**Using Transcranial Magnetic Stimulation to Investigate the Acute Effects of Translingual Neurostimulation in Individuals with Multiple Sclerosis**

by © Abby E. Blaney, A Thesis submitted

to the School of Graduate Studies in partial fulfillment

of the requirements for the degree of

MSc. Med. (Neuroscience) Faculty of Medicine

Memorial University of Newfoundland

May 2024

St. John's Newfoundland and Labrador

# Abstract

**Introduction**: Non-invasive neuromodulation techniques have emerged as a promising treatment to facilitate rehabilitation for individuals with Multiple Sclerosis (MS). One neuromodulation method, translingual neurostimulation (TLNS), involves electrical stimulation of the tongue and when paired with physiotherapy, is proposed to improve gait and balance in individuals with MS. Studies reporting the efficacy of TLNS for improving gait and balance in several neurological disorders are not congruent and the actual mechanisms underlying TLNS is not fully understood. Non-invasive brain stimulation devices such as transcranial magnetic stimulation (TMS) can help elucidate how TLNS may work to influence plasticity and recovery.

**Methods**: Participants were recruited from a clinical trial in which individuals with multiple sclerosis (MS) were randomized to receive either a real or modified TLNS device. TMS variables, including resting motor threshold (RMT), active motor threshold (AMT) and recruitment curves (excitatory and inhibitory) were measured pre and post a 20-minute TLNS stimulation.

**Results**: A repeated measures ANOVA using mixed models was conducted to investigate changes in corticospinal excitability between the TLNS stimulation and sham groups pre and post stimulation. Comparing pre and post RMT, AMT and REC values, there were no significant differences in maximum stimulator output (%MSO), motor evoked potential (MEP) amplitude latency or cortical silent period (CSP) between the stimulation and sham groups ($p > 0.1$).

**Conclusion**: Our analysis of the TMS variables, RMT, AMT and recruitment curves, indicate that 20 minutes of TLNS did not increase corticospinal excitability or decrease inhibition in individuals with MS. Future research will interrogate overall brain activation through changes in cerebral blood flow.

# Lay Summary

Multiple Sclerosis (MS) is a condition that affects the brain and spinal cord causing various symptoms. Individuals commonly report walking and balance problems as two of the most challenging and burdensome symptoms. Current drug treatments treat the progression of the disease but do not directly help with restoring walking and balance function. A treatment option called translingual neurostimulation (TLNS) uniquely uses the tongue to send signals to the brainstem and cerebellum – brain regions that control movement and balance. Pairing TLNS with physical therapy is suggested to improve walking and balance in individuals with MS, however the brain and spinal systems involved remains unclear. Our goal was to use a technique known as transcranial magnetic stimulation (TMS) to measure changes in the overall motor pathway from the brain to the spinal cord to the muscle after TLNS stimulation. However, we report no short-term effects of TLNS on the overall motor pathway.

# Acknowledgements

First and foremost, I want to sincerely thank my supervisor, Dr. Michelle Ploughman for her guidance, expertise, unwavering support, and insightful feedback on all my work. Dr. Ploughman is truly an inspiration and helped make my masters an incredibly valuable experience. I would also like to thank the members of my committee, Dr. Craig Moore and Dr. Sarah Donkers for taking the time to review my thesis and provide valuable feedback.

I also want to give a massive shoutout to all the students and staff at the Recovery and Performance Lab. I am so lucky to have been surrounded by many amazingly talented, bright, and kind individuals for the past two years. I am also grateful to all the participants who graciously volunteered their time and energy, whom without the research would not have been possible. I would also like to thank MS Canada for the opportunity to attend the endMS summer school and to Memorial University and CIHR for providing me with funding.

Lastly, a special thank you to my family and friends who have supported me from the beginning. I wouldn't be anywhere near where I am today without my amazing family and friends.

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# List of Abbreviations

AMPA: Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AMT: Active Motor Threshold

CNS: Central Nervous System

CSP: Cortical Silent Period

EEG: Electroencephalography

EMG: Electromyography

FDA: U.S Food and Drug Administration

fMRI: Functional Magnetic Resonance Imaging

fNIRS: Functional Near-Infrared Spectroscopy

GABA: Gamma Aminobutyric Acid

LTP: Long Term Potentiation

MEP: Motor Evoked Potential

MRI: Magnetic Resonance Imaging

MS: Multiple Sclerosis

NDMA: N-Methyl- d-aspartate

PoNS: Portable Neuromodulation Stimulator

RMT: Resting Motor Threshold

TLNS: Translingual Neurostimulation

TMS: Transcranial Magnetic Stimulation

# Chapter 1: Overview and Introduction

## 1.1 Multiple Sclerosis

### 1.1.1 Overview of MS

Multiple sclerosis (MS) is classified as a chronic inflammatory and demyelinating disease of the central nervous system (CNS) (Noseworthy et al., 2009). MS is a neurological disease that is most prevalent among young adults, having an onset typically between the ages of 20 to 40, with women being preferentially affected (Evans et al., 2013). More than 90, 000 people in Canada and 2.8 million people worldwide live with MS and prevalence rates are expected to increase over time (Walton et al., 2020). In MS, the immune system attacks the neuron myelin sheath causing inflammation and resulting in scarring (sclerosis), known as lesions. These lesions disrupt neuronal communication which can cause the various MS symptoms (Radtke et al., 2007). Symptoms of MS are variable but typically involve some degree of motor and/or cognitive deficit depending on the number, size and location of the lesions (Eran et al., 2018).

### 1.1.2 Epidemiology

According to the 3rd edition of the Atlas of MS (MSIF, 2020) — an extensive worldwide study on the epidemiology of MS — the prevalence of MS has increased in every world region since 2013 when the 2nd edition was published. Globally, the average age of diagnosis is 32 years old with women (69%) being 3 times more likely to be diagnosed than men (31%). Many studies have demonstrated a positive correlation between latitude and MS prevalence, suggesting that regions closer to the poles have higher rates of MS (Sabel et al., 2021). However, MS is a multifactorial disease with many genetic and environmental underpinnings. A longitudinal study by Bjornevik et al. (2022) investigated the relationship between Epstein-Barr virus and MS in a

cohort of over 10 million US military members. Investigators reported that 955 military members were diagnosed with MS during their time in service and they found a 32-fold increase in risk of MS after EBV infection. Genetic and heritability studies demonstrate that MS is associated with several genetic factors and not by a single gene mutation (International Multiple Sclerosis Genetics Consortium et al., 2007; Sawcer et al., 2011). The International Multiple Sclerosis Genetics Consortium (2019) conducted a genome-wide association study in MS that analyzed genetic data from 47,429 MS individuals and 68,374 control individuals. Results of the study identified 233 distinct risk variants (32 major histocompatibility complex (MHC), 200 autosomal non-MHC and one X chromosome), however these genetic links only explain about 40% of the difference in risk between the individuals with MS and the controls. Although several environmental and genetic factors have been shown to increase the risk of MS, the actual cause of disease remains largely unknown.

### 1.1.3 Pathogenesis

Disruption of the blood-brain barrier and immune cell infiltration are hallmarks of MS pathogenesis (Lassmann, 2018). Occurrence and progression of the disease is a result of both inflammatory and neurodegenerative processes. The blood-brain barrier is a tightly regulated, protective barrier made of endothelial cells that separates the blood vessels from the CNS (Daneman & Prat, 2015). In a healthy brain, the selective and regulated movement of ions, molecules, and cells (e.g., immune cells) between the blood vessels and CNS ensures homeostasis and normal neural function. On the other hand, a disrupted or leaky blood-brain barrier can result in different pathologies, such as MS. Studies suggest that with MS, leukocytes (white blood cells) – including T cells, B cells and macrophages – infiltrate the endothelium of

the blood-brain barrier and enter the CNS (Balasa et al., 2021; van Langelaar et al., 2020). However, there is much debate on the initiation of MS pathogenesis and is generally disputed from two standpoints: the "outside-in" model or the "inside-out" model (Titus et al., 2020). The outside-in model argues that the primary pathogenesis begins in the periphery and elicits autoimmune inflammation, followed by myelin degradation. Conversely, the inside-out model suggests pathogenesis begins with a neurodegenerative event resulting in oligodendrocyte injury/myelin destabilization followed by activation of a reactive inflammatory response. Regardless of the model, an immune response cascade is initiated, resulting in focal inflammation and subsequent destruction of oligodendrocytes – the myelinating cell of the CNS. The myelin sheath insulating the axon of neurons acts to increase nerve conduction speed and efficiency (action potentials) (Stadelmann et al., 2019). Therefore, for individuals with MS, the immune-mediated destruction of myelin in the CNS decreases neural communication and can form lesions in the brain and spinal cord, resulting in the various symptoms of disease.

### 1.1.4 Types of MS and Disease Course

There are four recognized types of MS which are characterized according to the disease course: clinically isolated syndrome (CIS), relapsing-remitting (RRMS), primary-progressive (PPMS) and secondary-progressive (SPSS) MS (Loma & Heyman, 2011). Clinically isolated syndrome refers to the first episode or clinical presentation of MS symptoms. However, not all patients who are diagnosed with CIS will develop clinical MS (Efendi, 2015). Relapsing-remitting MS is the most prevalent type (80-85% of individuals) and is characterized by unpredictable attacks/flareups where symptoms of disease may worsen, followed by a period of remission where individuals typically regain some or all lost function. The frequency and intensity in which individuals experience flareups as well as the recovery during the remission

period are variable, however over time, in general the symptoms of disease accumulate. Most individuals with relapsing-remitting MS will eventually transition into the secondary-progressive stage in which individuals typically experience steady worsening of disease with fewer relapses. Lastly, primary-progressive MS is characterized by consistent worsening of disease with no relapses. Given the several types of MS, the variability of attacks, unpredictable recovery during remission and the potential to affect any part of the CNS, the symptoms and severity of disease are highly individual.

### 1.1.5 Neurodegeneration and Lesion Formation

As mentioned previously, inflammation and demyelination can result in heterogenous lesion formation anywhere in the brain and spinal cord, including both white and gray matter regions. MS was previously considered a white matter disease, but many studies have since shown lesion formation in grey matter and the correlation with disease progression (Klaver et al., 2013; Lassmann, 2018). A recent systematic review found a strong and consistent association between white and grey matter lesions, especially in relapsing-remitting MS and further suggested that grey matter degeneration is mostly secondary to white matter damage (Lie et al., 2022). In addition, other studies found a significant increase in grey matter atrophy in advanced disease stages and a greater association (De Stefano et al., 2003; Eshaghi et al., 2018; Fisniku et al., 2008) with cognitive dysfunction compared to white matter lesions (Rothstein, 2020). According to the McDonald Criteria (2017) – a tool used by clinicians to diagnose individuals with MS – the presence of two or more lesions is required for an official MS diagnosis. Magnetic resonance imaging (MRI) is essential for identifying these demyelinating regions of the CNS to not only aid clinical diagnoses but to longitudinally monitor disease progression (Filippi et al.,

2019). Using MRI, images can be weighted (using T1 or T2-weighted imaging) to contrast specific features, including fat or cerebrospinal fluid (CSF). Given that myelin is a fatty membrane, a lesion where myelin damage has occurred has a focal decrease in fat and increase in cerebrospinal fluid which can be identified using MRI methods (Trip & Miller, 2005). Although lesions can form anywhere in the CNS, the most common locations are white matter areas such as periventricular (around the lateral ventricles) and juxtacortical (touching or within the cortex) regions, corpus collosum, infratentorial areas (pons and cerebellum) and spinal cord (cervical segment) (Filippi et al., 2019). In 1916, histologist James W. Dawson first described periventricular lesions as finger-like demyelinating regions extending from the surface of ventricles along central veins (J. W. Dawson, 1916). This pattern of lesion formation is now termed "Dawson's fingers" and a study in 2014 reported Dawson's fingers in approximately 78-92% of individuals with MS (Raz et al., 2014). The experienced symptoms of MS depend on the heterogenous nature of lesion formation, including, size, location, and the number of lesions in the CNS.

### 1.1.6 Symptoms of MS

Experienced symptoms of MS are highly variable not just within an individual but also across individuals. Some of the most common symptoms of MS are: fatigue, vision problems, numbness/tingling, pain, motor impairment (spasticity, gait, balance, etc.) and cognitive deficits (memory, information processing etc.) (Wajda & Sosnoff, 2015). Common first symptoms are optic neuritis – inflammation of the optic nerve – in which individuals report blurred vision or blind spots, pain with eye movement and altered colour vision (Malik et al., 2014) and numbness or tingling, usually affecting the legs (Ghasemi et al., 2017). A study involving a North American database consisting of 25,728 individuals with MS, revealed a pattern in symptom

presentation and accumulation over a 30-year disease course (Fox et al., 2015). Within the first year of disease onset, the most common symptoms were sensory (85% of individuals) and fatigue (81%). Vision impairment and mental illness (anxiety/depression) were commonly reported early in the disease course but did not increase in prevalence or severity with disease duration. Conversely, other symptoms such as mobility impairment, bowel/bladder function and spasticity gradually increased in prevalence over the 30-year period. Symptoms of disease do not always reflect evidence of MRI lesions and depend on the level of repair and neuroplasticity (Tafti et al., 2023).

### 1.1.7 Gait Dysfunction

Gait impairment is perceived as one of the most burdensome symptoms of MS and is associated with diminished quality of life (Sutliff, 2010). Over their lifetime, ~75% of individuals with MS will experience reduced mobility that is derived from impaired walking ability (Kister et al., 2013). In addition, ~50% of individuals with MS require assistance with walking (e.g., a cane) within 15 years of disease onset (Souza et al., 2010). Unlike other neurological disorders that can exhibit consistent gait patterns (e.g., Parkinson's disease), individuals with MS lack a characteristic gait dysfunction pattern. Gait impairment in MS can be caused by a variety of abnormalities such as ataxia, weakness, spasticity, and fatigue (Frohman, 2003). Studies have shown that these gait abnormalities can result in reduced gait speed and step length, reduced range of motion of leg joints, increased double-limb support (less time with only one foot in contact with the ground) and reduced dynamic stability. Data from the North American Research Committee on Multiple Sclerosis revealed that gait and mobility impairment

can have significantly negative effects on individuals daily living, socioeconomic status, and employment (Coleman et al., 2013).

### 1.1.8 Balance Dysfunction

Along with increased gait impairment in individuals with MS, balance problems are also a frequently reported concern (Ploughman et al., 2014). In MS, balance dysfunction is typically categorized by a decreased ability to maintain position (e.g., increased postural sway), limited/slowed movement towards limits of stability (e.g., difficulty attaining stable boundaries) and delayed responses to postural displacements and perturbations (e.g., autonomic postural response) (Cameron & Nilsagard, 2018). Due to spatial intra-lesion heterogeneity, imbalance in individuals may be a result of impairment to visual, somatosensory, vestibular, motor, or cognitive systems (Cameron et al., 2008). Impaired balance can result in falls or injury which has been demonstrated by Mazumder et al. (2014), where over a 6-month period falls were reported in 71% of individuals with MS compared to 41% of age matched healthy controls. Mobility concerns, falling and fear of falling can result in activity curtailment, social isolation, and further immobility and disability accumulation (Peterson et al., 2007). Although, there is currently no cure for MS, there are available treatments to help slow the progression of the disease and manage patient symptoms (e.g., gait and balance dysfunction).

### 1.1.9 Treatment of MS

Depending on the type and intensity of the symptom, the most common treatments are disease-modifying therapies, symptomatic drug therapy and rehabilitation (e.g., physiotherapy, occupational therapy). Disease-modifying therapies target the pathology of MS with the goal of

reducing the number and severity of relapses. Symptomatic drug therapy treats the symptoms of disease such as pain without targeting underlying issues. Rehabilitation techniques aim to restore lost function and improve quality of life (Amatya et al., 2019). Over the past few decades there has been a surge in the development of drug treatments for MS and according to the MS society of Canada, there are currently 18 disease-modifying therapies approved by Health Canada. Administration of the medications includes oral, injection or infusion and can be modified over the course of the disease. Although disease-modifying therapies are vital in disease management, medication alone does not promote functional recovery (Ploughman et al., 2022). Given that gait and mobility impairment can significantly negatively impact the quality of life of individuals with MS, it is imperative to optimize neuroplasticity and functional recovery. The remission stage offers an opportunity to restore functions lost during an MS flareup, such as walking or balance, and can be augmented by interventions such as rehabilitation.

### 1.1.10 Neurorehabilitation

Rehabilitation interventions have been shown to not only promote functional recovery but also act as a neuroprotectant (Lozinski & Yong, 2022). Several studies found that exercise regimes such as resistance training or aerobic exercise reduces brain volume loss in areas including the cortex and deep gray matter and improves aerobic capacity, functional mobility, memory, fatigue, and quality of life in individuals with MS (Devasahayam et al., 2017; Feys et al., 2019; Kjølhede et al., 2018). Studies using an experimental autoimmune encephalomyelitis (EAE) model of MS – a commonly used animal model that presents pathologically and clinically similar to MS – have shown that the rats undergoing an exercise paradigm (e.g. running or swimming), exhibited delayed onset of clinical signs, lower duration of first relapse (Le Page et

al., 1994), and a reduction in demyelination (Shahidi et al., 2020). Due to the variability and unpredictability of MS flareups, lesion formation, experienced symptoms, and overall disease course, treating the disease remains a persistent challenge. However, as technology progresses, novel approaches to treating symptoms of MS are also emerging, such as neuromodulation. Novel interventions are essential to supplement disease-modifying therapies in order to maximize function and neuroprotection in individuals with MS.

## 1.2 Neuromodulation

### 1.2.1 Classification and Applications

Many individuals across the globe unfortunately live with neurological or neuropsychiatric disorders that stem from abnormal neural circuitry of the nervous system (Ressler & Mayberg, 2007; Xiong et al., 2023). Neuromodulation is an encompassing term to describe several different techniques that stimulate different areas of the nervous system to alter or modulate this disturbed neural activity (Luan et al., 2014). Neuromodulation techniques deliver electrical or pharmaceutical agents to the brain, spinal cord, or peripheral nerves and exploit the inherent electrophysiological properties of neurons to directly modulate excitability and neural firing.

With rising rates of neurological and psychiatric disorders, the neuromodulation field of research and industry worth is rapidly expanding (Denison & Morrell, 2022). The first official application of neuromodulation in the 1960's used deep brain stimulation to treat chronic pain, which was shortly followed by spinal cord stimulation in 1967 and has since widely expanded to many other techniques, targets, and disorders (Denison & Morrell, 2022). The US Food and Drug Administration (FDA) has approved several neuromodulation devices as effective

treatments for different disorders and symptoms of the CNS such as Parkinson's disease, seizures, pain, depression etc. Neuromodulation techniques range from non-invasive such as transcranial magnetic stimulation (TMS) to invasive such as implanted spinal cord stimulation and deep brain stimulation (Luan et al., 2014). Cranial nerve stimulation is an additional neuromodulation technique and can be invasive such as vagus nerve stimulation or non-invasive such as translingual neurostimulation (TLNS). When comparing invasive and non-invasive neuromodulation techniques, there is a trade-off between specificity of location, invasiveness, and patient comfort. Invasive techniques require surgery but have greater spatial specificity, whereas non-invasive techniques are less risk-averse but may impede on patient comfort. Overall, selecting a suitable neuromodulation treatment depends on the neurological condition, area of the CNS, severity of disease and targeted symptoms of disease.

### 1.2.2 Cranial Nerve Stimulation

Cranial nerve stimulation techniques have been used to treat a variety of CNS disorders and include targets such as the vagus, cochlear, trigeminal, and facial cranial nerves (Borsody & Sacristan, 2016; Boughen et al., 2021; Kohlberg & Samy, 2020). Vagus nerve (cranial nerve X) stimulation is one of the most common cranial nerve treatments that was approved by the FDA in 1997 to treat refractory epilepsy (Howland, 2014). Since 1997, vagus nerve stimulation has also been approved by the FDA to aid in stroke rehabilitation and the treatment of migraines. Cochlear nerve (cranial nerve VIII) stimulation through a cochlear implant was the first available cranial nerve stimulator, dating back to 1972 (Bai et al., 2019). Cochlear implants are surgically implanted devices that treat severe-to-profound hearing loss through bypassing damaged cochlear hairs and directly stimulating the cochlear nerve via the spiral ganglion. Although

stimulation of the vagus and cochlear nerves have demonstrated to be effective treatments for various CNS disorders, they require an implantable device and are therefore invasive treatments with accompanied additional risks. Stimulation of the trigeminal and facial cranial nerves through TLNS is a non-invasive technique that offers minimal risk and can be used in rehabilitation paradigms for individuals with neurological disorders.

## 1.3 Translingual Neurostimulation (TLNS)

### 1.3.1 Background and Purpose

TLNS uses a portable neuromodulation device (PoNS$^{TM}$, Helius Medical Technologies) to deliver electrical stimulation the anterior surface of the tongue, stimulating the trigeminal (V) and facial (VII) cranial nerves. TLNS has become increasingly popular and promising as a treatment option when used in conjunction with physical therapy to improve motor function for individuals with certain neurological disorders. The first TLNS prototype was developed in 1999 with The Tongue Display Unit (Kaczmarek, 2011), which evolved into the Electrotactile Vestibular Substitution System (Vuillerme et al., 2011) and has now developed into the modern PoNS$^{TM}$ device. TLNS has several advantages in neurorehabilitation: 1) as indicated by the name, the device is portable which allows at home usage as well higher frequency rehabilitation regimes; 2) treatment is non-invasive and there are currently no reports of serious adverse events; and 3) the device can be used for gait and balance rehabilitation in a variety of neurological conditions (Boughen et al., 2021).

### 1.3.2 TLNS Combined with Targeted Physical Therapy

The efficacy of synergistically pairing TLNS with targeted physical therapy has been studied as a treatment for gait and balance dysfunction in several CNS disorders including traumatic brain injury, chronic balance dysfunction, stroke, and multiple sclerosis. In traumatic brain injury research, two randomized control trials examined the efficacy of pairing TLNS with physical therapy to treat chronic balance and gait deficits. Tyler et al. (2019) (N = 44) found that after 14 weeks of treatment, both traumatic brain injury groups (the high and low frequency TLNS paired with physical therapy) had significant improvements in gait and balance scores and effects were sustained for 12 weeks after treatment discontinuation. Analogous to Tyler et al., Ptito et al. (2021) (N = 122) found, that in persons with traumatic brain injury, after a 5-week treatment protocol, both groups had significant and clinically meaningful improvements in the same gait and balance outcome measures. Although both studies reported improvement in gait and balance in individuals with traumatic brain injury, there were no significant between-group differences (high and low frequency TLNS paired with physical therapy). A pilot study in individuals with subacute stroke found a significant increase in balance scores but not gait between the intervention and control groups (Galea et al., 2017). Tyler et al. (2014) was the first study to investigate the efficacy of TLNS in an MS population in which 20 individuals with MS (expanded disability status scale (EDSS) 3.5-6) were randomly assigned to an intervention (real TLNS) or control (minimally perceivable TLNS) group paired with physical therapy for 14 weeks (2x/day). Although this was a pilot study, post 14-week treatment, there were significant between-group differences with the intervention group displaying improved gait scores compared to the control group. Contrary to Tyler et al., Leonard et al. (2017) (N = 14) found no difference in gait scores between the treatment and control groups post 14-week treatment (90 minutes 2x/day) in individuals with MS (EDSS 3-6). However, researchers did report a

significant increase in balance scores in the treatment group compared to the control group. It is interesting to note that in the study by Tyler et al. (2014) the mean EDSS score for the intervention group was 5.25 while in the study by Leonard et al. (2017), the mean EDSS was 4.2. Initial disease severity or EDSS scores may impact positive outcomes of TLNS paired with physical therapy. Based on the above studies, there is a lack of consensus on the efficacy of TLNS paired with physical therapy on gait and balance in individuals with different neurological disorders.

### 1.3.3 TLNS – Proposed Mechanism of Action

The intent of TLNS via a PoNS$^{TM}$ device is to deliver electrical stimulation of the anterior superior surface tongue to stimulate the trigeminal (V) and facial (VII) cranial nerves (Danilov et al., 2015) and enhance neuroplastic changes that occur with physical rehabilitation. The pulse sequence of the device is designed to produce a continuous, comfortable, buzzing-like sensation on the tongue and deliver ~ 25,740,000 stimulation pulses through gold-plated electrodes during a standard 20-minute session (Kaczmarek, 2017). The tongue offers a unique stimulation target as branches of the trigeminal and facial nerves innervate the anterior region (Sanders, 2010). The trigeminal nerve is the largest cranial nerve and is divided into three divisions: ophthalmic, maxillary, and mandibular. The mandibular division includes an afferent branch called the lingual nerve and supplies sensation to the anterior two-thirds of the tongue. An afferent branch of the facial nerve called the chorda tympani joins the lingual in innervating the anterior two-third of the tongue and carries taste and pain signals. The tongue is proposed to be an ideal stimulation target because it is a controlled environment, and it contains a high nerve fiber density; the lingual nerve has ~ 10,000 - 33,000 fibers while the chorda tympani has ~ 3000 - 5000 fibers. Receptor density follows a negative gradient from the anterior portion of the tongue

to the posterior, hence the positioning of the electrodes toward the anterior region of the tongue (Spence, 2022).

The postulated general mechanism is that TLNS ultimately increases activation in the reticular activating systems, brainstem, and cerebellum – areas of the brain involved in modulating attention, movement, posture, balance, and coordination. In a pilot study by Wildenberg (2010), individuals with unspecified chronic balance (various underlying etiologies) (N = 12) and controls (N = 9) received nine stimulation sessions over a five day period in which functional magnetic resonance imaging (fMRI) measures were collected at baseline and on day five. They found an increase in blood oxygen level dependent (BOLD) signal within the dorsal pons after TLNS. A follow-up study by Wildenberg et al. (2011) also used fMRI and optic flow to study the effects of TLNS before and after 19 stimulation sessions over 10 days in individuals with unspecified chronic balance dysfunction (N = 9) and healthy controls (N = 9). The healthy controls underwent baseline fMRI and did not receive any stimulation treatment. They found that stimulation of the lingual and chorda tympani branches via TLNS increased activation in the brainstem projections trigeminal and solitary nuclei, respectively and increased activation of the cerebellum. It is suggested that activation of brainstem nuclei initiates a neural cascade in nearby nuclei, for example, the vestibular nuclei complex located immediately adjacent to trigeminal and solitary nuclei (Buisseret-Delmas et al., 1999; Wildenberg et al., 2010). The functional interaction between the vestibular and trigeminal nuclei may be strengthened by the stimulation. The vestibular nuclei are highly inter-connected and projects to different areas of the brain (e.g., cerebellum) involved in movement, balance, gait, awareness and breathing (Diep et al., 2021). Overall, TLNS may facilitate broader neuronal networks to permit controlled movement despite the presence of lesions as found in individuals with MS. However, additional objective and

measurable evidence using functional brain imaging tools is essential to support the proposed mechanism of action, help resolve the discrepancies in gait and balance outcome measures and ultimately inform and educate the individuals in which the device is intended to benefit.

A few studies have been conducted using functioning brain imaging tools such as fMRI and electroencephalogram (EEG) to help bridge the gap between gait and balance outcomes measures of TLNS and the underlying mechanism of action. Leonard et al. (2017) randomized 14 individuals with MS to receive either an active or sham TLNS stimulation paired with physical therapy. After the 14-week intervention, the fMRI results show a significant increase in blood oxygen level dependent signal in the left motor cortex as well as the dorsolateral prefrontal cortex (involved in working memory, cognitive flexibility, and planning) and the dorsal anterior cingulate cortex (associated with executive control, learning, adjustment, and self-control) in the active group. Another study using EEG found acute changes in microstates (e.g., attentional) between active or sham TLNS in healthy individuals (Frehlick et al., 2019). Activation of the above brain regions along with the brainstem and cerebellum via TLNS could influence cognitive and motor performance/learning. Despite the recent studies using functional brain imaging, the mechanism of action of TLNS, the potential effects of brainstem activation on cortical activity and the translation to subsequent effects on gait and balance parameters remains unclear.

## 1.4 Biomarkers

### 1.4.1 Importance of Biomarkers

Biomarkers are characteristics that can be objectively measured as an indicator of medical state and are important for diagnosis, prognosis, monitoring of disease course and

measuring response to treatments in basic and clinical research as well as clinical practice (Strimbu & Tavel, 2010). In MS, biomarkers can be classified into four categories: 1) predictive biomarkers can help identify individuals at risk for developing the disorder; 2) diagnostic biomarkers can be used to differentiate MS from other neurological disorders or healthy individuals; 3) disease activity biomarkers can monitor disease activity and help distinguish different stages of MS progression (e.g., relapsing-remitting vs. secondary-progressive MS); and 4) treatment-response biomarkers can indicate the outcome of a new treatment in a research setting as well as monitor drug effectiveness in clinical care (Paul et al., 2019). An ideal biomarker should be non-invasive, accurate, reproducible, and easily detectable in individuals.

### 1.4.2 Types of Biomarkers

Molecular and brain imaging biomarkers are the most known and considered biomarkers in MS. Molecular biomarkers are derived from fluid such as blood or cerebrospinal fluid and identify markers of inflammation. For example, cerebrospinal fluid immunoglobulin G (IgG) oligoclonal bands (OCBs) are found in the cerebrospinal fluid of > 95% of individuals with MS and neurofilament light chain (cFfL) concentration is increased during relapse (Yang et al., 2022). As mentioned earlier, MRI is an essential clinical tool for disease diagnosis, activity, and treatment response. MRI can be used as a biomarker to identify hyperintense T2 weighted lesions and atrophy of the gray matter, whole brain and spinal cord which have different implications for disease severity and prognosis (Paul et al., 2019). An additional useful biomarker, especially as a treatment-response biomarker, are functional brain imaging and electrophysiological techniques.

## 1.5 Functional Brain Imaging and Electrophysiological Techniques

### 1.5.1 Classification/Types and Applications

Functional brain imaging and electrophysiological techniques can be used to assess the effects of brain injury/disease on brain function and treatment related changes in brain systems. Functional neuroimaging and electrophysiological tests have huge implications for rehabilitation research and include non-invasive techniques such as fMRI, functional near infrared spectroscopy (fNIRS), EEG, magnetoencephalography (MEG), positron emission tomography (PET) and transcranial magnetic stimulation (TMS) (Crosson et al., 2010). fMRI and fNIRS are similar in that they both measure the hemodynamic response – changes in blood flow/hemoglobin concentrations in response to changes in neural activity – however, fMRI relies on the paramagnetic properties of hemoglobin, while fNIRS relies on the optical properties of cerebral blood flow to infer brain activity (Scarapicchia et al., 2017). Two other similar techniques, EEG and MEG, measure changes in the electric currents of the brain to infer neuronal activity using changes in electrical activity and magnetic fields respectively. PET uses radioactive tracers to measure changes in metabolic activity to deduce neuronal activity. Lastly, TMS uses magnetic pulses and electromyography (EMG) to measure changes in corticospinal excitability and probe the integrity of the corticospinal tract (Snow et al., 2019).

### 1.5.2 Transcranial Magnetic Stimulation (TMS) as a Biomarker

TMS is a non-invasive and painless brain stimulation tool that can be used as potential biomarker for CNS (dys)function and as a measure of response to treatment. TMS can be administered as a single-pulse, paired pulse or repetitive TMS. Single- and paired-pulse paradigms can be used to measure properties of the corticospinal tract whereas repetitive TMS is

used therapeutically and not as a biomarker. TMS delivers a series of brief electromagnetic pulses through an insulated coil positioned over the scalp which stimulates cortical motor neurons of the targeted stimulation site (e.g., primary motor cortex – M1) (Barker et al., 1985; Chou et al., 2022). The principle of TMS is derived from Faraday's law of electromagnetic induction in which a rapidly alternating magnetic field induces an electric current in the adjacent conductive material – for TMS, the cerebral cortex. Extracerebral tissues such as scalp and bone obstruct the induced current, however the pulse is still sufficient to depolarize superficial axons and activate the cortex (Klomjai et al., 2015). In addition, the magnetic field decreases exponentially with distance from the coil and therefore subcortical structures such as the basal ganglia and thalamus cannot be directly stimulated using TMS. Stimulation of the primary motor cortex (single- or paired-pulse) via an electromagnetic pulse using an insulated coil depolarizes motor neurons beneath the coil and induces descending volleys down the pyramidal tract and peripheral motor pathway to elicit a motor evoked potential (MEP) in the contralateral muscle under investigation (e.g., first dorsal interosseous (FDI) muscle) (Antczak et al., 2021; Chaves et al., 2021). Once the neural signal reaches the target muscle, TMS-derived MEPs can be recorded using electromyography (EMG) and used to assess excitatory and inhibitory properties of the motor system (Figure 1.1). Damage to the CNS such as brain or spinal cord lesions found in many individuals with MS can act as barriers to the transmission of a signal from the brain to the target muscle, resulting in decreased strength and speed of the transmitted and detected signal. As a result, from the TMS-derived MEPs we can measure changes in different variables including motor thresholds, MEP amplitude and latency as well as overall excitation and inhibition in response to therapeutic intervention.

## 1.6 TMS Variables and CNS Function

### 1.6.1 Motor Thresholds

One of the most measured TMS variable is the motor threshold which is defined as the lowest TMS intensity (magnetic field) required to reliably elicit a motor evoked potential (MEP) with a peak-to-peak amplitude of more than 50 μV in at least 5 out of 10 consecutive trials (Chaves et al., 2021; Rossini et al., 2015). Motor thresholds can be assessed during complete relaxation of the target muscle (resting motor threshold – RMT) or during slight tonic contraction of the target muscle (e.g., first dorsal interosseous muscle) with ~20% of the maximal strength (active motor threshold – AMT). In general, a decreased motor threshold is associated with a higher cortical excitability and the inability to obtain a MEP may indicate poorer integrity of the corticospinal tract. However, motor thresholds can be variable between and within individuals due to factors such as age, wakefulness, pharmacological influences etc. and is therefore imperative to consider and minimize confounding factors.

The variables derived from the resting and active motor thresholds – % maximum stimulation output (%MSO), MEP amplitude, latency, and cortical silent period (CSP; a measure of inhibition of the corticospinal projections) – can be used to assess changes in corticospinal excitability/inhibition in response to treatment. Maximum stimulation output is the required TMS stimulation intensity (%), MEP amplitude is measured peak-peak (μV), latency is the time (ms) from TMS stimulation to MEP onset and cortical silent period is the interruption of EMG activity (ms) following a suprathreshold TMS stimulation (Chaves et al., 2021). Compared to healthy controls, individuals with MS and stroke typically exhibit reduced MEP amplitudes, increased latency, increased % maximum stimulator output and prolonged cortical silent period (Table 1.1) (Nantes et al., 2016; Neva et al., 2016; Rossini et al., 2015; Snow et al., 2019).

Chaves et al. (2020) conducted a 10-week walking exercise training program in individuals with MS and found increased corticospinal excitability (greater MEP amplitude) and decreased intracortical inhibition (shorter cortical silent period) post treatment period. Although resting and active motor thresholds are useful TMS variables, measures derived from excitatory and inhibitory recruitment curves provide a more comprehensive and robust evaluation of corticospinal excitation and inhibition.

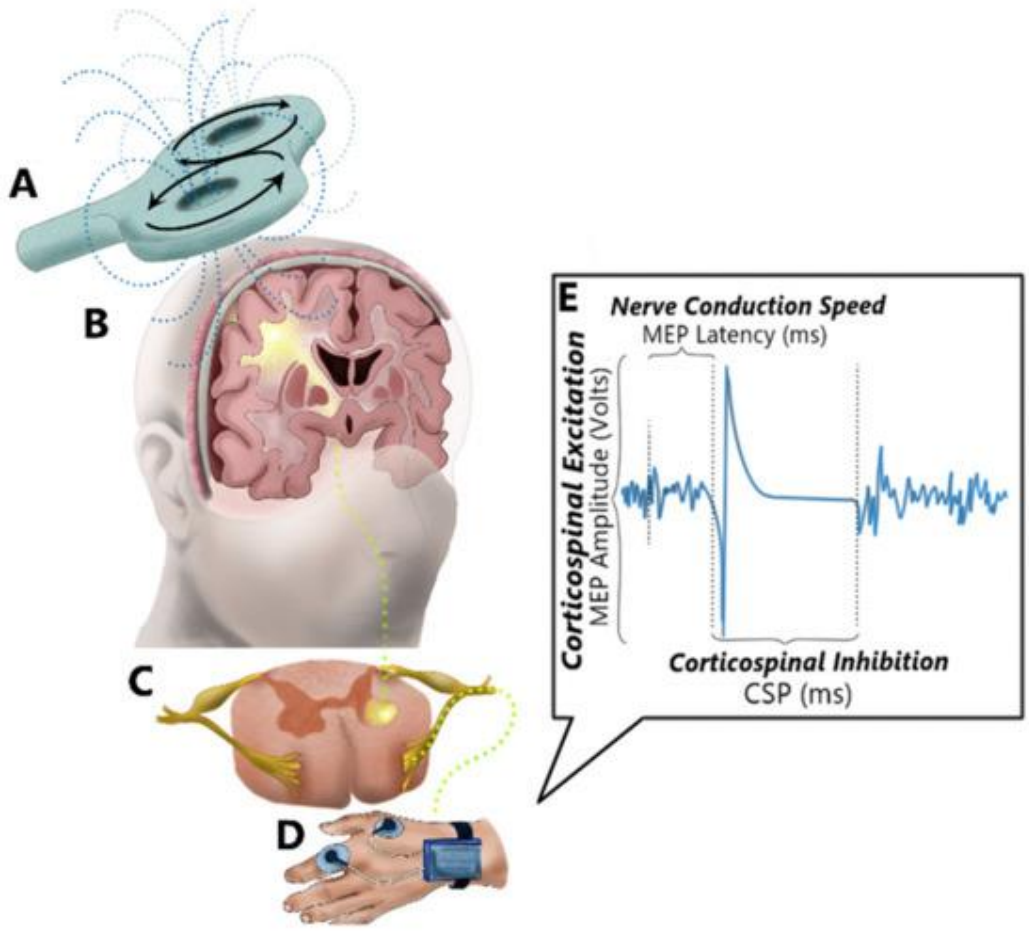


**Figure 1.1 Transcranial magnetic stimulation (TMS) neurophysiology**
Electromagnetic stimulation via an insulated coil (e.g., figure eight coil) (A) activates underlying cortical motor neurons in the motor cortex (B) which elicits descending corticospinal volleys down the pyramidal tract and peripheral motor pathway (C) and elicits a motor evoked potential (MEP) in the contralateral muscle under investigation (e.g., first dorsal interosseous (FDI) muscle) (D). TMS-derived MEPs are recorded using electromyography (EMG) and used to assess variables such as MEP latency, amplitude, and cortical silent period (CSP) (E).

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### 1.6.2 Recruitment Curves: Excitatory (eREC) and Inhibitory (iREC)

MEP recruitment curves provide useful neurophysiological measures and describe the relationship between stimulus intensity and MEP amplitude or cortical silent period (Rossini et al., 2015). The recruitment curve involves incrementally increasing TMS stimulation intensity which in general, should induce stronger descending volleys and a gradual increase in MEP amplitude and cortical silent period (Groppa et al., 2012; Rossini et al., 2015). Recruitment curves, also referred to as "input-output curve" or "stimulus-response curve", are more indicative of overall corticospinal excitation and inhibition as they provide information about neurons that are less excitable or spatially further from the targeted motor hotspot (Neva et al., 2016). Recruitment curves generally follow a sigmoid curve – beginning with a flat line, followed by a linear increase (between intensities 120-140%) reflecting MEP amplitude or cortical silent period gain which then reaches a plateau with no further increase in amplitude or cortical silent period despite an increase in stimulation intensity (Groppa et al., 2012) (Figure 1.2). From the excitatory and inhibitory recruitment curves, different variables such as area under the curve (AUC), slope and $R^2$ can be calculated and interpreted as total excitability/inhibition, recruitment gain and accuracy respectively (Table 1.1) (Kimiskidis et al., 2005; J.-P. Lefaucheur et al., 2012; Rossini et al., 2015). In addition, the stimulus-response relationship can be measured by calculating the ratio of the amplitude/cortical silent period obtained at 140% to that obtained at 120% (the linear portion/gain of the sigmoid curve) (J. P. Lefaucheur et al., 2006). The excitatory recruitment curve (MEP amplitude) is mediated by glutamatergic neurotransmission whereas the inhibitory recruitment curve (cortical silent period) is GABAergic-mediated (Stagg

et al., 2011). A study using proton magnetic resonance spectroscopy found that the slope of the excitatory recruitment curve (MEP amplitudes) positively correlates with cortical glutamate levels in the primary motor cortex (Stagg et al., 2011). In summary, the recruitment curves can provide accurate, comprehensive, and clinically relevant measures to not only infer overall integrity of the corticospinal tract but to also measure treatment-related changes.

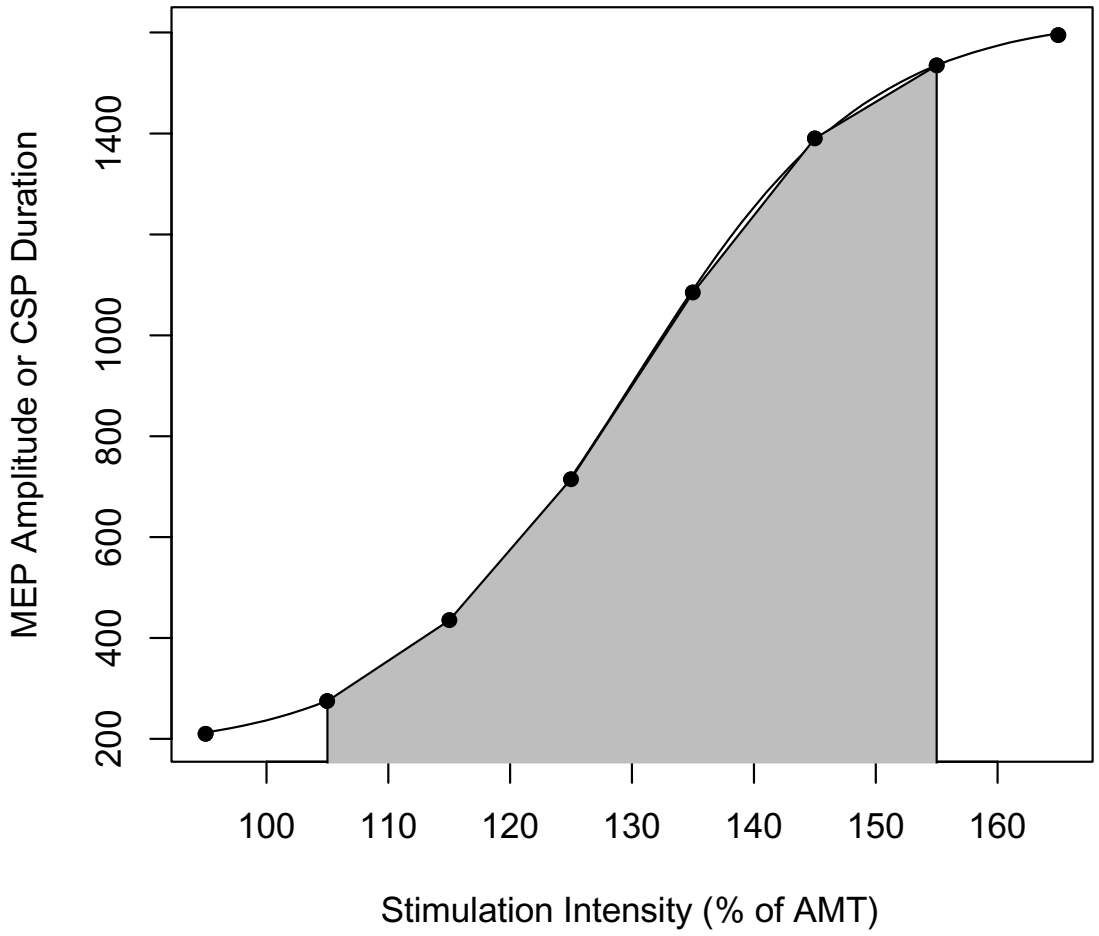


**Figure 1.2** Model of the ideal excitatory (MEP amplitudes) or inhibitory (cortical silent period - CSP duration) recruitment curves. Recruitment curves plot stimulation intensity (105-155% of active motor threshold - AMT) versus MEP amplitudes or cortical silent period duration and generally follows a sigmoid curve (boltzmann's equation). Variables such as area under the curve (highlighted in grey) can be calculated from the recruitment curves to indicate total excitability (MEP amplitudes) or inhibition (CSP duration) of the corticospinal tract. Original figure © Abby Blaney.

### 1.6.3 Excitation and Inhibition of the Corticospinal Pathway

As mentioned, TMS can be used as a biomarker to measure changes in corticospinal excitability and overall corticospinal pathway. More specifically, TMS activates a local concentration of pyramidal neurons in the underlying motor cortex including corticospinal neurons (gives rise to the corticospinal tract, which projects to the spinal cord and synapses onto motor neurons that innervate muscles), corticopontine neurons (project from the cortex to the pontine nuclei of the pons and contributes to motor coordination) and corticocortical neurons (project from one cortical area to another) (Kohn et al., 2020; Suter et al., 2013). Motor thresholds (including variables maximum stimulator output, amplitude and latency) are proposed to be mediated by glutamate neurotransmitters – the primary excitatory neurotransmitter in the CNS (Pal, 2021). Pyramidal neurons are excitatory neurons which express several types of glutamate receptors on their cell membrane including ionotropic and metabotropic receptors. Ionotropic glutamate receptors are ligand-gated ion channels and include α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and *N*-methyl-D-aspartate (NMDA) receptors (Karakas et al., 2015). The activation of pyramidal neurons, glutamate-mediated synaptic transmission, and subsequent activation of its receptors such as AMPA and NMDA are associated with long-term potentiation (LTP) and neuroplasticity (Reznikov et al., 2009).

Conversely, the inhibitory recruitment curve (iREC) comprised of cortical silent period (CSP) values is proposed to be mediated by gamma-aminobutyric acid (GABA) neurotransmitters – the primary inhibitory neurotransmitter in the CNS (Paci et al., 2021). The two main receptors that bind to GABA are $GABA_A$ (ligand-gated) and $GABA_B$ (G-protein coupled receptor). GABAergic inhibitory interneurons are important in modulating excitatory

pyramidal neurons and motor output. CSP as an indicator of corticospinal inhibition is only visible during slight tonic contraction of the muscle (e.g., first dorsal interosseous muscle) and following a TMS pulse. As mentioned, TMS stimulation of the motor cortex activates pyramidal neurons, however, GABAergic interneurons are also activated (Poston et al., 2012). Activation of $GABA_B$ receptors in particular results in the hyperpolarization of pyramidal neurons to generate an inhibitory postsynaptic potential (IPSP) and subsequent inhibition of the muscle activity as demonstrated by the MEP cortical silent period (de Leon & Tadi, 2023). Balance between excitatory and inhibitory processes is important for normal neuronal signaling and motor control. TMS studies have shown an imbalance between corticospinal excitation and inhibition in individuals with MS (Chaves, Wallack, et al., 2019; Mandolesi et al., 2015; Nantes et al., 2016).

### 1.6.4 Single-Pulse TMS as a Treatment-Response Biomarker

Single-pulse TMS outcome variables can be used to track treatment induced changes in corticospinal excitability. TMS has been utilized as a treatment-response biomarker in many neurodegenerative disease studies. In stroke research, studies reported that a single session of high frequency rTMS decreased RMT (Massie et al., 2013) and AMT (Khedr et al., 2009) and increased MEP amplitude (Hanafi et al., 2018; Kim et al., 2006) in the affected primary motor cortex. In multiple sclerosis research, a study found that a single bout of aerobic exercise enhances upper extremity corticospinal excitability in individuals with MS – reduction in inhibition (CSP) and an increase in excitation (MEP amplitude) (Chaves et al., 2020). In line with the above studies, in the case of the present study, TLNS is proposed to increase overall

brain excitability which may be reflected in a change in the corticospinal pathway of the upper extremity.

Overall, TMS can be a useful tool to probe corticospinal tract integrity by assessing the excitatory and inhibitory properties of the motor cortex and brain connectivity. Abnormalities in TMS variables (motor thresholds and recruitment curve parameters) may be indicative of abnormal neural transmission throughout the corticospinal system resulting from CNS injury such as demyelination or lesion formation as found in individuals with MS. Changes in TMS-derived MEP variables can provide evidence for treatment-related changes in the corticospinal output in individuals with neurological disorders including MS.

**Table 1.1** TMS-derived neurophysiological measures and the clinical relevance in individuals with multiple sclerosis.

| Variable | Characterization | Reported as (Units) | Physiological Significance | Clinical Significance: MS Populations vs. Healthy Controls |
|---|---|---|---|---|
| Resting Motor Threshold (RMT) | Minimum TMS intensity required to elicit 5/10 MEPs of $\geq 50$ μV during complete relaxation of FDI muscle | %MSO (1-100) | Excitability/local concentration of excitatory interneurons and corticospinal neurons (glutamatergic) | Increased RMT |
| Active Motor Threshold (AMT) | Minimum TMS intensity required to elicit 5/10 MEPs of $\geq 200$ μV during slight tonic contraction of the FDI muscle at ~20% of maximum muscle strength | %MSO (1-100) | Similar physiology to RMT with the potential contribution of fast-propagating pyramidal neurons (glutamatergic) | Increased AMT |
| MEP Amplitude | MEP peak-to-peak amplitude recorded by EMG | Microvolts (μV) | Integrity and excitability of the | Decreased amplitude and harder to |

| | | | | |
|---|---|---|---|---|
| | | | corticospinal tract (glutamatergic) | facilitate despite increasing TMS stimulation intensity |
| MEP Latency | Time from TMS stimulation to MEP onset | Time (ms) | Corticospinal nerve impulse transmission/conduction | Increased Latency |
| MEP Cortical Silent Period (CSP) | The interruption of EMG activity following a suprathreshold TMS stimulation | Time (ms) | Corticospinal inhibition (GABAergic) | Prolonged CSP |
| Excitatory Recruitment Curve (eREC) | Incremental increase in TMS stimulation using six suprathreshold intensities ranging from 105-155% of the AMT | MEP amplitudes (μV) | Recruitment of less excitable or spatially further neurons (glutamatergic) | AUC decreased; slope decreased |
| Inhibitory Recruitment Curve (iREC) | Incremental increase in TMS stimulation using six suprathreshold intensities ranging from 105-155% of the AMT | Cortical silent period (CSP – ms) | Recruitment of less excitable or spatially further neurons (GABAergic) | AUC increased; slope increased |

**Note:** AUC, area under the curve; GABA, γ-Aminobutyric acid; FDI, first dorsal interosseous; MEP, motor evoked potential; %MSO, percent maximal stimulator output (TMS stimulation intensity); TMS, transcranial magnetic stimulation.
Adapted from "Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee" by Rossini et al., 2015, *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *126*(6), 1071–1107; "Transcranial Magnetic Stimulation as a Potential Biomarker in Multiple Sclerosis: A Systematic Review with Recommendations for Future Research" by Snow et al., 2019, *Neural Plasticity*, *2019*; "Clinical diagnostic utility of transcranial magnetic stimulation in neurological disorders. Updated report of an IFCN committee" by Vucic et al., 2023, *Clinical Neurophysiology*, *150*, 131–175.

## 1.7 Thesis Objective

The aim of the present study was to use transcranial magnetic stimulation (TMS) to investigate the acute effects of TLNS via a portable neuromodulation stimulator (PoNS™) on

CNS function in individuals with MS. More specifically, I used single-pulse TMS as a biomarker to probe potential acute TLNS-induced changes in upper extremity corticospinal excitability and inhibition. Current studies using functional brain imaging suggest TLNS increases activation of subcortical brain regions (brainstem and cerebellum) associated with movement, gait, balance, and coordination, however the extent and mechanism by which activation of the brainstem and cerebellum propagate to cortical regions remains unclear. TLNS is postulated to induce global excitability changes in the brain which can be detected using TMS as a treatment-response biomarker. In addition, there remains discrepancies in clinical studies reporting gait and balance as outcome measures of TLNS paired with physical therapy. TMS may help to elucidate the effects of TLNS on the corticospinal tract as well as the connection between changes in overall brain and spinal cord activation/connectivity and the subsequent translation to neural transmission through the corticospinal tract to the target muscle.

## 1.8 Co-Authorship Statement

This research was conducted under the supervision of Dr. Michelle Ploughman, who devised the study and provided guidance to all aspects of the project. This project was completed in conjunction with a collaborative clinical trial with Helius Medical Company by Dr. Michelle Ploughman and Dr. Sarah Donkers (University of Saskatchewan). AB independently conducted the literature survey, performed data pre-processing and analysis, and interpreted and communicated findings. Co-authors Syed Raza, Caitlin Newell, Ganeswara Rao Melam, Syamala Buragadda and Isabella Burry assisted with participant recruitment, data collection and data extraction/analysis. This manuscript is in preparation to be submitted to the journal of clinical neurophysiology.

# Chapter 2: Manuscript

## 2.1 Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system that affects about 2.8 million people worldwide (Evans et al., 2013). The immune system attacks the myelin sheath, causing degeneration of the white and gray matter. Although symptoms of disease are variable, over 70% of individuals with MS experience motor impairment such as gait and balance dysfunction (Ploughman et al., 2014). The onset of disease is typically between the ages of 20 to 40 years with the cumulative burden of symptoms being associated with lower health-related quality of life (Amtmann et al., 2018; Gil-González et al., 2020; McCabe & McKern, 2002). As the prevalence rate of MS continues to increase, preferentially affecting individuals in their most productive years of life, the need for novel treatment options to aid daily functioning is essential.

Current treatments include disease-modifying therapies (DMTs), symptomatic drug therapy and rehabilitation (e.g., physiotherapy, occupational therapy). DMTs are crucial as they target the pathology of the disease with the goal of reducing disease activity and clinical progression, however, DMT's alone do not directly induce long-term neuroplastic changes and functional recovery (Ploughman et al., 2022; Rae-Grant et al., 2018). Neurorehabilitation, such as training-based interventions, has been shown to promote motor recovery for neurological disorders such as stroke, traumatic brain injury and MS (Devasahayam et al., 2017; Ploughman et al., 2019; Sandroff et al., 2020). Although neurorehabilitation interventions are effective, typically not all function is restored. Combining specific treatment interventions to create a synergistic effect, offers potential options to maximize recovery for individuals with motor impairments.

Neuromodulation is a therapeutic method that delivers electrical or chemical agents directly to a neurological site, allowing the modulation of specific networks (Abboud et al., 2017). Neuromodulation techniques range from non-invasive, such as transcranial magnetic stimulation, to invasive, such as implanted spinal cord stimulation and deep brain stimulation. Studies using a type of neuromodulation called cranial nerve stimulation, have shown that stimulation of the vagus nerve (J. Dawson et al., 2021; Neren et al., 2016) or the trigeminal and facial nerves (via the tongue) can help facilitate rehabilitation for individuals with traumatic brain injury, stroke and MS. One particular cranial nerve stimulation technique called translingual neurostimulation (TLNS) delivers electrical stimulation to the tongue to modulate the brainstem and cerebellum (Wildenberg et al., 2010) – locations of the brain associated with balance and movement. A 14-week pilot study by Tyler et al. (2014) (N = 20) showed that TLNS in conjunction with physical therapy improved gait and balance in individuals with MS. Another study found an increase in blood oxygen level dependent signal in the left motor cortex, the dorsolateral prefrontal cortex and the dorsal anterior cingulate cortex (areas involved in motor control, learning, planning and executive control) after 14 weeks of TLNS combined with physical therapy in individuals with MS (N = 14) (Leonard et al., 2017). However, the mechanism in which TLNS directly influences cortical activation, and the corticospinal pathway is not known. In addition, several brain imaging studies investigating TLNS have small sample sizes (D'Arcy et al., 2020; Leonard et al., 2017), were conducted in non-clinical populations (Frehlick et al., 2019) or lacked a placebo stimulation paradigm (Wildenberg et al., 2010).

The integrity of the corticospinal pathway can be probed using Transcranial Magnetic Stimulation (TMS), a non-invasive electrophysiological technique that uses magnetic pulses to induce neuronal activation in the underlying cortex, producing TMS-induced motor evoked

potentials (MEP) (Rossini et al., 2015). TMS has several applications in clinical research including as a biomarker (single- or paired-pulse TMS) of disease state and to measure neurophysiological effects of therapeutic interventions (Chaves et al., 2021). Single-pulse TMS protocols derive information about the overall integrity of the corticospinal pathways; lower motor thresholds, higher TMS-induced peak MEP amplitude, shorter latency, and shorter cortical silent period (CSP) indicate a healthier corticospinal tract (Chaves et al., 2020; Chaves et al., 2021). Single-pulse TMS has been widely used as a prognostic, progression, and response measure for stroke recovery (Di Pino et al., 2014) and neurodegenerative diseases such as Parkinson's disease (Fisher et al., 2008; J.-P. Lefaucheur, 2005), Alzheimer's disease (Antczak et al., 2021; Mimura et al., 2021), ALS, and MS (Caramia et al., 2004; Snow et al., 2019). Findings confirm that changes in corticospinal excitability correlate with changes in disease severity and recovery. In MS research, studies have found increased motor thresholds (resting and active) (Vucic et al., 2012; Zipser et al., 2018), decreased latency (Nantes, et al., 2016; Neva et al., 2016; Perretti et al., 2004) and increased CSP (Nantes, et al., 2016; Tataroglu et al., 2003) in individuals with MS compared to healthy controls. Although most previous work has been cross-sectional, TMS has the potential to detect neuromodulation-induced changes of the excitatory and inhibitory properties of the motor cortex and corticospinal pathway, important information in neuromodulation clinical trials.

The goal of this study was to investigate the acute/immediate effects of 20 minutes of TLNS via a portable neuromodulation stimulator (PoNS™, Helius Medical Technologies), or a sham device, on upper extremity corticospinal excitability in individuals with MS participating in a clinical trial of neuromodulation plus PT for gait and balance (Ploughman et al., 2023). We

hypothesized that only individuals receiving TLNS stimulation (not sham stimulation) would exhibit increased brain excitability and decreased brain inhibition.

## 2.2 Methods

### 2.2.1 Study Design

The current study was conducted in conjunction with an ongoing clinical trial investigating the effects of TLNS paired with targeted physical therapy on gait and balance in individuals with MS (Ploughman et al., 2023) (NCT05275049). In brief, the randomized control trial was quadruple-blinded (participants, physical therapists, assessors, and investigators), in which individuals with MS were randomized to receive either real TLNS stimulation or a sham stimulation device. Both groups received 14 weeks of individualized physical therapy. Following study approval from the Human Research Ethics Board (Newfoundland and Labrador: HREB#2012.085; Saskatchewan: HREB Bio 2578), participants were recruited and gave written informed consent per the Declaration of Helsinki. During the baseline period, we collected demographic and disease-related variables, a list of medications and co-morbid conditions. Additionally, we screened for potential contraindications for TLNS stimulation and TMS assessments before subsequently conducting the TMS testing. Before beginning the trial intervention, to measure the immediate effects of TLNS, we used a repeated measures design in which we measured TMS variables before and after one 20-minute stimulation. During the 20-minute stimulation, participants in both groups (TLNS stimulation and sham) were requested to remain seated, with the device turned on, and perform their breathing and awareness training (20 minutes of mindfulness/meditation with the PoNS$^{TM}$ device) which they were taught prior to trial initiation.

2.2.2 Participants

According to the trial criteria, we included participants who were: (1) between the ages 18-70 years; (2) confirmed MS diagnosis using the Revised McDonald Criteria (Thompson et al., 2018); and (3) mild-moderate gait impairment due to symptoms of MS. We excluded participants who had: (1) contraindications to TLNS (as per manufacturers' instructions); (2) relapse within the previous 90 days; and (3) ongoing physical rehabilitation treatment or had been characterized as functional community ambulators (gait speed > 120cm/s) (Ploughman et al., 2023). For this sub-study using TMS, we further excluded those participants who had contraindications to TMS (Rossini et al., 2015), did not consent to TMS testing, or whose TMS data was incomplete). We extracted neurologist-scored Expanded Disability Status Scores (EDSS), disease duration (years) and disease course (e.g., relapsing remitting) from health records.

**Table 2.1** Demographic and disease related information for both the TLNS stimulation and sham groups.

| Variable | Stimulation (n = 14) | SHAM (n = 8) | p-value |
|---|---|---|---|
| Age (years)* | 49.6 ± 9.3 | 45.6 ± 6.5 | 0.31 |
| EDSS** | 5 ± 3.12 | 2.25 ± 2.62 | 0.39 |
| Disease Duration (years) * | 15.3 ± 7.1 | 11.6 ± 6.7 | 0.29 |
| Sex (F/M)† | 8/2 | 5/2 | 0.68 |
| MS Type (RRMS, SPMS, PPMS, PRMS)† | RRMS = 5<br>SPMS = 2<br>PPMS = 2<br>PRMS = 1 | RRMS = 5<br>Unknown = 2 | 0.15 |

Note: Values are mean ± SD or number. EDSS reported as median and IQR.
Abbreviations: EDSS: Expanded Disability Status Scale; MS: multiple sclerosis; PPMS: primary-progressive MS; PRMS: progressive-relapsing MS; RRMS: relapsing-remitting MS; SPMS: secondary-progressive MS
* Student t-test
** Wilcoxon Test
† Chi-squared test

2.2.3 Translingual Neurostimulation

TLNS was delivered via the Portable Neuromodulation Stimulation (PoNS™) device which delivers amplitude-controlled, biphasic pulses to the anterior superior surface of the tongue through gold-plated electrodes on a polyimide substrate for minimal tissue irritation (Figure 2.1) (Kaczmarek, 2017). The sham device looks identical to the commercial PoNS™; however, the two device configurations deliver different stimulation intensities. The stimulation intensity on both device configurations was set to a fixed level for the study duration – the real pons device delivered pulses in 5 ms intervals with 150 pulses/s and the sham device had 12.5 s intervals with 0.08 pulses/s (real/sham stimulation ratio 1875:1). To avoid bias, all participants were seated comfortably in the same chair and given identical instructions for device operation and intensity (devices set to level four), including that they may or may not feel the stimulation.

**Figure 2.1** Portable neuromodulation stimulator (PoNS™) device mouthpiece that is populated with electrodes. Photo © Abby Blaney.

2.2.4 Transcranial Magnetic Stimulation

We used single-pulse TMS to measure the effects of TLNS on upper extremity corticospinal excitability and inhibition. The BiStim $200^2$ stimulator, equipped with a 70 mm figure-of-eight coil (Magstim Co., Whitland, UK), delivered monophasic pulses to the motor cortex region corresponding to the first dorsal interosseous muscle of the weaker hand. Studies have shown that the hemisphere corresponding to the weaker hand has lower excitability and higher inhibition compared to the stronger hand and therefore more sensitive to acute or long-term changes in the corticospinal tract (Chaves et al., 2020). The weaker hand was determined from combined pinch and grip strength tests using dynamometers (B&L Engineering, Santa Ana, CA and Lafayette Instruments, Lafayette, IN, respectively). Electromyography surface electrodes (Kendall 200 Coviden, Mansfield, MA, USA) were used to measure the elicited MEPs in the contralateral first dorsal interosseous muscle. To ensure recording of both the MEP and CSP, electromyography activity was sampled from 100 ms pre-stimulation to 800 ms post-stimulation and transmitted to a recording system (Brainsight™, Rogue Research, Montreal, QC, Canada; 3 kHz sampling, 2,500 V/V amplification, 600 V/V gain, bandwidth of 16–550 Hz). Brainsight™ was also used as a neuronavigation tool to ensure consistent orientation and angle of the coil over the participants scalp and to locate the hotspot. The coil was held tangential to the participants scalp with the coil handle oriented posterolaterally at a 45° angle to the midsagittal line to deliver posterior-anterior pulses in the primary motor cortex (Rossini et al., 2015). The hot spot was defined as the optimal location over the motor cortex that elicits the largest MEP amplitude in the muscle with minimum stimulator intensity. Electromyographic recording data for each session were exported to and analyzed using Signal software v6.06 (Cambridge Electronic Design, Cambridge, UK). From each TMS-evoked MEP, we extracted peak-peak amplitude,

latency (time (ms) from TMS stimulation to MEP onset) and CSP (the interruption of electromyographic activity following a suprathreshold TMS stimulation).

### 2.2.5 Motor Thresholds

Motor threshold is the lowest stimulator output to induce a TMS-evoked motor potential. Higher motor thresholds indicate decreased corticospinal tract excitability (Chaves et al., 2021). To measure the resting and active motor thresholds for each participant, we determined the minimum TMS intensity (maximum stimulator output required to elicit at least 5/10 MEP amplitudes of $\geq 50$ μV during complete relaxation (resting motor threshold) and $\geq 200$ μV during slight tonic contraction of the muscle at ~20% of maximum muscle strength (active motor threshold during a pinch grip) (Rossini et al., 2015). When measuring the active motor threshold, it is recommended to use and maintain low-level contractions (e.g., 20% maximum muscle strength) to avoid muscle fatigue (Vucic et al., 2023). For each threshold we determined the maximum stimulator output and measured peak-peak MEP amplitudes (μV) and latencies (ms).

### 2.2.6 Excitatory and Inhibitory Recruitment Curves

As stimulator intensity is increased, there is a corresponding increase in neuronal recruitment until a plateau is reached (Rossini et al., 2015). When stimulator intensity is plotted against the MEP amplitudes and CSP durations, the resulting recruitment curves index overall excitability or inhibition of the corticospinal pathway, respectively (Chaves et al., 2021; Snow et al., 2019). To produce the recruitment curves, we incrementally increased TMS stimulation (increments of 10%) using six suprathreshold intensities ranging from 105-155% of the active motor threshold in a randomly generated order. For each suprathreshold intensity, we delivered 3-6 stimulations to calculate the average MEP amplitude (μV) and CSP (ms) and created excitatory (eREC) and inhibitory (iREC) recruitment curves respectively. For the eREC, we

plotted MEP amplitude against TMS intensities and calculated the total excitability of the corticospinal pathway (area under the curve; AUC). Similarly, for the iREC we plotted CSP duration against TMS intensity and calculated the overall inhibition (AUC). AUC was calculated using the trapezoidal rule $\Delta X \times (Y1 + Y2)/2$, with the X-values being the TMS intensity and the Y-values being MEP amplitude or CSP duration (Chaves et al., 2021; Snow et al., 2019).

### 2.2.7 Statistical Analysis

All statistical analyses were performed using RStudio (R Core Team 2023). To compare differences pre- and post-stimulation, within and between TLNS/sham devices, we used two-way mixed model analysis of variance (ANOVA) with repeated measures – group (stimulation or sham) as the between-subject factor and time (pre- and post-stimulation) as the repeated measure. The dependent variables were resting and active motor thresholds (MSO%) and excitatory and inhibitory recruitment curves (MEP amplitudes, latencies, CSP, eREC AUC and iREC AUC). To compare the demographic variables between the stimulation and sham groups, we used Student's t-test (quantitative variables) and chi-square test (categorical variables). Statistical significance was predetermined at $p < .05$. Testing the assumptions revealed all variables were normally distributed and there was homogeneity of variances ($p > 0.05$), as assessed by Shapiro-Wilk's test of normality and Levene's test respectively.

## 2.3 Results

### 2.3.1 Participants

Out of 26 recruited participants, four were excluded from the study due to contraindications with TMS (n = 2; recent seizure and metal implant), unable to obtain a MEP (n = 1) or dropped out of the study (n = 1). Twenty-two participants with MS completed the study,

14 TLNS stimulation and 8 sham (Table 2.1). There were no significant differences in the demographic variables, EDSS, age, sex, disease duration and MS type between the TLNS stimulation and sham groups ($p > 0.1$).

During data collection, MEPs at higher suprathreshold stimulations (135-155% AMT) could not be collected in four participants due to required TMS output being greater than the machine capacity (stimulation: n = 2; sham: n = 1) or participant reporting of pain (stimulation: n = 1). We were also unable to collect recruitment curve data for two stimulation participants due to coil overheating. In addition, some participants did not display a quantifiable MEP (stimulation: n = 1; sham: n = 1) or CSP (stimulation: n = 2; sham: n = 1) during 105-135% AMT. Therefore, during data processing, the individuals with incomplete excitatory (eREC) (stimulation: n = 6; sham: n = 2) and incomplete inhibitory (iREC) (stimulation: n = 7; sham: n = 2) recruitment curve measures were not included in the statistical analysis (Table 2.2).

### 2.3.2 Resting and Active Motor Thresholds

A two-way ANOVA revealed there was no significant interaction between group (stimulation or sham) and time (pre and post) on resting ($F(1,19) = 0.040$, $p = 0.844$) (Figure 2.2A) or active motor threshold ($F(1,20) = 0.561$, $p > 0.1$) (Figure 2.2B). Simple main effects analysis showed that neither group nor time had a statistically significant effect on thresholds ($p > 0.1$).

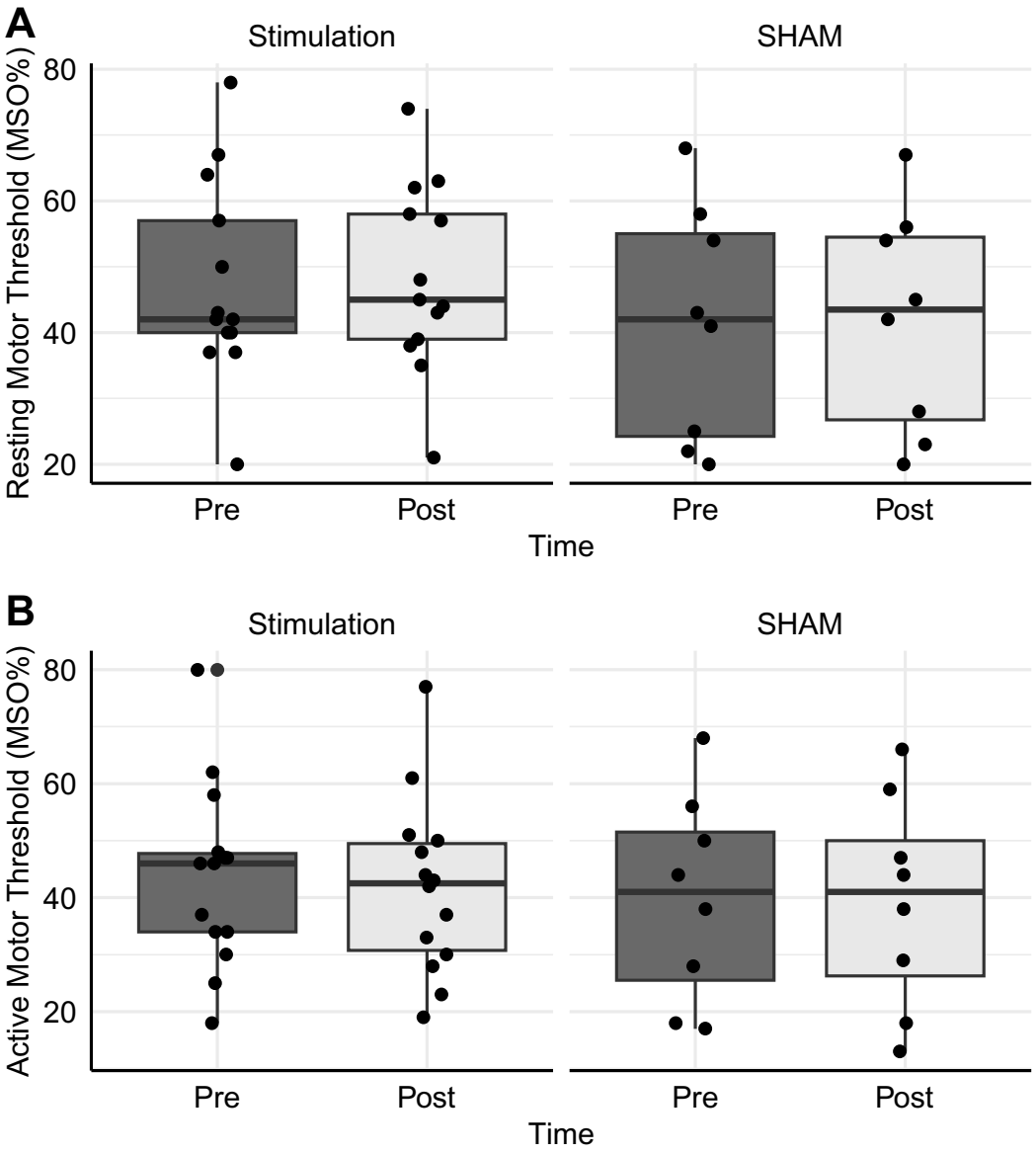


**Figure 2.2** Effects of a 20-minute stimulation using real TLNS stimulation or sham TLNS on resting (A) and active (B) motor thresholds (maximum stimulator output – MSO%). Boxplots include individual data points, medians (horizontal line), interquartile ranges (boxes) and 95% confidence interval (error bars).

3.3 Excitatory Recruitment Curve

A two-way ANOVA revealed there was no significant interaction between group and time on overall corticospinal excitation (eREC AUC; Figure 2.3) ($F(1,11) = 0.005$, $p = 0.824$). Simple main effects analysis showed that group and time did not have a statistically significant effect on eREC AUC ($p > 0.1$) (Figure 2.4).

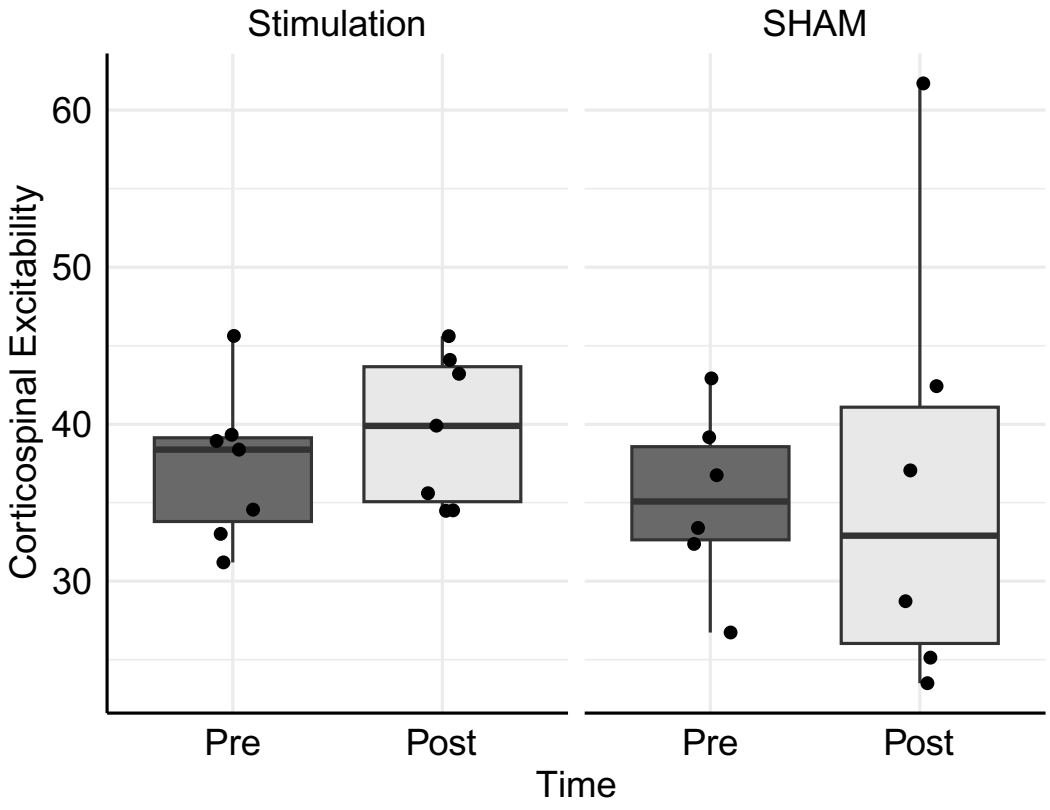


**Figure 2.3** Effects of a 20-minute stimulation using real TLNS stimulation or sham TLNS on upper extremity corticospinal excitability (excitatory recruitment curve area under the curve). Boxplots include individual data points, medians (horizontal line), interquartile ranges (boxes) and 95% confidence interval (error bars).

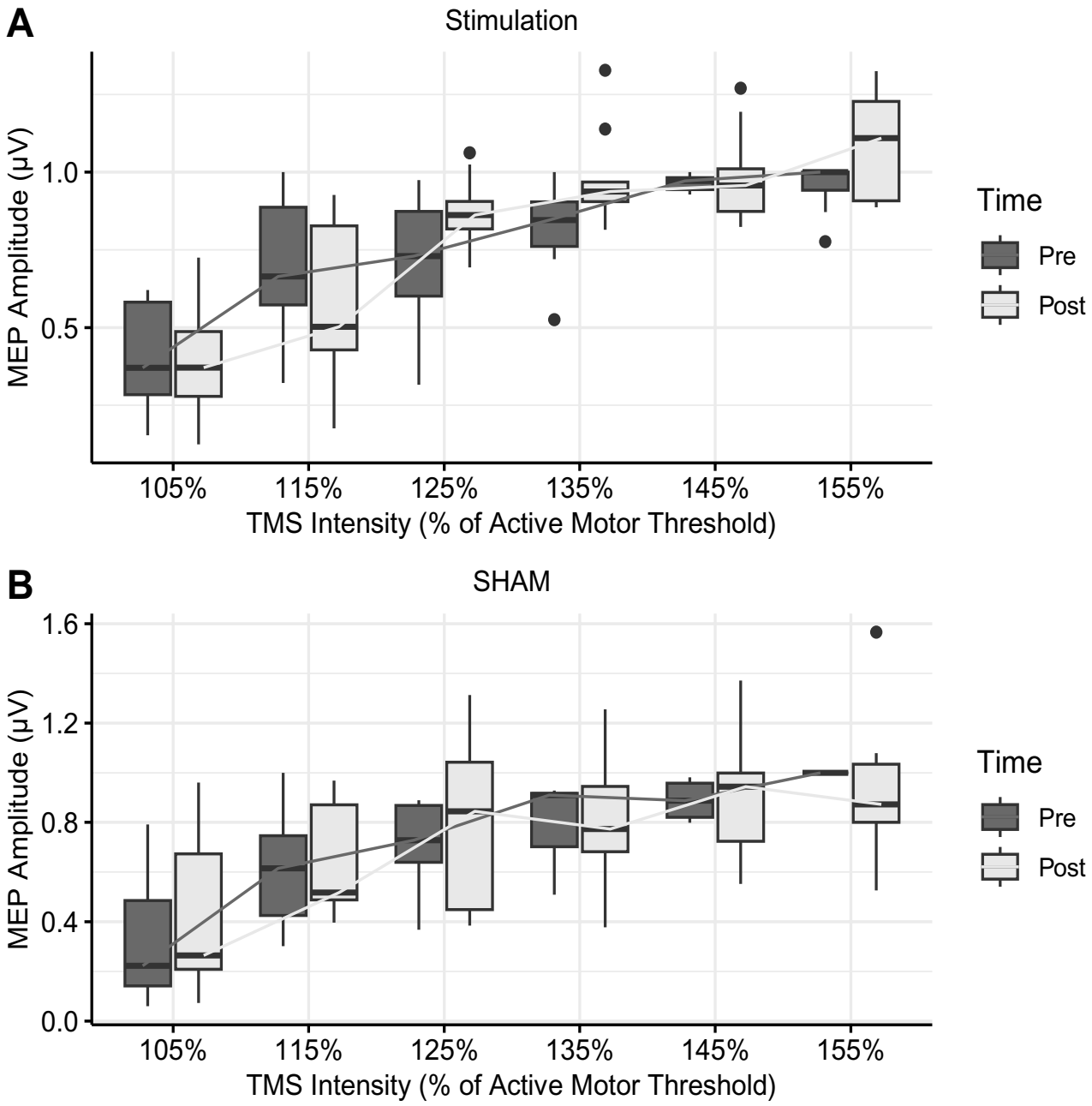


**Figure 2.4** Effects of a 20-minute stimulation using (**A)** real TLNS stimulation or (**B**) sham TLNS on brain excitability (motor evoked potential (MEP) amplitudes). The excitatory recruitment curve was created by delivering suprathreshold TMS stimulations (105-155% of active motor threshold; x-axis) pre- and post-stimulation and recording the corresponding MEP

amplitude. Boxplots include medians (horizontal line), interquartile ranges (boxes) and 95% confidence interval (error bars).

3.4 Inhibitory Recruitment Curve

Two-way ANOVA analysis showed no significant interaction between group (stimulation or sham) and time (pre and post) on overall corticospinal inhibition (Figure 2.5; Figure 2.6) ($F(1,10) = 0.005$, $p = 0.947$. Simple main effects analysis showed that group ($p = 0.046$) had a statistically significant effect on corticospinal inhibition but not time ($p > 0.1$). Considering the Bonferroni adjusted p-value, the simple main effect of group was not significant at pre ($p = 0.13$) or post ($p = 0.116$).

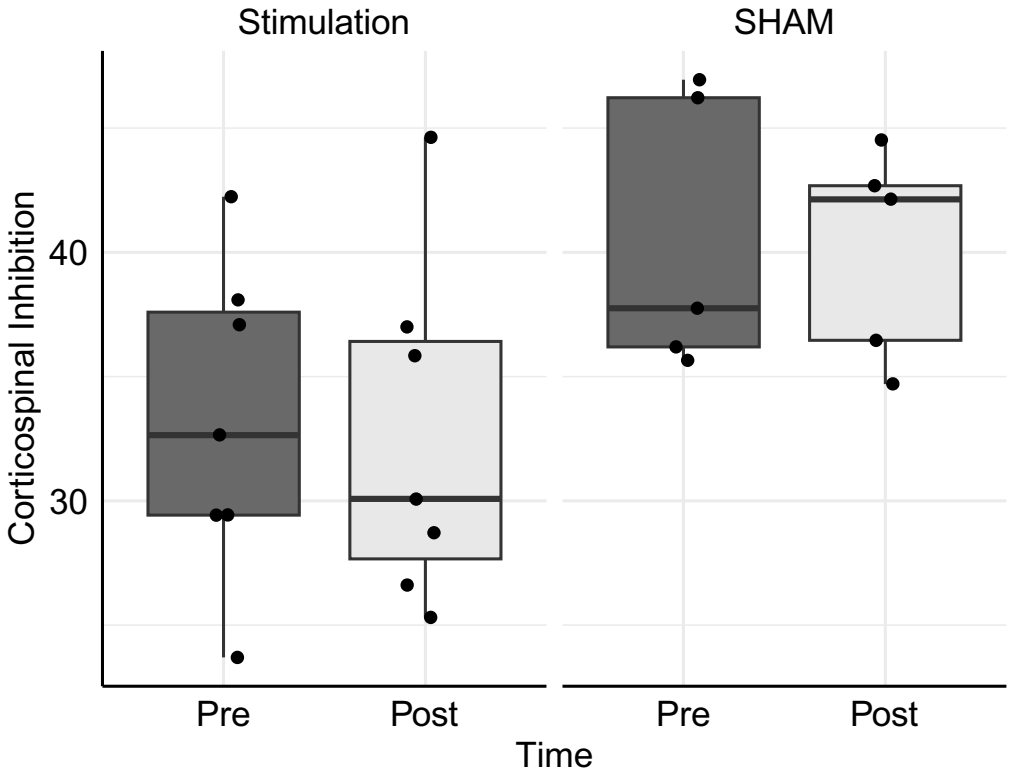


**Figure 2.5** Effects of a 20-minute stimulation using real TLNS stimulation or sham TLNS on corticospinal inhibition (inhibitory recruitment curve area under the curve). Boxplots include

individual data points, medians (horizontal line), interquartile ranges (boxes) and 95% confidence interval (error bars).

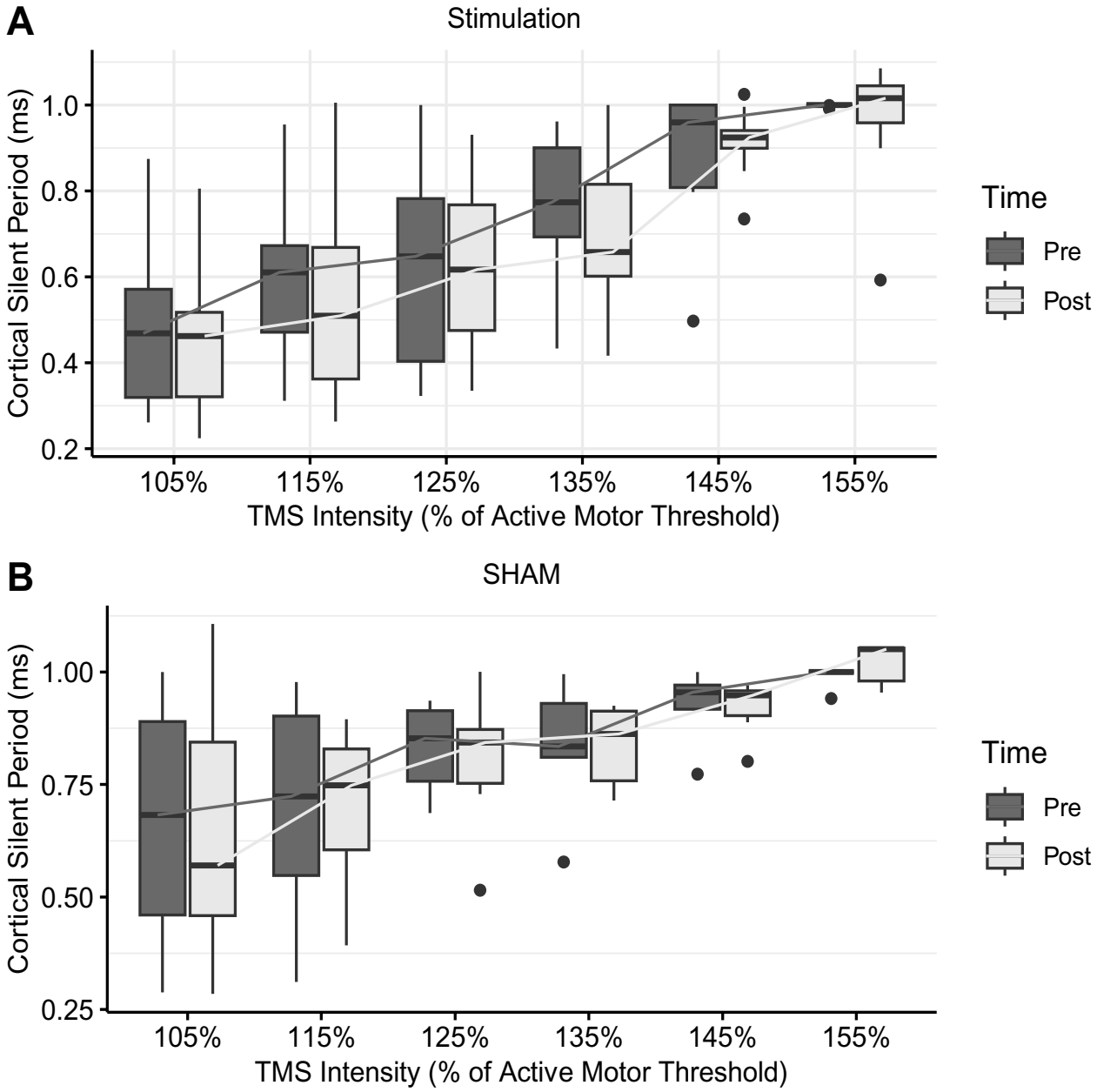


**Figure 2.6** Effects of a 20-minute stimulation using (A) real TLNS stimulation or (B) sham TLNS on brain inhibition (cortical silent period - CSP). The inhibitory recruitment curve (iREC) was created by delivering suprathreshold TMS stimulations (105-155% of AMT; x-axis) pre- and

post-stimulation and recording the corresponding CSP for both the TLNS stimulation (A) and sham (B) groups. Boxplots include medians (horizontal line), interquartile ranges (boxes) and 95% confidence interval (error bars).

**Table 2.2** Transcranial magnetic stimulation (TMS) variables and corresponding values for both the real TLNS stimulation and sham groups.

| | N | | Pre | | Post | |
|---|---|---|---|---|---|---|
| TMS Variable | Stimulation | SHAM | Stimulation | SHAM | Stimulation | SHAM |
| RMT (%MSO)* | 14 | 8 | 47.5 ± 15.4 | 41.4 ± 17.9 | 48.2 ± 14.2 | 41.9 ± 17.0 |
| RMT Amplitude (μV)* | 13 | 8 | 101.0 ± 38.6 | 96.1 ± 23.6 | 109.0 ± 37.1 | 93.6 ± 30.4 |
| RMT Latency (ms)* | 13 | 8 | 0.026 ± 0.003 | 0.027 ± 0.005 | 0.026 ± 0.004 | 0.028 ± 0.005 |
| AMT (%MSO)* | 14 | 8 | 43.7 ± 16.0 | 39.9 ± 18.2 | 41.9 ± 15.5 | 39.2 ± 18.7 |
| AMT Amplitude (μV)* | 13 | 7 | 315.0 ± 110 | 335.0 ± 187 | 334.0 ± 101 | 342.0 ± 177 |
| AMT Latency (ms)* | 13 | 7 | 0.025 ± 0.004 | 0.025 ± 0.005 | 0.024 ± 0.004 | 0.025 ± 0.006 |
| eREC AUC* | 8 | 6 | 35.0 ± 7.8 | 34.1 ± 5.9 | 37.6 ± 8.7 | 37.1 ± 13.2 |
| iREC AUC* | 7 | 6 | 32.2 ± 7.2 | 38.3 ± 7.4 | 31.2 ± 8.4 | 39.4 ± 4.2 |

Note: Values are mean ± SD.
Abbreviations: AMT: active motor threshold; AUC: area under the curve; CSP: cortical silent period; eREC: excitatory recruitment curve; iREC: inhibitory recruitment curve; MEP: motor evoked potential; MSO%: maximal stimulator output percentage; RMT: resting motor threshold.
*indicates Group:Time interaction $p > 0.1$.

## 2.4 Discussion

The goal of this sub-study, within a randomized controlled trial, was to use TMS to determine whether 20 minutes of TLNS stimulation via a portable neuromodulation stimulator (PoNS™) or a sham device (combined with breathing and awareness training) had immediate effects on upper extremity corticospinal excitability and inhibition in individuals with MS having

gait and balance problems. For both TLNS and sham groups, based on the measured TMS variables, we found no significant effect of TLNS on upper extremity corticospinal excitability or intracortical inhibition, measured directly after stimulation. Notably there were issues regarding data quality and feasibility of using TMS for this purpose, which are discussed below.

### 2.4.1 TMS as a Suitable Measure for TLNS Induced Changes in CNS and Neuroplasticity

The efficacy of TMS is dependent on factors such as coil geometry, stimulus intensity and the depth of the target area (Hardwick et al., 2014). Considerations of coil type is essential as different brain regions and pathways can be preferentially targeted. However, there is a trade-off between stimulation depth and focality (Ueno & Sekino, 2021). The figure-eight coil provides superficial focal stimulation and is useful in targeting specific cortical regions such as the primary motor cortex. On the other hand, the double-cone coil is designed to stimulate deep brain regions such as the cerebellum, however, increased depth is likely accompanied by decreased focality (Hardwick et al., 2014). Based on the proposed mechanism of action of TLNS, we expected global changes in brain excitability that could be detected using the figure-eight coil.

The TMS derived measures, motor thresholds (resting (RMT) and active (AMT) motor thresholds), MEP amplitudes and latency can provide information about the excitability of the motor cortex as well as the overall integrity of the corticospinal pathway. Several studies have reported increased motor thresholds (maximum stimulator output) and MEP latency and decreased MEP amplitudes in individuals with MS compared to healthy controls (Caramia et al., 2004b; Jacques et al., 2022; Neva et al., 2016), as well as an association with clinical disability (higher disability, less excitability) as measured by the expanded disability status scale (EDSS)

(Mori et al., 2013; Neva et al., 2016). As previously mentioned, the motor thresholds and MEP amplitudes and latency are primarily mediated by glutamatergic signalling and the interaction with excitatory receptors such as NMDA and AMPA. After administering TLNS stimulation, any changes in the TMS-derived excitatory variables could indicate corticospinal modulating effects (e.g., changes in excitatory neuronal signalling).

TLNS is postulated to increase activation in the brainstem and cerebellum leading to the upregulation of cortical activity (e.g., motor cortex, bilateral anterior cingulate – areas of the brain involved in modulating movement, posture, balance, and coordination. Current studies suggest that TLNS stimulates the trigeminal and facial cranial nerves which sends neural signals along their respective branches (lingual and chorda tympani) to projections in the brainstem and cerebellum which then initiates a neural cascade potentially activating cortical structures (Kaczmarek, 2017; Leonard et al., 2017; Sanders, 2010; Wildenberg et al., 2010a, 2011). In addition, the manufacturers of the device state that TLNS paired with physical therapy can induce neuroplastic changes in the corticospinal pathways. Based on the proposed mechanisms of action and intended benefit of the device, we hypothesized that TLNS would increase the strength and efficiency of neural transmission/communication (increase corticospinal excitability) from the motor cortex to the contralateral muscle (e.g., first dorsal interosseous muscle), meaning a decrease in motor thresholds and MEP latency and an increase in MEP amplitudes. However, we found that 20 minutes of TLNS stimulation did not have a significant impact on motor thresholds (resting or active motor threshold %MSO), MEP amplitudes (excitatory recruitment curve) or latency in individuals with MS. Overall, we postulate that these findings could be attributed to lack of short-term retention of TLNS effects on upper extremity

corticospinal excitability/unable to simultaneously administer TMS and TLNS or lack of TLNS-induced cortical activation (activation restricted to subcortical regions).

### 2.4.2 Effect of TLNS on Motor Thresholds

Results of this study indicate that acute TLNS does not influence resting or active motor thresholds (RMT and AMT). Resting and active motor thresholds are among the most reported TMS variables and are defined as the minimum TMS intensity (maximum stimulator output; MSO%) required to elicit the desired MEP response in the contralateral muscle either during complete relaxation (resting motor threshold) or during slight tonic contraction of the muscle (active motor threshold) (Rossini et al., 2015). Overall, the resting and active motor thresholds provide similar yet complementary physiological information about the corticospinal excitability and pathway integrity. The resting motor threshold reflects baseline excitability and local density of neurons while the active motor threshold additionally includes the contribution of cortical and spinal neurons involved in muscle contraction, making the resting motor threshold values typically higher than active motor thresholds (Chaves et al., 2021; Rossini & Rossi, 2007). In the present study, 20 minutes of TLNS did not significantly change the resting or active motor thresholds (maximum stimulator output) values. These results suggest that TLNS (paired with breathing and awareness training) does not subsequently enhance excitatory synaptic transmission or upper extremity corticospinal excitability in this sample of individuals with MS. However, the clinical relevance of the motor thresholds has been questioned due to the high variability among study results (Chaves et al., 2019; Snow et al., 2019). More thorough TMS variables are required to deduce the effects of TLNS on upper extremity corticospinal excitability, such as the excitatory recruitment curve (eREC).

### 2.4.3 Effect of TLNS on MEP Amplitudes and the Excitatory Recruitment Curve (eREC)

Similar to the motor thresholds, we found no changes in the excitatory recruitment curve post TLNS stimulation. As previously mentioned, the excitatory recruitment curve is constructed by incrementally increasing TMS intensity and recording the corresponding MEP amplitudes (Chaves et al., 2021). In general, increasing TMS intensity induces stronger descending volleys resulting in faster temporospatial summation at cortico-motoneuronal synapses and an increase in MEP amplitudes (R. Chen et al., 2008; Rossini & Rossi, 2007; Vucic et al., 2023). Compared to the motor thresholds, the recruitment curves index the excitability of neurons that are less excitable or spatially distant from the TMS stimulation site (e.g., primary motor cortex). The excitatory recruitment curve can measure overall excitability of the corticospinal tract and is a more comprehensive measure than the motor thresholds (Rossini et al., 2015). Excitatory recruitment curves can be altered by neurological disorders such as MS due to demyelination, axon damage, and lesion formation in the corticospinal tracts (Neva et al., 2016). From the excitatory recruitment curve, we can derive different parameters such as the area under the curve (AUC) which indicates the total excitability of the corticospinal pathway. We hypothesized that TLNS would increase upper extremity corticospinal excitability in individuals with MS, resulting in increases in MEP amplitudes and area under the curve. However, we found that TLNS stimulation did not affect the total excitability of the corticospinal pathway (AUC). These results further suggest that TLNS (paired with breathing and awareness relaxation) does not enhance upper extremity corticospinal excitability, measured directly afterwards, in individuals with MS.

### 2.4.4 Effect of TLNS on MEP Latency

The results of this study also indicate that acute TLNS does not influence MEP latency. Latency reflects the signal transmission time from the motor cortex to the contralateral muscle

following a TMS stimulation (Šoda et al., 2023). Compared to healthy controls, individuals with MS exhibited delayed MEP latencies which indicates abnormal conduction along the corticospinal tract (Jacques et al., 2022; Kale et al., 2009; Neva et al., 2016). We hypothesized that TLNS would decrease MEP latency in individuals with MS. However, we found that TLNS did not increase subsequently measured MEP latency and therefore had no effect on corticospinal neural transmission. Calculating the strength (motor thresholds and excitatory recruitment curve) and efficiency (latency) of neural transmission along the corticospinal tract can aid in elucidating the effects of TLNS on upper extremity corticospinal excitability.

### 2.4.5 Effects of Translingual Neurostimulation (TLNS) on Corticospinal Inhibition in MS

Results of the current study indicate that TLNS does not affect CSP duration or the inhibitory recruitment curve. The cortical silent period is the interruption of EMG activity following a suprathreshold TMS stimulation. Opposite to the TMS-derived variables motor thresholds (RMT and AMT), MEP amplitude and latency which indicate corticospinal excitability, the cortical silent period indicates GABAergic-mediated corticospinal inhibition (Chaves et al., 2021). Increased corticospinal inhibition requires stronger TMS stimulation intensity (higher motor thresholds) to elicit a motor evoked potential. Studies have reported prolonged cortical silent period duration in individuals with MS (Rossini et al., 2015; Tataroglu et al., 2003) and that treatment paradigms such as 10 weeks of exercise training (Chaves et al., 2020) can reduce the cortical silent period duration. Similar to the excitatory recruitment curve (MEP amplitudes), the cortical silent period duration gradually increases as the TMS stimulation intensity increases and can be used to construct an inhibitory recruitment curve (iREC) to evaluate the overall effects of TLNS on corticospinal inhibition (area under the curve). In the present study, we hypothesized that there would be a decrease in the area under the inhibitory

recruitment curve post TLNS stimulation. However, we found no change in the area under the curve value, indicating TLNS did not influence overall corticospinal inhibition.

The duration of the cortical silent period involves cortical and spinal inhibitory networks (R. Chen et al., 1999). The initial portion of the CSP (~50 ms) involves spinal mechanisms while the latter portion involves the motor cortex and is mediated by GABAergic neurotransmitters and $GABA_B$ receptors (Hallett, 2007; Rossini et al., 2015). The overall duration of the cortical silent period mainly indicates the level of slower inhibitory postsynaptic potentials (IPSPs) mediated by $GABA_B$ receptors within the primary motor cortex and is usually altered by cortical mechanisms (Özyurt et al., 2019; Paci et al., 2021). Given that the present study found no TLNS-induced changes in the inhibitory recruitment curve (cortical silent period durations), we can also infer there was no alteration in the inhibitory mechanisms within the primary motor cortex. As stated earlier, the spatial resolution of TLNS may be primarily limited to activation of the brainstem and cerebellum regions, with limited propagation to the cortical regions. Results from the inhibitory recruitment curve support this hypothesis and further emphasizes the need for additional research regarding the proposed connection between TLNS-induced subcortical changes yielding cortical changes.

### 2.4.6 Spatial Resolution of TLNS - Neural Networks and the Corticospinal Pathway

The current research supporting the postulation that TLNS activates broad neuronal networks such as cortical structures, and the overall corticospinal pathway is limited and not well supported. An fMRI study by Leonard et al. (2017) reported a significant increase in blood oxygen level-dependent signal post TLNS treatment in only the left motor cortex but not the other regions (right motor or bilateral premotor regions). However, the sham group also showed

a significant increase in blood oxygen level dependent signal in bilateral premotor regions post treatment. D'Arcy et al. (2020) reported changes in right and left hand EEG and MEG motor activation post TLNS treatment, however the study was a case study in a person having traumatic brain injury and therefore limits the ability to draw definitive conclusions. Combining the results of the present study, which indicates a lack of effect of TLNS on upper extremity corticospinal excitability, with the studies that evaluated cortical activation post treatment, the overall potential effects of TLNS on cortical activity remains inconclusive.

It is possible that the spatial resolution of TLNS is limited to the brainstem and cerebellum region, as more convincingly demonstrated in the literature. Studies have shown an increase in blood oxygen level dependent signal within brainstem (dorsal pons) (Wildenberg et al., 2010) including increased activation in the brainstem projections trigeminal and solitary nuclei and increased activation of the cerebellum post TLNS treatment in individuals with chronic balance dysfunction (Wildenberg et al., 2011). The flat figure-of-eight TMS coil used in the present study is typically used to stimulate superficial cortical regions and not deep brain structures such as the brainstem and cerebellum (Ueno & Sekino, 2021). Alternative TMS coil types such as the double-cone or batwing coils can effectively stimulate cerebellar targets and may be more useful to detect TLNS-induced brainstem and cerebellum neuronal contributions. Overall, the route and extent by which the activation of the brainstem and cerebellum propagate to cortical regions remains unclear. Longitudinal measures of TLNS using TMS and other functional brain imaging devices (such as near infrared spectroscopy - fNIRS) are needed to understand potential TLNS-induced cortical changes and subsequent behavioural effects (e.g., gait and balance). In addition, different coil types should be used to stimulate cerebellar targets and measure changes in corticobulbar tract excitability.

### 2.4.7 Long-Term Potentiation and Synaptic Plasticity

Long-term potentiation (LTP), a form of neuroplasticity, involves the strengthening of synapses over time (Cooke & Bliss, 2006). LTP can be divided into early-phase LTP which is independent of protein synthesis and a late-phase LTP which is dependent on the activation of transcription factors and protein synthesis (Baltaci et al., 2019). As mentioned previously, neuromodulation involves applying electrical or chemical agents to a neurological site to regulate/modulate neural circuits (Abboud et al., 2017). Studies using plasticity-inducing interventions (neuromodulation techniques) such as transcranial electrical and magnetic stimulation (e.g., tDCS and rTMS) indicate that single-session protocols using high pulse frequency and duration can induce changes in cortical excitability that outlast the stimulation period for an hour or longer (Nitsche & Paulus, 2001; Ziemann et al., 2008). The goal of these single session stimulation protocols is to induce and measure transient early-phase LTP. LTP typically occurs with repeated stimulation of the presynaptic neurons resulting in changes such as the upregulation of glutamate receptors (especially the receptor subtypes AMPA and NDMA) or changes in the efficacy of glutamate release (Agboada et al., 2020). Repeated modulation of nerve activity can have an array of effects such as changes in neuronal membrane potential, synaptic transmission, voltage-gated ion channels etc. In theory, the activation of neurons in the brainstem via TLNS could strengthen synaptic connections by promoting membrane depolarization and the generation of action potentials and subsequently increasing the release of glutamate. This strengthening of synaptic connections and efficient neural communication (early-phase or long-phase LTP) could potentially be detected using TMS-derived variables (maximum stimulator output, MEP amplitude and MEP latency). Conversely, increased

inhibition and the activity of $GABA_A$ and $GABA_B$ receptors supresses neuron depolarization and inhibits LTP. A TLNS-induced decrease in the cortical silent period duration could indicate reduced corticospinal inhibition and LTP facilitation.

In the case of this present study, a single 20-minute TLNS session is more likely to induce transient early-phase LTP which translates to changes in TMS-derived measures including an acute decrease in motor thresholds, MEP latency, and cortical silent periods and an increase in MEP amplitudes. However, after administering high frequency translingual electrical stimulation for a prolonged duration (~ 25,740,000 stimulation pulses via the PoNS ™ device during a 20-minute period), we were unable to detect any acute changes in upper extremity corticospinal excitability (early-phase LTP) post stimulation. It is possible that the potential effects of TLNS on corticospinal excitability and CNS function are either not retained immediately after a single session or the overall neuromodulating effects on the corticospinal tract are not strong enough to be detected via TMS. It is also possible that TLNS induced changes in synaptic connections and corticospinal excitability may only be detectable longitudinally (inducing late-phase LTP) after repeated treatment sessions with a PoNS ™ device.

### 2.4.8 Efficacy of Translingual Neurostimulation (TLNS)

Although TLNS treatment is feasible and safe, there is an overall lack of understanding of the neuromodulating mechanism of action as well as the efficacy of TLNS in individuals with gait and balance impairment. Current findings demonstrate improvements in gait and balance after repeated pairings of TLNS and targeted physical therapy (Leonard et al., 2017; Ptito et al., 2021; M. Tyler et al., 2019; M. E. Tyler et al., 2014). However, the control groups also exhibited gait

and balance improvements, and several studies report no differences in variables between groups. Functional brain imaging studies report increased blood oxygen level-dependent signal in the cerebellum and brainstem (especially the dorsal pons) nuclei (Wildenberg et al., 2010) and left primary motor cortex (Leonard et al., 2017) post TLNS treatment. However, the connection between subcortical activation of the cerebellum and brainstem via TLNS and changes in cortical regions and functional outcome measures (e.g., gait and balance) is not well supported. In addition, many studies lack a sufficient sample size and a placebo stimulation paradigm. A major challenge when investigating the efficacy of TLNS treatment, is identifying an appropriate control condition. For example, comparing the TLNS device to no device would not account for placebo effects while comparing the TLNS device to a sham device may underestimate the true effect since the sham device still delivers a small level of stimulation. Results of the present study, in which we found no TLNS-induced changes in upper extremity corticospinal excitability, support the demand for additional TLNS research. Overall, the mechanism of action of TLNS is largely unclear which warrants advisement on expanding indications.

### 2.4.9 Feasibility of TMS in Clinical Populations

Using TMS as a biomarker in clinical populations is challenging due to variation within and between individuals (Snow et al., 2019). The present study was limited by a small sample size and wide variability in the TMS outcome measures. In individuals with MS, the corticospinal tract can be compromised due to demyelination, axon damage, and lesion formation which translates to higher TMS stimulation intensities required to transmit neural signals from the motor cortex to the contralateral muscle. In this study, the excluded individuals with incomplete recruitment curves also had the highest motor thresholds, meaning more stimulation

was required to elicit a MEP. Studies have shown a mild to moderate correlation between MEP amplitude, MEP latency and CSP duration with measures of disease severity (Nantes et al., 2016; Tataroglu et al., 2003; Vucic et al., 2012). In addition, these studies found significant differences in MEP amplitude, MEP latency and motor thresholds between relapsing-remitting MS and progressive MS. As a result, problems with data quality and variability can arise when conducting TMS in clinical populations. Future studies should stratify treatment groups based on disease severity/MS type.

### 2.4.10 Limitations

The current study has some limitations to consider. First, we were unable to obtain complete data sets for each TMS variable for all participants. The resulting reduction in sample size was due to contraindications to TMS (n = 2), inability to locate a motor hotspot (n = 1) dropped out of study (n = 1). The overall sample size was limited to 22 participants with MS (14 TLNS stimulation and 8 sham) with further exclusions for recruitment curve analysis which likely reduced the statistical power to detect meaningful effects. Other studies measuring comparable TMS variables have also reported challenges in TMS-based measures in MS populations compared to healthy populations (Neva et al., 2016). Second, although TMS can be used to measure the immediate effects of TLNS, because the TLNS device contains metal components, we were unable to simultaneously employ TMS (magnetic field contraindication) and TLNS, meaning we could not capture the instantaneous effects of TLNS on CNS function.

Future work should investigate the longitudinal effects of TLNS on upper extremity corticospinal excitability using TMS. Since we were unable to detect acute changes in brain excitability or inhibition using TLNS, longitudinal deviations in TMS variables could indicate

neuroplasticity and LTP. Lastly, the instantaneous effects of TLNS should be investigated using functional brain imaging devices such as functional near infrared spectroscopy (fNIRS). Similar to fMRI, fNIRS measures changes in blood oxygenation, however fNIRS uses differences in optical absorption and can therefore be paired simultaneously with TLNS to capture instantaneous effects (W.-L. Chen et al., 2020).

## 2.5 Sources of Funding

This study was supported by an investigator-led grant (MP and SD) from the manufacturer of the PoNS device, Helius Medical. The company had no involvement in the design, analysis, interpretation or presentation of this work. Equipment to conduct this study was provided by the Canada Foundation for Innovation (MP). The study was also supported by trainee fellowships from the Faculty of Medicine, Memorial University (AB), the Canada Graduate Scholarship - Master's, Canadian Institutes of Health Research (CIHR) (AB), and the School of Graduate Studies, Memorial University (AB).

## 2.6 Conflict of Interest

The authors report no conflicts.

# Chapter 3: Discussion

## 3.1 Overview/Key Findings

The purpose of this study was to use TMS to measure the acute effects of TLNS via a portable neuromodulation stimulator (PoNS™) on the corticospinal pathway in individuals with MS. Using TMS, we measured upper extremity corticospinal excitability and inhibition before and after 20-minutes of TLNS stimulation. As a proxy for corticospinal excitability and inhibition, we analyzed the TMS-derived variables % maximum stimulator output (%MSO), MEP amplitude, MEP latency and cortical silent period (CSP). Based on the indication of the device provided by the manufacturers, we hypothesized TLNS would increase upper extremity corticospinal excitability (decrease maximum stimulator output and MEP latency and an increase in MEP amplitude) and decrease inhibition (cortical silent period) post-stimulation compared to pre-stimulation. Additionally, we hypothesized that there would be between group differences post-stimulation, with the real TLNS stimulation group having higher corticospinal excitability and lower inhibition compared to the sham group. However, we found no significant differences in any of the corticospinal excitability and inhibition variables within groups (pre vs. post) or between groups (TLNS stimulation vs. sham). To interpret the results of the present study, we proposed several ideas, including that the effects of TLNS may not be retained immediately following a single session whereas longitudinal investigation could detect TLNS-induced changes in the TMS variables. Additionally, TLNS may primarily activate subcortical regions (pons and cerebellum) with less modulation effects on cortical regions in which case single-pulse TMS is not sensitive enough to measure. Lastly, the breathing and awareness training could confound a TLNS-induced increase in corticospinal excitability. Importantly, this study was accompanied by limitations and challenges, which are discussed below.

## 3.2 Limitations/Challenges

Although single-pulse TMS is a useful technique to probe the integrity of the whole corticospinal tract (speed and strength of descending pyramidal volleys from the motor cortex to the contralateral muscle), there are some associated limitations/challenges. As reported in the present study, in some cases we were unable to elicit a MEP or obtain recruitment curve variables. The inability to obtain a MEP/recruitment curve variables may indicate an impaired corticospinal tract, one that could be too damaged to respond to the TLNS. In addition, clinical populations are more likely to have contraindications to TMS (such as a history of seizures or metal implants as reported in the present study) and therefore must be excluded from testing. As a result, these factors resulted in a reduced sample size of 22 participants with MS (14 TLNS stimulation and 8 sham), with further exclusions for recruitment curve analysis. Compared to the calculated sample size of 26 participants, the current study has decreased statistical power which limits our ability to detect the true effect.

As mentioned previously, a standard recruitment curve generally follows a sigmoid curve, however we were unable to fit the data of this study to the sigmoid curve. As stated, individuals with neurological disorders such as MS can have abnormal MEPs which can result in variable recruitment curve shapes that don't adhere to the ideal sigmoid shape. In addition, in the literature there is a discrepancy in recruitment curve data collection and analysis methods. Other studies have fitted recruitment curve data with linear models and reported various slope coefficients such as sigmoid 'm', peak slope, linear slope and Boltzmann 'k' (Massie & Malcolm, 2013). From the fitted curve (linear or sigmoidal), studies have reported additional recruitment curve variables such as slope and $R^2$ to further describe recruitment gain and accuracy respectively. As such, for the present study we were unable to report slope and $R^2$

variables. Future studies should investigate the best model for analyzing recruitment curve data for clinical populations, such as MS, at various disease stages.

The use of TMS as a diagnostic measure in clinical populations, such as MS, can be challenging and highly variable. To mitigate variability, we used neuronavigation to ensure consistent coil orientation and stimulation location within the motor cortex. A study by Bastani & Jaberzadeh (2012) found that increasing the number of MEPs increased intra-session and inter-session reliability. We therefore collected six MEPs for each intensity of the recruitment curve to account for variability. Lastly, we requested that all participants refrain from excitatory inducing activities such as exercise or consuming caffeine prior to the testing session.

Lastly, although we were able to measure corticospinal excitability immediately after TLNS stimulation, we were unable to simultaneously administer TMS and TLNS to collect the instantaneous effects of the device on CNS function. Simultaneously administering TMS and TLNS is not feasible as TMS is also considered a neuromodulation technique (mainly repetitive TMS) and metal objects such as a TLNS device jeopardize the safety of the participants (Rossi et al., 2021). In addition, although combining TLNS with breathing and awareness training is standard practice and has been used in other studies (D'Arcy et al., 2020; Frehlick et al., 2019; Leonard et al., 2017; M. Tyler et al., 2019; Tyler et al., 2014), in the present study it is possible that breathing and awareness training imposes relaxation effects and negate the excitatory effects of TLNS on corticospinal excitability as measured using TMS.

## 3.6 Future Directions

Future studies should use TMS to measure the longitudinal effects of TLNS on upper extremity corticospinal excitability as well as collect additional TMS variables. The present study was unable to detect acute TLNS-induced changes in upper extremity corticospinal excitability, however, longitudinal changes in TMS variables could indicate synaptic (late phase LTP) and neuroplasticity. Single-pulse TMS can also be used as a motor mapping tool to measure motor cortical representations over time (Sondergaard et al., 2021). Along with the motor thresholds, MEP amplitude and latency, motor mapping may be useful to detect TLNS-induced changes in the motor cortex. In addition, TMS paired-pulse paradigms deliver two consecutive pulses which can promote inhibitory or facilitatory effects depending on stimulus intervals and intensity (Sollmann et al., 2020). Paired-pulse variables such as short interval intracortical inhibition (SICI), long interval intracortical inhibition (LICI) and intracortical facilitation (ICF), can probe potential TLNS-induced changes in intracortical inhibition and facilitation (Jannati et al., 2022). However, paired-pulse protocols can be lengthy which in a clinical population can provoke additional TMS challenges with fatigability and spasticity. Given that single pulse TMS stimulation of the motor cortex measures the entire corticospinal pathway (brain and spinal cord together), it may also be important to measure the contribution of the spinal cord alone. Cervicomedullary stimulation activates similar descending pathways as TMS and can be used to measure the effects of TLNS on the spinal cord motor output (Taylor, 2006). It is however important to note that spinal cord stimulation is not typically highly tolerated or accepted by participants. Additional TMS measures such as cerebellar brain inhibition using a double-cone coil or lower limb TMS could provide important insights to potential TLNS-induced changes in cerebellar activity and cortical excitability of lower limb musculature, respectively.

With regards to investigating the effects of TLNS in MS populations, future studies should stratify treatment groups based of MS subtypes/disease stage (e.g., relapsing-remitting vs. secondary-progressive MS). Other studies have reported differences in TMS variables between MS types (relapsing-remitting, secondary-progressive and primary progressive) (Nantes et al., 2016; Vucic et al., 2012), however, given that relapsing-remitting is the most common form of MS, recruiting sufficient sample sizes of other subtypes may be a challenge.

Lastly, the instantaneous effects of TLNS should be investigated using functional brain imaging devices such as functional near infrared spectroscopy (fNIRS). As mentioned, fNIRS is similar to fMRI in that they both measure changes in hemoglobin concentrations to infer brain activity, however, fNIRS relies on the optical properties of cerebral blood flow (León-Carrión & León-Domínguez, 2012). Therefore, fNIRS can be simultaneously used with TLNS to measure instantaneous changes in cerebral blood flow. Overall, additional research is required to understand the TLNS mechanism of action as well as the efficacy to ultimately benefit the individuals in which the device is designed to aid.

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# Appendices

Appendix 1: Ethics Approval

*Department of Research*
*5th Floor Janeway Hostel*
*Health Sciences Centre*
*300 Prince Philip Drive*
*St. John's, NL A1B 3V6*
*Tel: (709) 752-4636*
*Fax: (709) 752-3591*

October 6, 2021

Dr. Michelle Ploughman
100 Forest Road
St. John's, NL
A1A 1E5

Dear Dr. Ploughman,

Your research proposal *HREB Reference #: 2021.085 "Neuromodulation in Multiple Sclerosis using Translingual Stimulation: an RCT"* was reviewed by the Research Proposals Approval Committee (RPAC) of Eastern Health October 5th, 2021, and we are pleased to inform you that the proposal has been granted full approval.

The approval of this project is subject to the following conditions:

- The project is conducted as outlined in the HREB approved protocol;
- Adequate funding is secured to support the project;
- In the case of Health Records, efforts will be made to accommodate requests based upon available resources. If you require access to records that cannot be accommodated, then additional fees may be levied to cover the cost;
- A progress report being provided upon request.

If you have any questions or comments, please contact [redacted], Manager of the Patient Research Centre at 777-7283 or by email at [redacted]

Sincerely,

[redacted]
Regional Director, Research and Innovation
Co-Chair, RPAC

FM/rg

Appendix 2: Ethics Renewal



**HREB - Approval of Ethics Renewal 576818**
administrator@hrea.ca
**Sent:** Friday, June 24, 2022 1:40 PM
**To:** Ploughman, Michelle
**Cc:** Hreaadministrator

Researcher Portal File #: 20220178

Dear Dr. Michelle Ploughman:

This e-mail serves as notification that your ethics renewal for study HREB # 2021.085 – Neuromodulation in Multiple Sclerosis using Translingual Stimulation: an RCT – has been **approved**. Please log in to the Researcher Portal to view the approved event.

Ethics approval for this project has been granted for a period of twelve months effective from July 19, 2022 to July 19, 2023.

Please note, it is the responsibility of the Principal Investigator (PI) to ensure that the Ethics Renewal form is submitted prior to the renewal date each year. Though the Research Ethics Office makes every effort to remind the PI of this responsibility, the PI may not receive a reminder. The Ethics Renewal form can be found on the Researcher Portal as an "Event".

The ethics renewal was reviewed by the Health Research Ethics Board at their meeting dated June 23, 2022.

Thank you,

Research Ethics Office

(e) info@hrea.ca
(t) 709-777-6974
(f) 709-777-8776
(w) www.hrea.ca
Office Hours: 8:30 a.m. – 4:30 p.m. (NL TIME) Monday-Friday

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