 seconds. The lowest intensity that produces an EMG response will be recorded as an indication of your responsiveness. If no response is observed after about 15 attempts (about 3 minutes), we will then record that no response was found. If we are unable to find a response, you cannot participate further in the study. However, you will still receive compensation for this visit.
- Next, electroencephalogram (EEG) testing will occur to determine the time between a stimulation to the nerve at your wrist and when that signal arrives at your brain. For this you will wear an EEG cap. Stimulation electrodes will be placed at the wrist of your preferred hand (targeting the median nerve). A strong electrical stimulus will be applied to the median nerve using an electrical stimulator. There may be some slight discomfort with this nerve stimulation at the wrist. If we are unable to find a response to this stimulation, you cannot participate further in the study. However, you will still receive compensation for this visit.
- Investigators will then conduct the baseline test, which will consist of approximately 20 TMS pulses at an intensity just above the threshold determined earlier, but they will still be painless. We will measure the excitability of the brain by determining the size of the muscle response to these 20 pulses on the EMG screen. This concludes the baseline test.
- Investigators will apply one of four different interventions that involve pairs of stimulation (i.e. stimulation to the median nerve followed by stimulation to the brain). You will receive all four interventions, but only one will be applied during each visit. There will be a one week break between each visit. Thus, the duration of your

participation will be four weeks. For each of these interventions, the following procedures will take place:
    - With the median nerve stimulation electrodes in place, we will apply a fairly strong stimulus to the nerve, which could be mildly painful.
    - The brain stimulation that follows will occur at an intensity that was the same for baseline testing (not painful).
    - You will receive a series of these paired stimulations for a total of 30 minutes. This concludes the intervention applied on each of the four days you come in.
- Posttesting will begin immediately after the intervention. The same procedures as described for baseline testing (collection of 20 responses following single TMS pulses) will occur at 0, 10, 20, 30, 40, 50 and 60 minutes following interventions.
- Immediately following this visit, investigators will assess your response to the intervention. If your response is the same as 10 other people in your randomly assigned study group, you cannot participate further in the study. However, you will still receive compensation for this visit.

Visits 2-4:

These visits will be very similar to your first visit, but you will not have to spit into a tube or fill out a form to determine your preferred hand.

- Investigators will measure your blood pressure, heart rate and your weight.
- Investigators will ask you a set of questions to see how you have been feeling since your last visit.
- Investigators will use EMG, Briansight and TMS to determine the responsiveness of the same location on your brain as the first visit. This procedure will be identical to your first visit.
- All following procedures (i.e. EEG, baseline testing, intervention and posttesting) will be identical to your first visit as described above.

Risks of Study Participation

Likely Risks for TMS testing (30-40%)

Headache

The TMS testing and/or the EEG cap that you wear during the visits may cause a mild headache. If this happens, please inform the study investigators and/or doctors. We will stop the session if this pain occurs.

Unlikely Risks for TMS testing (< 5%)

Seizure

People with brain injury may have a higher risk of seizure from TMS testing. If seizure does occur, an immediate 911 phone call will be placed and study personnel will take measures to ensure immediate safety. All of the test sessions will occur in the Clinical Translational Science Institute, where there is ready access to life-support equipment including oxygen, suction, blood pressure monitoring, CPR equipment, and antiepileptic drugs.

Fainting

The TMS testing may cause fainting possibly associated with jerking movements of the limbs (i.e. convulsive syncope). We will measure your blood pressure at the start of each visit. To avoid fainting, we will encourage you to eat a full meal and drink extra decaffeinated fluids before study visits. You will also sit in a reclining chair. If you feel faint or lightheaded, we will stop the testing and lay you on a flat, comfortable surface. Once you feel better, you may return home.

Hearing Impairment

The TMS testing makes a clicking sound, which could cause permanent hearing loss without protective measures. To prevent this, you will wear earplugs during TMS testing. We will monitor the position of your earplugs but, in addition, you should immediately report to the investigator any loosening or detachment of an earplug during TMS. We will immediately stop TMS if you report or if an investigator observes that an earplug has loosened or has fallen out and we will resume TMS once the earplug has been re-secured.

Temporary numbness or twitching of the face

The TMS may cause temporary numbness or twitching of the face for up to one hour. The investigators will watch your face closely for any signs of twitching. We will ask you to let us know right away if you have any changes in your face, like sensation, during the stimulation. If this occurs, we will stop the stimulation immediately.

Temporary mania or intense mood

Past studies reported mood swings in patients being treated with TMS for bipolar disorder, post-traumatic stress disorder, and depression. Symptoms varied across patients and included feelings of joy, sensitivity to criticism, anger, restlessness, elevated confidence, high-flying ideas, and reduced sleeping. The duration of these symptoms lasted for hours up to five days.

Temporary thinking problems

Past studies reported difficulty in concentrating in patients receiving TMS for bipolar disorder, post-traumatic stress disorder, and depression. Symptoms lasted for hours up to five days.

Temporary difficulty with movement or motor control impairment

Possible movement problems include a tingly feeling, stiffness, or twitching of muscles in the arm that may last minutes to hours.

Temporary neck pain

Stiffness or a dull ache in the neck may last for minutes to hours.

Temporary visual changes

One study reported on two cases of people who had impaired vision before beginning TMS that was applied to the front part of the left side of the brain which is directly connected to the visual system. This was done to study the effects of TMS for major depression. One case involved a temporary worsening of her near and distance vision after TMS, whereas the other case involved a temporary improvement in her visual field. We will minimize the risk of any visual changes in our study by applying TMS to a different region of the brain that is not directly connected to the visual system.

<u>Rare Risks for TMS (< 1%)</u>

Dental pain

One person receiving TMS treatment for depression experienced pain in the teeth of the left upper jaw. The pain stopped after the treatment.

Risks with Pregnancy

The effect of TMS on the unborn fetus is not known and participating females should not be pregnant. Women of child-bearing potential will have pregnancy tests at the start of each visit. If you become pregnant, you may no longer participate in this study.

Other risks

There may be other risks associated with TMS that are not known at this time. Risks of surface EMG are rare and minimal, but may include redness or allergic reactions of the skin caused by the tape or electrode gel used in this study. As with any testing, there may be unanticipated side effects. It is important to talk to the research doctor if you wish to stop participating in the study so that you may stop the study safely. Answering some of the interview questions might make you feel uncomfortable. If there are any questions that you do not want to answer, let us know and we can skip them. Feel free to ask the study team any questions that you have about the possible side effects and risks involved in your participation in this study.

Social implications of seizure and convulsive syncope

Because of the loss of consciousness and associated convulsions (jerking movements) that occur with a seizure or convulsive syncope, the possibility exists that others may erroneously interpret such features as epilepsy. This could lead to risk of loss or denial of employability, motor vehicle licensure and insurability. To minimize this risk, if a seizure or convulsive syncope does occur, we will provide you with a letter stating that the event was experimentally produced.

Discontinuation without Subject Consent

The possibility exists that under certain conditions, we will discontinue your participation in the study without consent. These conditions would be for your safety. One condition would be if your behavior/mood becomes abnormal during TMS (confused, nonresponsive, fainting, etc). Another condition would be if you show abnormal muscle activity on the EMG screen during the TMS test session. Normally, the EMG screen will show no activity or some sporadic activity. But bursting activity or continuous activity that cannot be stopped when we request so, may signal that a seizure could soon occur; thus, we would need to stop the study immediately and discontinue future participation.

Benefits of Study Participation

If you agree to take part in this study, there may or may not be direct medical benefit to you.

Research-Related Injury

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment, and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to you or your insurance company. If you think that you have suffered a research-related injury, please let us know right away.

Compensation

For completing the total of four weeks in the study (one day per week), you will receive $100 (in Visa Gift Cards) in compensation for time/travel/parking. If you do not complete all visits, you will receive $25 for each visit that you attend (i.e. if you attend 3 visits, you will receive $75 in Visa Gift Cards). You will not be charged for any of the tests.

Confidentiality

The records of this study will be kept private; however, the U.S. Food and Drug Administration may inspect subjects' records to insure safety. In any publications or presentations, we will not include any information that will make it possible to identify you as a subject. Your record for the study may, however, be reviewed by departments at the medical center with appropriate regulatory oversight. This information will not be recorded in your medical record. To these extents, confidentiality is not absolute.

Protected Health Information (PHI)

Your PHI created or received for the purposes of this study is protected under the federal regulation known as HIPAA. Refer to the attached HIPPAA authorization for details concerning the use of this information.

Voluntary Nature of the Study

Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect current or future relations with the University or the University of Minnesota Medical Center-Fairview. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Genetic Testing

We will ask you to spit into a tube. The saliva will be used for genetic analysis. This analysis will determine if you have a certain gene that might influence your response to TMS.

Genes are in your blood and tissue, and they are what make you different from anyone else. Some genes control things like the color of your hair or eyes. Other genes might make you more likely to get certain diseases or affect whether a drug helps you and/or gives you side effects.

You have to have this genetics testing if you want to be in the study.

The study personnel will not use your tissue for any other tests without your permission. No one other than the employees of the University of Minnesota Genomics Center will test your samples.

What are the risks of genetic testing?

The risks to you and your family from genetic research are very low. Your samples will be identified only with your study code number. In the event of an unexpected breach of confidentiality, a recent federal law (Genetic Information Non-Discrimination Act, GINA) will help protect you from health insurance or employment discrimination based on genetic information obtained about you through research such as this. If you have questions about GINA or the risks of research on genetic information, plase ask study staff.

Will getting this genetic testing help me?

This testing will not help you. Information from this testing may help researchers understand brain activity and how people respond to TMS, and come up with new ways to help others in the future.

Costs and payment

You will not have to pay for the genetic testing. You will also not be paid for having this testing done.

Where will my samples go? How long will they be kept?

Your tissue sample will be stored in a secure laboratory at the University of Minnesota Genomics Center. Your sample will be stored for no more than 2 years, and then the study personnel and/or employees of the University of Minnesota Genomics Center will destroy your sample.

Your saliva sample will have a code that connects the sample to you. The study personnel will be able to find your name from the code so they can destroy your sample or instruct the University of Minnesota Genomics Center to destroy your sample if you change your mind later. If you change your mind about being in this genetics study later, you must tell the investigator you want the University of Minnesota Genomics Center to stop testing your sample(s). The study personnel or employees of the University of Minnesota Genomics Center will then destroy your sample(s). If the University of Minnesota Genomics Center did any testing before you changed your mind, the study personnel will still use the test results.

If you tell the investigator you want the University of Minnesota Genomics Center to stop testing your tissue, this is not the same as canceling your authorization (permission) to use and share your records. You must follow the directions in the separate HIPAA document to cancel your authorization to use and share your records.

What results may be expected from this study?

NO results will be shared with you, even if a medically significant result should be discovered and even if the testing reveals information that could be used by you to make healthcare of lifestyle choices that could prolong your life or prevent or delay the development of a life threatening condition.

Photographs and Videos

Photographs and videos may be taken of you during your study participation. These may include photographs and videos of you while the EMG, EEG, peripheral nerve stimulator and/or TMS are

in place or in use. Only study personnel will be taking these photographs and videos. Photographs and videos are used to help describe the methods of this study. They may be used in publications or presentations at scientific or educational meetings.

To ensure your privacy, study personnel will take measures to avoid capturing your full face in photographs and videos. If your full face is captured, it will be blurred out or covered within one week of capture. If only a portion of your face (e.g. the side of your face) is captured, this may be left un-altered in the use of that image or video.

Photographs and videos will be de-identified within one week of capture. This means that your full face will not be identifiable and your name will not be attached to these images and videos. De-identified files will be kept as long as study personnel deem appropriate for the previously indicated uses.

You have the option to separately consent to the use of photographs and videos in the separate form entitled "Photography Release Statement." If you do not wish for study personnel to take and use photographs and videos of you, your participation in this study or future studies will not be affected.

Participation in Future Studies

We would like to know if you are interested in being contacted for any future studies. These studies may or may not involve TMS. Your answer will not affect your participation in this current study. Your answer will also not affect your relationship with the University of Minnesota. If you are contacted for future studies, you are not obligated to participate in those studies. Please indicate below whether or not you wish to be contacted in the future in regards to potential study participation.

□ Yes, I would like to be contacted as a potential participant in future studies

□ No, I would not like to be contacted as a potential participant in future studies.

Contact People

You may ask questions now. If you have questions later, you are encouraged to contact Dr. James Carey (612-626-2746) or Kate Frost (612-626-0637). If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the Fairview Research Helpline at telephone number 612-672-7692 or toll free at 866-508-6961. You may also contact this office in writing or in person at Fairview Research Administration, 2344 Energy Park Drive, St. Paul, MN, 55108.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. The specific identifier for this study is NCT02619643. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at anytime.

Subject signature:_____Date:_____Time of Day:_____

Subject printed name:_____

Signature of Person Obtaining Consent:_____-
_____Date:_____ Time of Day:_____

Appendix D. Edinburgh Handedness Survey

Edinburgh Handedness Inventory

Subject Initials: _____ **ID#:** _____

Date: _____

Indicate your preference in the use of hands
++ = The preference is so strong that you would never try to use the other hand unless absolutely forced.
+ = Your preference in use of hand.
If you are truly indifferent, put a + in both columns.
Leave blank if you have no experience in that activity.

		R	L
1	Writing		
2	Drawing		
3	Throwing		
4	Scissors		
5	Toothbrush		
6	Knife (without fork)		
7	Spoon		
8	Broom (upper hand)		
9	Striking Match (match)		
10	Opening box (lid)		

Handedness Index = $\frac{\text{(R +'s minus L +'s)}}{\text{(Total +'s)}}$ ☐

Appendix E. HIPAA Form

HIPAA[1] AUTHORIZATION TO USE AND DISCLOSE INDIVIDUAL HEALTH INFORMATION FOR RESEARCH PURPOSES

1. Purpose. As a research participant, I authorize Kate Frost MS, RCEP and Dr. James Carey PhD, PT, FAPTA and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research project entitled Primed vs. Unprimed Facilitatory and Depressive Paired Associative Stimulation , [1601M82561].

2. Individual Health Information to be Used or Disclosed. My individual health information that may be used or disclosed to conduct this research includes: medical history, medication lists, medical records, results of physical exams, imaging (CT, MRI) results.

3. Parties Who May Disclose My Individual Health Information.
The researcher and the researcher's staff may obtain my individual health information from other healthcare providers, such as laboratories, which are a part of this research, as well as healthcare providers that are not part of this research (other doctors, hospitals and/or clinics) for the purposes of carrying out this research study. I authorize these parties to disclose my individual health information to the researcher and the researcher's staff for the purposes of carrying out this research study.

4. Parties Who May Receive or Use My Individual Health Information. The individual health information disclosed by parties in item 3 and information disclosed by me during the course of the research may be received and used by Kate Frost, Dr. James Carey and the researcher's staff and co-investigators. Also, if I receive compensation for participating in this study, identifying information about me may be used or disclosed as necessary to provide compensation.

5. Right to Refuse to Sign this Authorization. I do not have to sign this Authorization. If I decide not to sign the Authorization, I may not be allowed to participate in this study or receive any research related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

6. Right to Revoke. I can change my mind and withdraw this authorization at any time by sending a written notice to Kate Frost at 420 Delaware St. SE, MMC 388, Minneapolis, MN, 55455 to inform the researcher of my decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.

7. Potential for Re-disclosure. Once my health information is disclosed under this authorization, there is a potential that it will be re-disclosed outside this study and no longer covered by this authorization. However, the research team and the University's Institutional Review Board (the committee that reviews studies to be sure that the rights and safety of study participants are protected) are very careful to protect your privacy and limit the disclosure of identifying information about you.

[1] HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information.

7A. Also, there are other laws that may require my individual health information to be disclosed for public purposes. Examples include potential disclosures if required for mandated reporting of abuse or neglect, judicial proceedings, health oversight activities and public health measures.

8. Suspension of Access. I may not be allowed to review the information collected for this study, including information recorded in my medical record, until after the study is completed. When the study is over, I will have the right to access the information again.

This authorization does not have an expiration date.

I am the research participant or personal representative authorized to act on behalf of the participant.

I have read this information, and I will receive a copy of this authorization form after it is signed.

_____	_____
signature of research participant or research participant's personal representative	date
_____	_____
printed name of research participant or research participant's personal representative	description of personal representative's authority to act on behalf of the research participant

Appendix F. Photograph Release Form

Photograph Release Statement

I, _____(participant), hereby give James Carey, PhD, PT and Kate Frost, PhD Candidate, permission to use photographs or a video of me performing tests or receiving treatment related to my participation in the study, "Primed vs. Unprimed Facilitatory and Depressive Paired Associative Stimulation." I understand that this information may be shown at a scientific meeting for the purpose of explaining the study to clinicians and scientists.

_____	_____	_____
(Printed Name Participant)	(Signature Participant)	(Date)

_____	_____	_____
(Printed Name Investigator)	(Signature Investigator)	(Date)

Appendix G. Genotyping: DNA Extraction Quality Control Results

Plate ID	Well	Sample ID	Date	NanoDrop Conc.(ng/ul)	PicoGreen Conc.(ng/ul)	Volume (ul)	mass (ng)	260/280	260/230	Special Notes
201703	A01	K01	#######	278.0	104.5	150	15675	1.94	1.55	Buccal, Oragene Tube
201703	B01	K02	#######	451.7	147.8	150	22177	1.87	1.43	Buccal, Oragene Tube
201703	C01	K03	#######	552.0	149.9	150	22486	1.92	1.59	Buccal, Oragene Tube
201703	D01	K04	#######	492.7	133.6	150	20045	1.74	1.13	Buccal, Oragene Tube
201703	E01	K05	#######	1885.0	176.5	150	26475	1.99	1.77	Buccal, Oragene Tube
201703	F01	K06	#######	223.5	132.7	150	19905	1.8	1.2	Buccal, Oragene Tube
201703	G01	K07	#######	543.2	141.0	150	21146	1.92	1.53	Buccal, Oragene Tube
201703	H01	K08	#######	493.7	157.4	150	23617	1.93	1.62	Buccal, Oragene Tube
201703	A02	K09	#######	631.5	140.0	150	21006	1.8	1.36	Buccal, Oragene Tube
201703	B02	K10	#######	235.7	112.6	150	16892	1.91	1.49	Buccal, Oragene Tube
201703	C02	K11	#######	216.4	116.4	150	17463	1.96	1.95	Buccal, Oragene Tube
201703	D02	K12	#######	770.0	147.7	150	22149	2.03	1.81	Buccal, Oragene Tube
201703	E02	K13	#######	803.1	172.7	150	25910	1.9	1.82	Buccal, Oragene Tube
201703	F02	K14	#######	1661.0	171.0	150	25648	2.04	2.03	Buccal, Oragene Tube
201703	G02	K15	#######	226.0	126.3	150	18940	1.9	1.43	Buccal, Oragene Tube
201703	H02	K16	#######	134.7	92.1	150	13816	1.83	1.06	Buccal, Oragene Tube
201703	A03	K17	#######	526.7	117.4	150	17614	1.91	1.36	Buccal, Oragene Tube
201703	B03	K18	#######	183.5	103.0	150	15445	1.88	1.4	Buccal, Oragene Tube
201703	C03	K19	#######	86.7	43.7	150	6552	1.7	0.62	Buccal, Oragene Tube
201703	D03	K20	#######	161.4	74.2	150	11132	1.94	1.5	Buccal, Oragene Tube
201703	E03	K21	#######	1774.0	181.6	150	27235	2.01	1.95	Buccal, Oragene Tube
201703	F03	K22	#######	621.1	155.5	150	23323	1.91	1.48	Buccal, Oragene Tube
201703	G03	K23	#######	408.5	139.3	150	20898	1.99	1.88	Buccal, Oragene Tube
201703	H03	K24	#######	578.7	181.9	150	27283	1.83	1.43	Buccal, Oragene Tube
201703	A04	K25	#######	604.0	133.3	150	20001	1.9	1.64	Buccal, Oragene Tube
201703	B04	K26	#######	420.9	144.8	150	21718	1.87	1.53	Buccal, Oragene Tube
201703	C04	K27	#######	368.7	130.4	150	19560	1.87	1.24	Buccal, Oragene Tube
201703	D04	K28	#######	674.5	109.4	150	16408	1.71	1.05	Buccal, Oragene Tube
201703	E04	K29	#######	233.2	133.8	150	20077	1.9	1.47	Buccal, Oragene Tube
201703	F04	K30	#######	476.8	173.9	150	26087	1.84	1.33	Buccal, Oragene Tube
201703	G04	K31	#######	29.1	17.2	150	2578	1.74	0.89	Buccal, Oragene Tube
201703	H04	K32	#######	396.3	159.4	150	23904	1.92	1.77	Buccal, Oragene Tube

Appendix H. Genotyping: Taqman Results

UMGC Sample Plate	Sample Well	Sample ID	Assay name	rs#	Call	Call (Sequence)	X	Y
Carey_Sample_005	A01	201703-1	BDNF-AS(01)T	rs6265	Both	GA	3.56	4.26
Carey_Sample_005	A02	201703-9	BDNF-AS(01)T	rs6265	XX	GG	5.20	2.78
Carey_Sample_005	A03	201703-17	BDNF-AS(01)T	rs6265	Both	GA	3.95	4.97
Carey_Sample_005	A04	201703-25	BDNF-AS(01)T	rs6265	XX	GG	5.17	2.84
Carey_Sample_005	A05	201703-32	BDNF-AS(01)T	rs6265	Both	GA	4.05	5.08
Carey_Sample_005	A06	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.98	2.51
Carey_Sample_005	A07	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.99	2.46
Carey_Sample_005	A08	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.94	2.50
Carey_Sample_005	A09	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.84	2.34
Carey_Sample_005	A10	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.87	2.34
Carey_Sample_005	A11	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.86	2.43
Carey_Sample_005	A12	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.94	2.37
Carey_Sample_005	B01	201703-2	BDNF-AS(01)T	rs6265	Both	GA	4.01	4.83
Carey_Sample_005	B02	201703-10	BDNF-AS(01)T	rs6265	Both	GA	3.77	4.70
Carey_Sample_005	B03	201703-18	BDNF-AS(01)T	rs6265	Both	GA	3.88	4.88
Carey_Sample_005	B04	201703-26	BDNF-AS(01)T	rs6265	Both	GA	3.86	4.74
Carey_Sample_005	B05	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.90	2.50
Carey_Sample_005	B06	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.91	2.02
Carey_Sample_005	B07	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.95	2.35
Carey_Sample_005	B08	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.86	2.33
Carey_Sample_005	B09	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.78	2.09
Carey_Sample_005	B10	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.78	2.17
Carey_Sample_005	B11	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.80	2.26
Carey_Sample_005	B12	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.79	2.24
Carey_Sample_005	C01	201703-3	BDNF-AS(01)T	rs6265	Both	GA	3.24	3.60
Carey_Sample_005	C02	201703-11	BDNF-AS(01)T	rs6265	XX	GG	4.76	2.42

Carey_Sample_005	C03	201703-19	BDNF-AS(01)T	rs6265	XX	GG	4.81	2.51
Carey_Sample_005	C04	201703-27	BDNF-AS(01)T	rs6265	XX	GG	4.69	2.36
Carey_Sample_005	C05	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.86	2.19
Carey_Sample_005	C06	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.92	2.17
Carey_Sample_005	C07	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.85	2.19
Carey_Sample_005	C08	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.86	2.24
Carey_Sample_005	C09	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.91	1.91
Carey_Sample_005	C10	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.82	1.96
Carey_Sample_005	C11	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.90	2.01
Carey_Sample_005	C12	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.78	2.01
Carey_Sample_005	D01	201703-4	BDNF-AS(01)T	rs6265	XX	GG	4.52	2.28
Carey_Sample_005	D02	201703-12	BDNF-AS(01)T	rs6265	XX	GG	4.65	2.34
Carey_Sample_005	D03	201703-20	BDNF-AS(01)T	rs6265	XX	GG	4.38	2.17
Carey_Sample_005	D04	201703-28	BDNF-AS(01)T	rs6265	XX	GG	2.31	2.22
Carey_Sample_005	D05	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	1.00	2.02
Carey_Sample_005	D06	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.82	2.15
Carey_Sample_005	D07	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.78	2.11
Carey_Sample_005	D08	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	1.46	1.82
Carey_Sample_005	D09	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.70	1.98
Carey_Sample_005	D10	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.74	1.88
Carey_Sample_005	D11	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.78	1.88
Carey_Sample_005	D12	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.84	2.02
Carey_Sample_005	E01	201703-5	BDNF-AS(01)T	rs6265	XX	GG	4.75	2.35
Carey_Sample_005	E02	201703-13	BDNF-AS(01)T	rs6265	Both	GA	3.78	4.60
Carey_Sample_005	E03	201703-21	BDNF-AS(01)T	rs6265	XX	GG	3.37	2.43
Carey_Sample_005	E04	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.75	2.06
Carey_Sample_005	E05	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.86	2.14
Carey_Sample_005	E06	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.81	2.15

Carey_Sample_005	E07	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.74	2.38
Carey_Sample_005	E08	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.80	2.15
Carey_Sample_005	E09	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.61	2.05
Carey_Sample_005	E10	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.80	1.92
Carey_Sample_005	E11	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.80	1.82
Carey_Sample_005	E12	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.72	1.97
Carey_Sample_005	F01	201703-6	BDNF-AS(01)T	rs6265	XX	GG	4.69	2.33
Carey_Sample_005	F02	201703-14	BDNF-AS(01)T	rs6265	XX	GG	4.58	2.28
Carey_Sample_005	F03	201703-22	BDNF-AS(01)T	rs6265	XX	GG	4.65	2.28
Carey_Sample_005	F04	201703-29	BDNF-AS(01)T	rs6265	XX	GG	4.87	2.30
Carey_Sample_005	F05	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.81	2.10
Carey_Sample_005	F06	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.82	2.11
Carey_Sample_005	F07	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.87	2.07
Carey_Sample_005	F08	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.86	2.04
Carey_Sample_005	F09	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.81	1.91
Carey_Sample_005	F10	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.77	1.95
Carey_Sample_005	F11	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.79	2.01
Carey_Sample_005	F12	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.80	2.24
Carey_Sample_005	G01	201703-7	BDNF-AS(01)T	rs6265	Both	GA	3.49	3.90
Carey_Sample_005	G02	201703-15	BDNF-AS(01)T	rs6265	XX	GG	4.03	2.04
Carey_Sample_005	G03	201703-23	BDNF-AS(01)T	rs6265	XX	GG	4.23	2.13
Carey_Sample_005	G04	201703-30	BDNF-AS(01)T	rs6265	Both	GA	3.32	4.13
Carey_Sample_005	G05	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	1.06	1.92
Carey_Sample_005	G06	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.68	2.10
Carey_Sample_005	G07	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.80	1.97
Carey_Sample_005	G08	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.75	2.06
Carey_Sample_005	G09	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.75	1.90
Carey_Sample_005	G10	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.57	1.89

Carey_Sample_005	G11	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.79	1.92
Carey_Sample_005	G12	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.71	1.96
Carey_Sample_005	H01	201703-8	BDNF-AS(01)T	rs6265	XX	GG	4.02	2.15
Carey_Sample_005	H02	201703-16	BDNF-AS(01)T	rs6265	XX	GG	4.36	2.23
Carey_Sample_005	H03	201703-24	BDNF-AS(01)T	rs6265	XX	GG	4.50	2.26
Carey_Sample_005	H04	201703-31	BDNF-AS(01)T	rs6265	Both	GA	3.43	4.05
Carey_Sample_005	H05	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	1.27	2.14
Carey_Sample_005	H06	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.81	2.06
Carey_Sample_005	H07	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.83	2.09
Carey_Sample_005	H08	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.76	1.99
Carey_Sample_005	H09	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.67	2.07
Carey_Sample_005	H10	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.75	2.02
Carey_Sample_005	H11	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.81	1.90
Carey_Sample_005	H12	Blank	BDNF-AS(01)T	rs6265	Undetermined	Undetermined	0.79	1.96

Appendix I. Participant Report of Symptoms

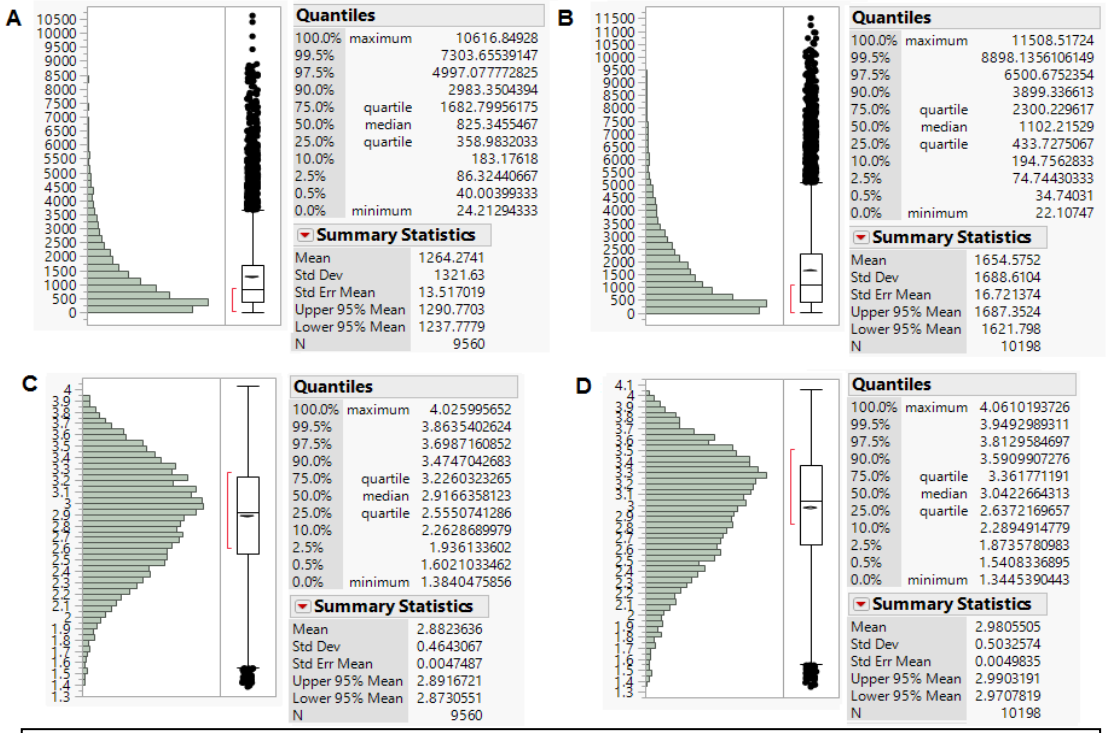
Visit 1: Participant Report of Symptoms

Participant initials: _____ Participant ID#: _____

Symptom	Before TMS/PAS	During TMS/PAS	Immediately After TMS/PAS
Seizure (Y/N)			
Headache (Y/N; 0-10)			
Neck Pain (Y/N; 0-10)			
Dental Pain (Y/N; 0-10)			
Hearing Abnormality (Y/N)			
Nausea (Y/N)			
Abnormal Muscle Contractions (Y/N)			
Dizziness (Y/N)			
Abnormal sleep (Y/N)			
Difficulty concentrating (Y/N)			
Abnormal Anxiety (Y/N)			
Abnormal Memory Problems (Y/N)			
Abnormal Mood (Y/N)			
Balance Issues (Y/N)			
Impaired Use of either hand			
Other (describe below)			

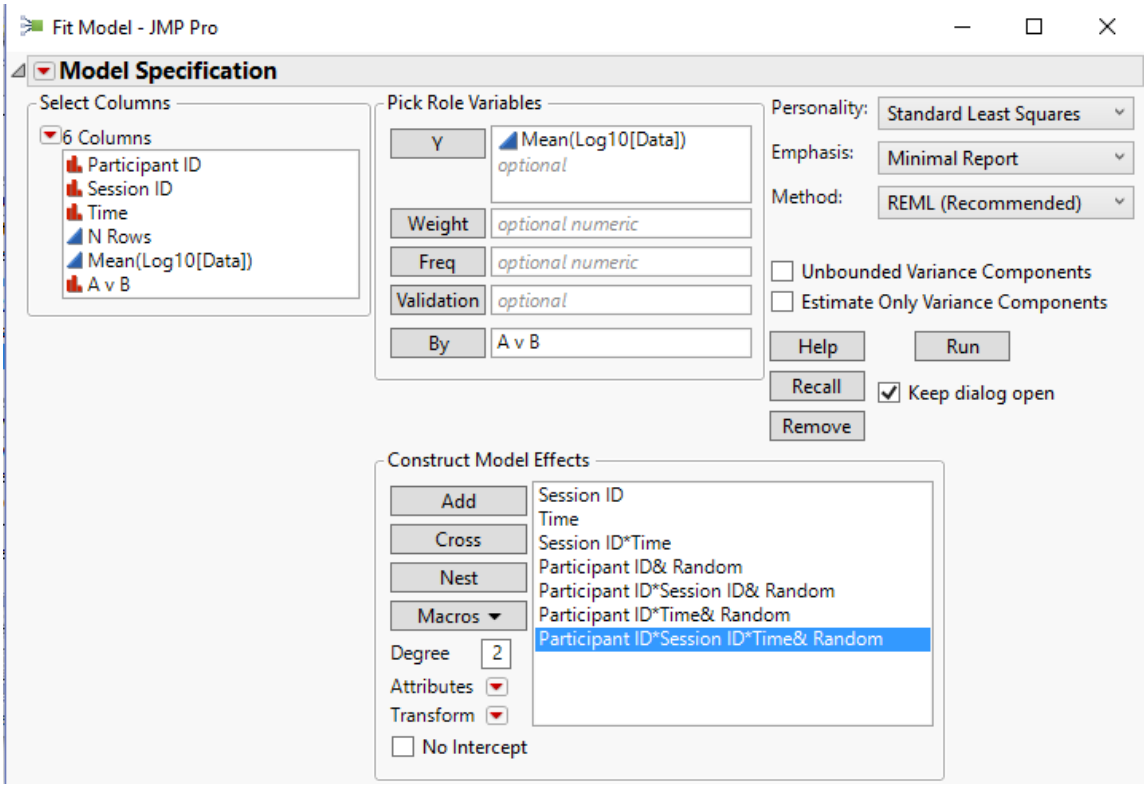
Comments:

Appendix J. MEP Variable Distributions

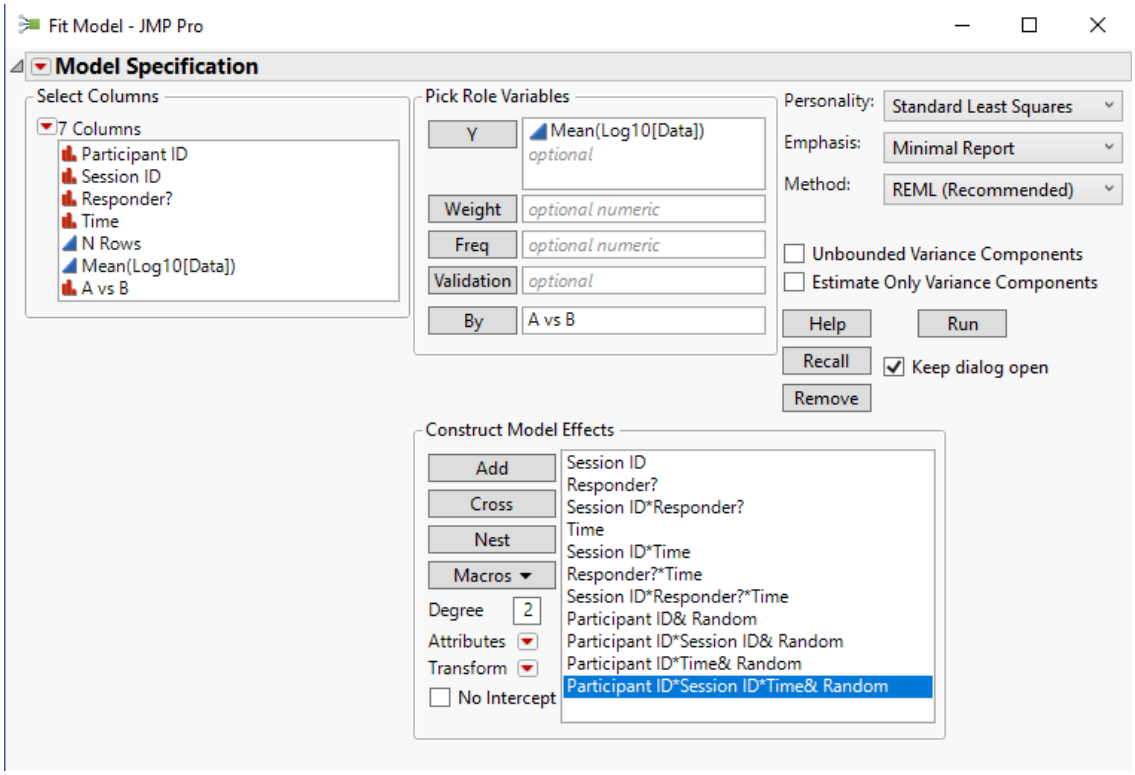


Distribution of raw peak-to-peak MEP amplitudes for (A) Experiment 1 and (B) Experiment 2. (C) and (D) show normalization of each distribution following Log_{10} transformation for each experiment respectively.

Appendix K. Methods: JMP Whole Group Fit Model Set-up



Appendix L. Methods: JMP Responder, Nonresponder Fit Model Set-up



Appendix M. Methods: Example JMP Contrast Test Detail & Results

Example contrast development using JMP to assess for statistical significance between interventions at a specific time point. This contrast is specific to Experiment 2, Whole Group and compares change from baseline values between $PAS_{SHAM} \rightarrow PAS_{LTP}$ (A1) and $PAS_{LTD} \rightarrow PAS_{LTP}$ (A2) at time point 0 minutes following intervention.

A v B=B - Fit Least Squares - JMP Pro

Response Mean(Log10[Data]) A v B=A

Effect Details

Session ID*Time

Contrast

Test Detail

A1,0	0.5
A1,10	0
A1,20	0
A1,30	0
A1,40	0
A1,50	0
A1,60	0
A1,Pretest	-0.5
A2,0	-0.5
A2,10	0
A2,20	0
A2,30	0
A2,40	0
A2,50	0
A2,60	0.5
A2,Pretest	0
A3,0	0
A3,10	0
A3,20	0
A3,30	0
A3,40	0
A3,50	0
A3,60	0
A3,Pretest	0
A4,0	0
A4,10	0
A4,20	0
A4,30	0
A4,40	0
A4,50	0
A4,60	0
A4,Pretest	0
Estimate	0.0259
Std Error	0.0435
t Ratio	0.5964
Prob>|t|	0.5512

The following is an example contrast created to assess the Whole Group linear trend for $PAS_{SHAM} \rightarrow PAS_{LTP}$ in Experiment 1.

A v B=B - Fit Least Squares - JMP Pro

Response Mean(Log10[Data]) A v B=A

Effect Details

Session ID*Time

Contrast

Test Detail

A1,0	0.5
A1,10	0.3333
A1,20	0.1667
A1,30	0
A1,40	-0.167
A1,50	-0.333
A1,60	-0.5
A1,Pretest	0
A2,0	0
A2,10	0
A2,20	0
A2,30	0
A2,40	0
A2,50	0
A2,60	0
A2,Pretest	0
A3,0	0
A3,10	0
A3,20	0
A3,30	0
A3,40	0
A3,50	0
A3,60	0
A3,Pretest	0
A4,0	0
A4,10	0
A4,20	0
A4,30	0
A4,40	0
A4,50	0
A4,60	0
A4,Pretest	0
Estimate	-0.049
Std Error	0.0401
t Ratio	-1.228
Prob>|t|	0.2203

The following is an example contrast that was set up to compare the grand average change from baseline between $PAS_{SHAM} \rightarrow PAS_{LTP}$ and $PAS_{LTD} \rightarrow PAS_{LTP}$ for Experiment 1.

A v B=B - Fit Least Squares - JMP Pro

Response Mean(Log10[Data]) A v B=A

Effect Details

Session ID*Time

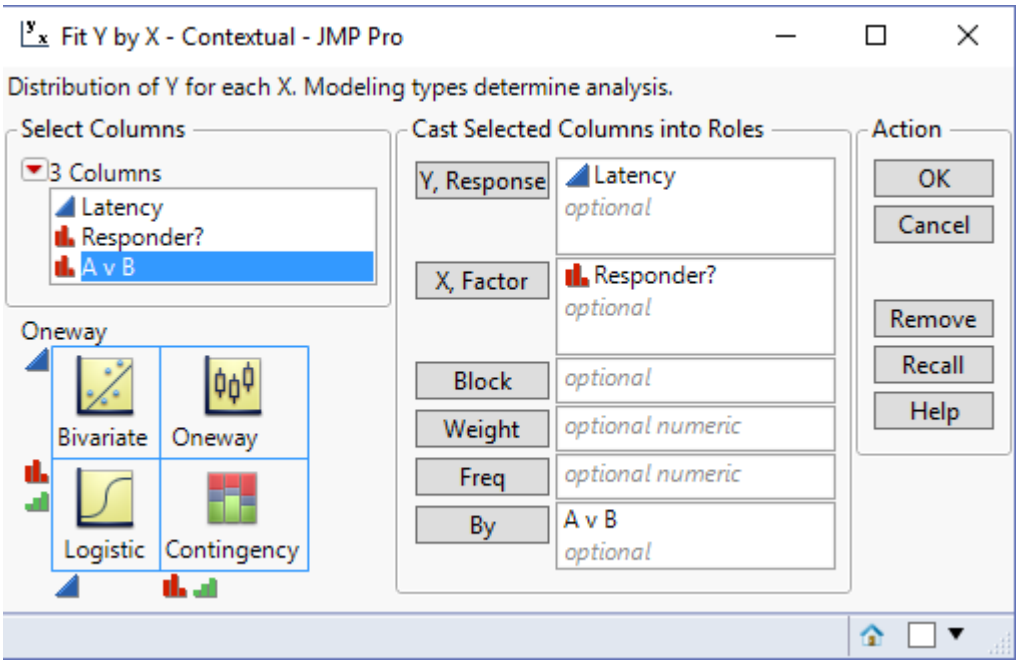
Contrast

Test Detail

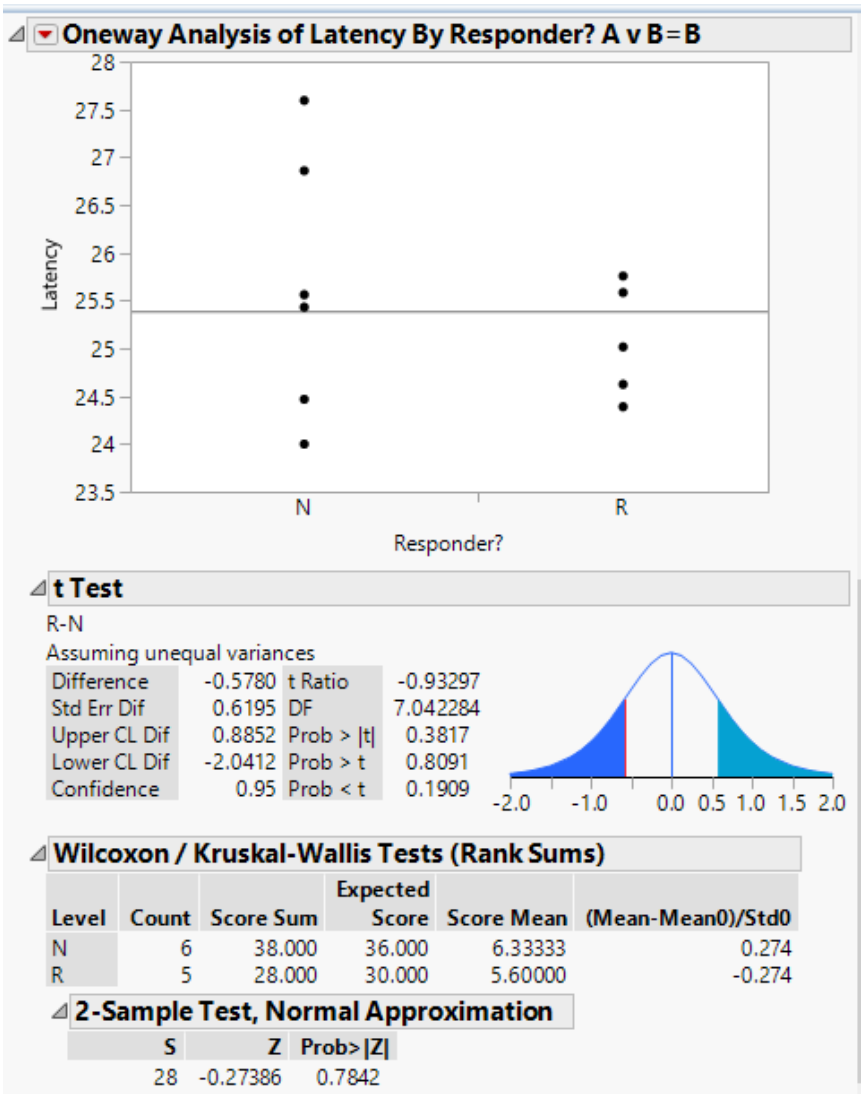
A1,0	0.0714
A1,10	0.0714
A1,20	0.0714
A1,30	0.0714
A1,40	0.0714
A1,50	0.0714
A1,60	0.0714
A1,Pretest	-0.5
A2,0	-0.071
A2,10	-0.071
A2,20	-0.071
A2,30	-0.071
A2,40	-0.071
A2,50	-0.071
A2,60	-0.071
A2,Pretest	0.5
A3,0	0
A3,10	0
A3,20	0
A3,30	0
A3,40	0
A3,50	0
A3,60	0
A3,Pretest	0
A4,0	0
A4,10	0
A4,20	0
A4,30	0
A4,40	0
A4,50	0
A4,60	0
A4,Pretest	0
Estimate	0.0209
Std Error	0.0307
t Ratio	0.6823
Prob>|t|	0.4956

Appendix N. JMP Input and Output for Mann-Whitney U (Wilcoxon Rank Sum) Test of MEP Onset Latency

JMP Input:



JMP Output for Experiment 1:



t Test

R-N

Assuming unequal variances

Difference	-0.5780	t Ratio	-0.93297
Std Err Dif	0.6195	DF	7.042284
Upper CL Dif	0.8852	Prob > |t|	0.3817
Lower CL Dif	-2.0412	Prob > t	0.8091
Confidence	0.95	Prob < t	0.1909

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

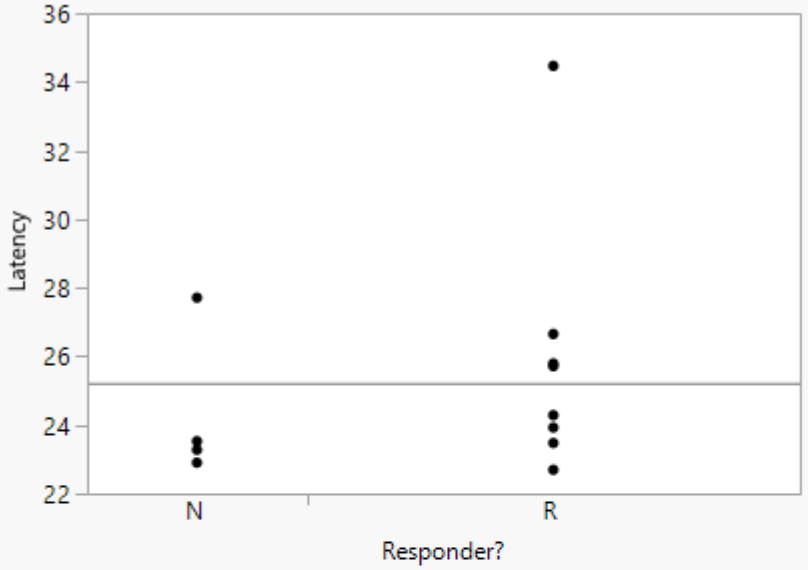
Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
N	6	38.000	36.000	6.33333	0.274
R	5	28.000	30.000	5.60000	-0.274

2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
28	-0.27386	0.7842

JMP Output for Experiment 2:

Oneway Analysis of Latency By Responder? A v B=A

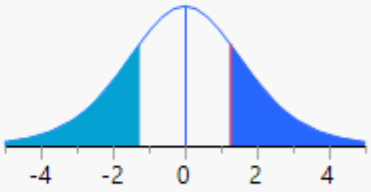


t Test

R-N

Assuming unequal variances

Difference	1.2534	t Ratio	0.765325
Std Err Dif	1.6377	DF	9.150867
Upper CL Dif	4.9489	Prob > |t|	0.4634
Lower CL Dif	-2.4421	Prob > t	0.2317
Confidence	0.95	Prob < t	0.7683



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

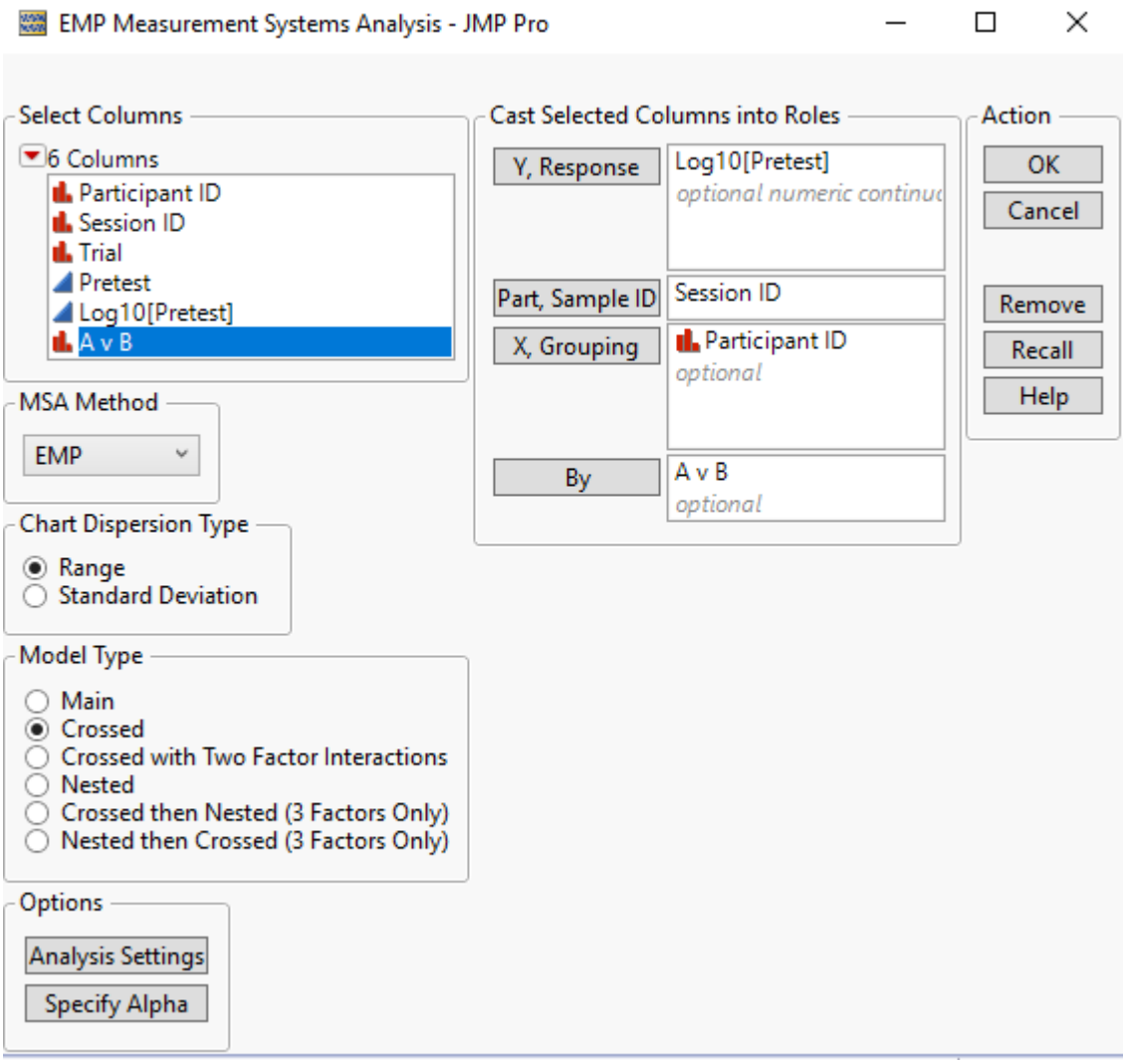
Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
N	4	23.000	28.000	5.75000	-0.694
R	9	68.000	63.000	7.55556	0.694

2-Sample Test, Normal Approximation

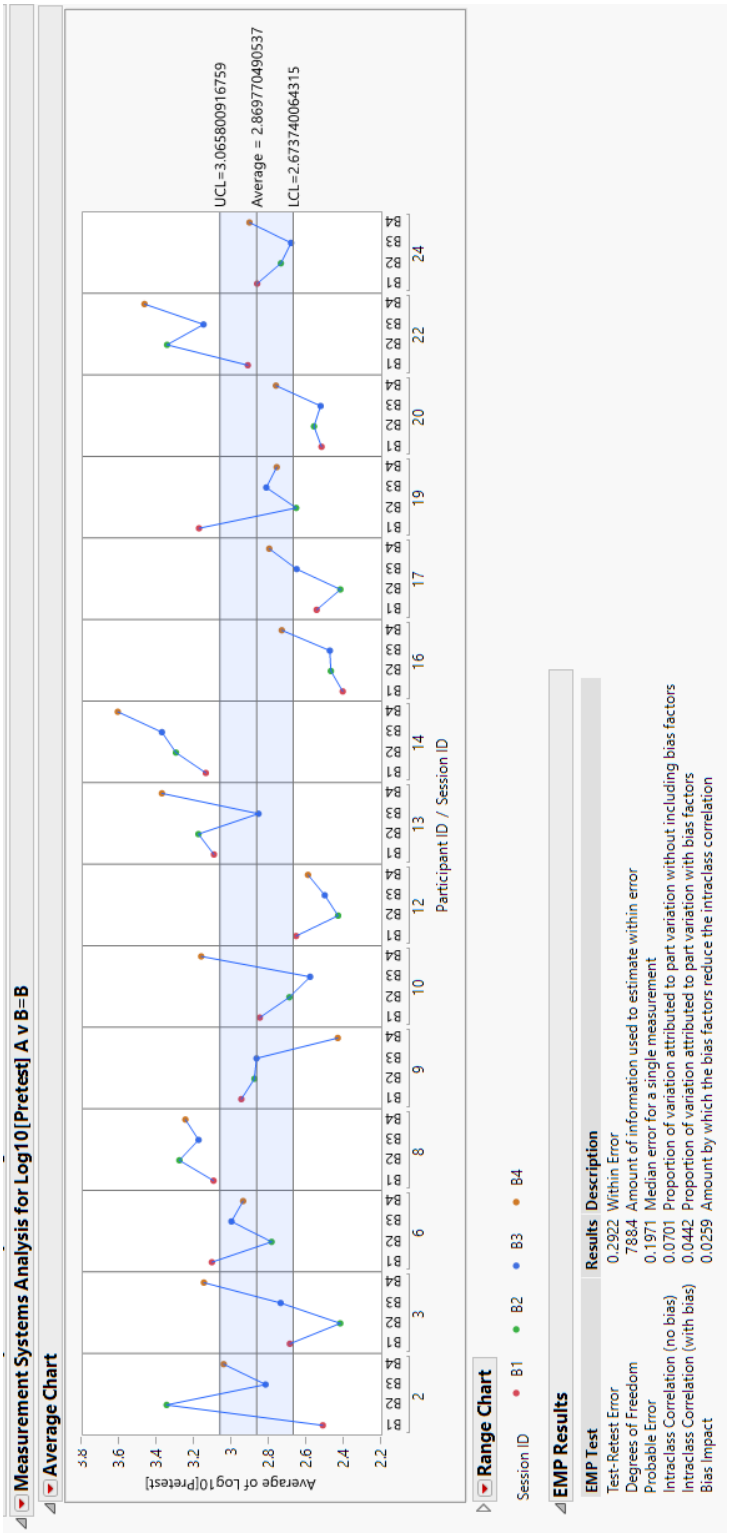
S	Z	Prob>|Z|
23	-0.69437	0.4875

Appendix O. JMP Input and Output for MEP Intraclass Correlation Coefficients

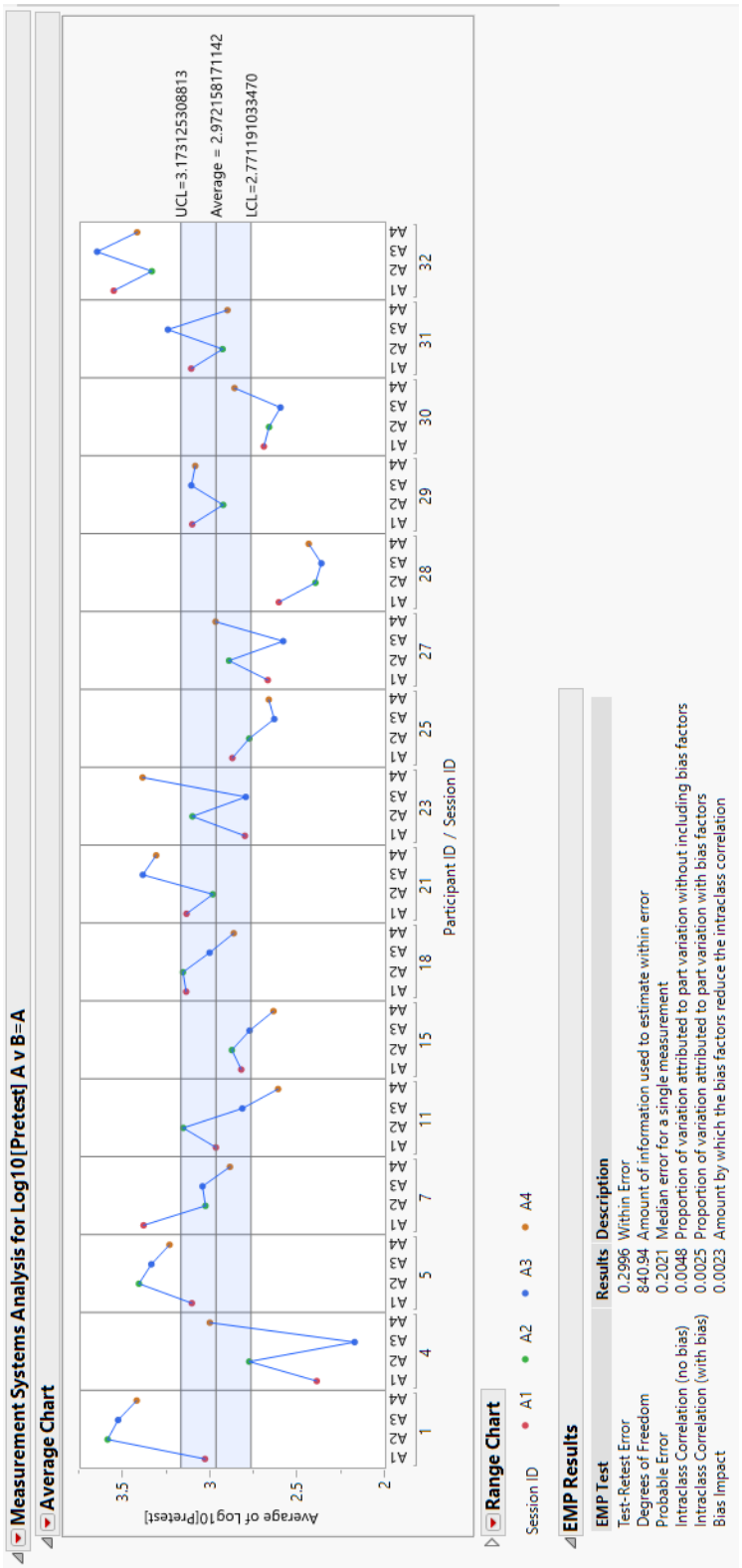
JMP Input:



JMP Output for Experiment 1:



JMP Output for Experiment 2:



Appendix P. Participant Demographics for Experiment 1

Table. Participant Demographics (Experiment 1 – $PPAS_{LTD}$)

Participant ID	Age (Years)	Gender	Handedness	Intervention	RMT (%MSO)	PT (mA)	N_{20} Latency (ms)
K02	24	F	R	0/-	36	2.7	19.4
				0/0	40	3	19.1
				-/-	41	2.9	19.1
				+/-	38	2.7	19.5
K03	19	F	R	0/-	50	2.8	18.9
				0/0	52	3.2	17.8
				-/-	49	2.6	19.2
				+/-	47	2.6	18
K06	24	F	R	0/-	60	2.3	17.8
				+/-	60	2.8	19.2
				-/-	59	1.9	18.1
				0/0	61	2.2	N/A
K08	21	M	R	0/-	50	4.6	19.5
				+/-	50	3	19.7
				-/-	50	3.7	19.4
				0/0	46	3.7	19.5
K09	21	M	R	0/-	39	2.4	18.4
				-/-	40	3.4	18.4
				0/0	43	2.9	18.1
				+/-	45	4.4	17.7

K10	24	F	L	0/-	44	1.9	18.1
				0/0	41	3.5	18.1
				-/-	41	2	17.8
				+/-	42	2	17.8
K12	24	F	R	0/-	34	2.9	17.8
				-/-	38	2.2	17.7
				+/-	37	3.2	18.1
				0/0	34	3.2	18.1
K13	25	M	R	0/-	33	2.7	19.7
				0/0	34	2.9	19.4
				+/-	33	3.7	19.5
				-/-	32	2.6	19
K14	24	F	R	0/-	29	3.6	18.9
				+/-	32	3	18.6
				0/0	33	3	18.4
				-/-	32	3	19.1
K16	27	M	R	0/-	55	2.7	18.9
				-/-	48	2.4	18.6
				0/0	44	2.5	18.9
				+/-	50	2.2	18.9
K17	29	M	R	0/-	47	3.8	20
				0/0	46	3.1	20
				-/-	51	2.8	19.7
				+/-	46	2.6	19.7

K19	24	F	R	0/-	45	2	17.3
				0/0	43	3.4	NA
				-/-	45	1.7	17.8
				+/-	45	1.8	17.3
K20	23	F	R	0/-	44	2.6	18
				-/-	43	2	17.7
				0/0	42	1.8	17.8
				+/-	37	2.6	17.5
K22	22	F	R	0/-	34	2.9	18.9
				+/-	35	2.2	18.8
				-/-	32	2.9	18.9
				0/0	33	2.4	18.8
K24	23	F	R	0/-	52	2.6	17.8
				0/0	49	2.6	18.4
				+/-	52	2.7	18.4
				-/-	49	2.8	18.1

RMT resting motor threshold, MSO maximum stimulator output, PT perceptual threshold

Appendix Q. Table of Participant Demographics for Experiment 2

Table. Participant Demographics (Experiment 2 – $PPAS_{LTP}$)

Participant ID	Age (Years)	Gender	Handedness	Intervention	RMT (%MSO)	PT (mA)	N_{20} Latency (ms)
K01	22	M	R	0/+	35	3	19.7
				-/+	37	2.6	18.4
				+/+	35	3.1	18.9
				0/0	36	3.7	18.1
K04	27	F	L	0/+	49	3.1	17.3
				+/+	49	2.2	17.3
				-/+	52	3	17.2
				0/0	54	3.3	17.8
K05	21	M	R	0/+	32	2.5	19.7
				-/+	37	3.6	19.8
				+/+	34	2.4	19.5
				0/0	31	3.1	19.5
K07	26	F	R	0/+	59	4.2	17
				0/0	59	2.6	16.9
				-/+	64	2.6	20.3
				+/+	60	3	17
K11	23	F	R	0/+	41	3	18.9
				-/+	36	4.6	18.1
				+/+	34	2.8	18.8
				0/0	38	3	18.4

K15	22	M	R	0/+	45	2.4	19.7
				0/0	52	3	NA
				-/+	48	2	19.8
				+/+	47	2.2	19.5
K18	23	F	R	0/+	47	4.2	18.9
				+/+	47	2.5	18.8
				0/0	48	4.2	18.9
				-/+	45	3.5	18.6
K21	23	F	R	0/+	46	3.3	18
				0/0	46	1.9	18.1
				-/+	42	2.5	18.6
				+/+	43	2.6	18.4
K23	23	F	R	0/+	45	3.4	19.1
				0/0	48	2.3	19.5
				-/+	39	3	19.2
				+/+	42	2.3	19.1
K25	22	F	R	0/+	39	2.5	17.3
				+/+	41	1.8	17.7
				-/+	42	2.1	18.4
				0/0	42	2	18
K27	25	M	R	0/0	33	2.4	19.8
				+/+	33	2.6	19.7
				0/+	32	2.3	19.4

				-/+	32	2.9	19.8
K28	23	M	R	0/0	43	2.3	18.9
				0/+	44	2.5	18.8
				-/+	45	2.4	19.4
				+/+	46	2.9	18.9
K29	25	F	R	0/+	28	2.1	18.3
				-/+	30	2.2	17.8
				0/0	29	1.6	17.7
				+/+	30	2	18
K30	26	M	R	0/+	40	3.4	18.6
				+/+	40	4	19.5
				0/0	39	4	19.4
				-/+	40	4	19.5
K31	18	F		0/+	36	3.2	17.0
				0/0	36	2.1	17.5
				-/+	35	2.3	17.3
				+/+	34	2.5	16.6
K32	20	M	R	0/+	40	2.3	18.9
				+/+	39	2.2	18.9
				-/+	40	2.8	19.2
				0/0	38	2.1	18.6

RMT resting motor threshold, MSO maximum stimulator output, PT perceptual threshold