Using Measures of Corticospinal Excitability to Map Symptom Severity in Multiple Sclerosis

by © Hailey Wiseman

A thesis submitted to the School of Graduate Studies in partial fulfillment

of the requirements for the degree of Master of Science

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Submitted May 2020

Abstract

Background: Transcranial magnetic stimulation (TMS) is a tool used to measure corticospinal excitability. To evaluate the usefulness of TMS as a biomarker in multiple sclerosis (MS), the first step is to examine how well variables derived using TMS align with clinical symptoms of MS.

Methods: Participants with MS (n=38) were assigned to motor, cognitive, sensory, or asymptomatic clinical group based on their Expanded Disability Status Scale (EDSS) assessment. Following recording of demographic information, subjective health and scoring of walking and cognition, TMS measures were collected from each brain hemisphere. We first examined whether TMS parameters (resting motor threshold (RMT), active motor threshold (AMT), and cortical silent period (CSP)) would differ among clinical groups. Next, we examined whether TMS parameters predicted severity of symptoms.

Results: CSP and AMT in the hemisphere corresponding to the weaker hand predicted measures of symptom severity among people with MS in the motor and cognitive profile groups. Longer CSP was the strongest predictor of slower walking speed ($F_{(1,17)}$=22.82, $p<0.001$). Higher AMT was the strongest predictor of cognitive impairment using the Montreal Cognitive Assessment ($F_{(1,17)}$==25.29, $p=0.001$) and perceived physical impact of MS using the Multiple Sclerosis Impact Scale-29 ($F_{(1,17)}$=30.63, $p<0.001$).

Conclusions: CSP and AMT in the hemisphere corresponding to the weaker hand predicted severity of symptoms among people with MS in the motor and cognitive groups. In these cases, TMS variables provided greater predictive value than the traditional EDSS, supporting the use of TMS outcomes as biomarkers in MS.

Keywords: TMS, Biomarker, CSP, AMT

Acknowledgements

I would like to acknowledge my supervisor, Dr. Michelle Ploughman whose passion in the field of Multiple Sclerosis research has always been an inspiration to me. Dr. Michelle Ploughman introduced me to the field of Multiple Sclerosis research and provided me with guidance, support and motivation throughout this degree. Dr. Michelle Ploughman, I am humbled by your confidence in my abilities, and I will be forever grateful to you for providing me with this opportunity.

I would also like to acknowledge the members of the Recovery & Performance Laboratory for their encouraging words and guidance throughout my project. Without the support of the laboratory staff who I now call my "lab family", this thesis would not have been possible. I would also like to thank the members of my committee, Dr. Craig Moore and Dr. Kevin Power, for taking the time to review my thesis and provide valuable feedback.

Lastly, I would like to thank my friends and family, who have supported me throughout this journey and been by my side throughout the ups and downs. There are not enough words to express my gratitude towards my parents who have instilled upon me a strong passion for learning and have done everything possible to lead me down a path of success. I would also like to say a special thank you to my boyfriend, who has stood by my side and been my ultimate supporter throughout this degree.

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List of Abbreviations

AMT: Active Motor Threshold

CNS: Central Nervous System

CSP: Cortical Silent Period

CST: Corticospinal Tract

EDSS: Expanded Disability Status Scale

EMG: Electromyography

GABA: γ-aminobutyric acid

GML: Grey Matter Lesion

MoCA: Montreal Cognitive Assessment

MRI: Magnetic Resonance Imaging

MS: Multiple Sclerosis

MSIS-29: Multiple Sclerosis Impact Scale-29

RMT: Resting Motor Threshold

SDMT: Symbol Digit Modality Test

TMS: Transcranial Magnetic Stimulation

WML: White Matter Lesion

β**:** Standardized regression coefficient

F: F-Statistic

p: Calculated probability

R_2: Square of the correlation coefficient

SD: Standard Deviation

Chapter 1: Introduction and Literature Review

1.1 GENERAL INTRODUCTION

Multiple Sclerosis (MS) is a neurodegenerative disease with an unpredictable pattern of progression, characterized by inflammation culminating in lesions throughout the central nervous system (CNS) (Compston et al., 2005). MS lesions are best described as ‘plaques’ or ‘scars’ and are the product of axonal demyelination. These sclerotic lesions vary in size and location within the brain and spinal cord, such that the patient experiences a diverse array of symptoms (Compston et al., 2005). In most cases, specific MS symptoms (i.e. sensory, motor, cognitive) reflect the areas within the CNS which are involved in demyelination and are often asymmetrical, as one side of the body (and one side of the brain) is more involved than the other (Reich, Smith et al., 2007). Although symptoms typically fall under three main categories (motor, cognitive and sensory), in some cases, patients may present with an initial attack, leading to their MS diagnosis, with symptoms resolving such that people can remain relatively symptom-free for many years (Zipoli, Goretti et al., 2010).

The Expanded Disability Status Scale (EDSS) is a measurement tool used by neurologists in order to rate MS disease severity which is a categorical ranking scale of 0 (no impact of MS) to 10 (death from MS). Although MS is a heterogeneous disease with each patient experiencing their own unique set of motor, cognitive and sensory symptoms, the EDSS scoring system relies heavily on walking capacity and the measurement of changes in other symptoms can be overlooked (Goodkin, Cookfair et al., 1992; Meyer-

Moock, Feng et al., 2014). Additionally, although individuals may detect declines in functional ability and symptom severity, the EDSS is somewhat insensitive to small changes in functional ability and/or symptom severity perceived by the patient and may underestimate progression (Li, Held et al., 2006). In the future, the effectiveness of new treatments will be determined by whether the treatments can halt symptom progression, stabilize underlying CNS changes and improve function as reported by the patients themselves; indicators that must be measured within clinical trials. Thus, there is a need to examine new outcome measures that demonstrate sensitivity and detect heterogeneity in MS symptoms in order to assess the benefits of emerging treatments.

A biomarker is a tool, which objectively measures and evaluates biological changes in disease pathology, or responses to therapeutic interventions (Mayeux, 2004). Transcranial magnetic stimulation (TMS) is a non-invasive technique used to assess excitability within the motor cortex and corticospinal tract (CST). For this reason, variables derived using TMS have been a useful biomarkers in other neurological disorders in which there is primarily motor dysfunction (stroke and Huntington's) (Lefaucheur, Ménard-Lefaucheur et al., 2006; Carter, Patel et al., 2012; Boyd, Hayward et al., 2017). Research investigating TMS as a biomarker in MS has shown questionable efficacy (Simpson & Macdonell, 2015). These variable findings could be attributed to the fact that some studies group patients who have multiple types of impairment together (motor and non-motor). For example, Neva and colleagues (2016), aimed to evaluate a TMS measure called cortical silent period (CSP) in MS participants and controls. The CSP has been widely adopted when evaluating motor cortex and corticospinal excitability in response to neurologic

changes (Khedr, Ahmed et al., 2011; Gray, Palmer et al., 2017). Neva and colleagues (2016) found no difference in the CSP duration between MS participants and controls. On the other hand, Tataroglu and colleagues (2003), found a significantly prolonged CSP in people with MS with motor related impairments (cerebellar) when compared to controls. Since MS is a heterogeneous disease, and TMS measures the integrity of a motor tract (not sensory or cognitive functions), it would be reasonable to believe that TMS may indeed be an effective biomarker when motor impairment is being exclusively investigated in MS. For example, several groups have endorsed TMS as a weak biomarker of disease severity in MS (Simpson & Macdonell, 2015; Snow, Wadden, Chaves & Ploughman, 2019). Because TMS non-invasively examines brain and spinal cord integrity, it has the potential to provide a 'window' on brain function in MS.

The work outlined in this thesis aimed to determine whether TMS parameters would distinguish groups of MS patients with different clinical profiles, such as motor, cognitive, sensory and asymptomatic, from one another. We then evaluated which TMS measures predicted symptom severity within the clinical groups compared to the current neurologist scored EDSS measure. These preliminary steps are needed to determine whether TMS could help detect declines in MS symptoms with better resolution than current measures. For the purposes of this thesis, the term TMS is used to describe both the device as well as the measures derived from TMS. Since TMS indicates underlying function (or dysfunction) of cortical networks, the results of our research are necessary in order to further understand whether TMS is a useful biomarker that can track disease progression and detect the effects of medication and rehabilitation treatments on neurophysiological processes.

This thesis is prepared in the traditional format with three chapters. Chapter One provides an overview of MS and associated symptoms, followed by an explanation of biomarkers within the field of MS including their limitations. TMS is then described, followed by previous research assessing the use of TMS in neurological disorders and the use of TMS and its importance within the field of MS. Chapter Two consists of materials and methods used to answer the two objectives of this research. The first objective was to compare TMS measures between clinical profiles of MS. Secondly, we aimed to determine if TMS measures could predict symptom severity and provide better sensitivity than the current disease severity measure, EDSS. Chapter Three consists of a discussion highlighting the major findings of this research, suggestions for future directions in the assessment of TMS as a biomarker for specific clinical profiles of MS, and the limitations of this study. The format of this Master's Thesis is APA style (6th edition).

1.2 OVERVIEW OF MULTIPLE SCLEROSIS

1.2.1 Epidemiology

MS is a disabling neurologic disease of the CNS that affects over 2.3 million people worldwide (Browne, Chandraratna et al., 2014). Most individuals diagnosed with MS are between 20 to 40 years of age, although, younger and older individuals can be diagnosed (Rolak, 2003). The cause of MS is still unknown, but evidence supports that environmental, lifestyle and genetic factors contribute to the disease. For example, the most common environmental factor found to increase the risk of MS is low levels of vitamin D (Cortese, Riise et al., 2015; Mokry, Ross et al., 2015). A prevailing lifestyle factor associated with a

higher risk of MS is smoking (Hedström, Hillert et al., 2013; Ramanujam, Hedström et al., 2015). Ramanuham and colleagues (2015) found that smoking tobacco not only increases an individual's risk of MS but has been shown to accelerate the progression of the disease (Ramanujam, Hedström et al., 2015). Lastly, the genetic contribution to MS is very diverse; however, gene variation on immune response molecules, known as major histocompatibility complex molecules, have a large role to play in the increased risk of the disease (Consortium, 2005; Ramagopalan, Knight et al., 2009). Thus, it appears that a combination of environmental, lifestyle and genetic factors impact a person's susceptibility to MS.

1.2.2 Clinical Course in MS

MS can be a challenging disease to recognize clinically due to its heterogeneity and unpredictable disease patterns. Typically, patients experience a clinically isolated symptom, such as unilateral optical neuritis or partial myelopathy (Thompson, Banwell et al., 2018). A clinically isolated symptom initiates further investigation by a patient's neurologists using the 2017 revised McDonald criteria, outlining a combination of criteria used for MS diagnosis (Thompson, Banwell et al., 2018). Such criteria include MRI imaging, cerebrospinal fluid analysis, blood tests to rule out other conditions, along with a neurological examination. Additionally, the revised McDonald criteria in 2017 introduced a new criterion to aid in MS diagnosis; the presence of oligoclonal bands which can be detected in the cerebrospinal fluid using MRI (Thompson, Banwell et al., 2018).

Even though patterns of disease progression are inconsistent, two main clinical forms that classify a patient's disease course are known as relapsing-remitting or progressive. The most common presentation of MS typically diagnosed is relapsing-remitting MS, characterized by clearly defined periods of relapse, with full or partial remission (Compston et al., 2005). Approximately 40-50% of people who have relapsing-remitting MS will transition into secondary progressive MS after 20 years of disease onset (Compston et al., 2005). Secondary progressive MS is characterized by continuous progressive decline with either no remissions or minor remissions (Rovaris, Confavreux et al., 2006). The second clinical form of MS diagnosed in 10% of patients is primary progressive MS. Primary progressive MS has a vague onset in which patients experience progressive decline from disease onset with occasional plateaus and possible minor improvements (Compston et al., 2005). In general, research has focused on comparing clinical biomarkers of disability between relapsing-remitting MS, secondary progressive MS, and primary progressive MS because they are well defined clinical courses of MS. Nevertheless, the symptoms of MS vary substantially from person to person and as a result, finding a biomarker that can distinguish patients by their profile of symptoms is challenging.

1.2.3 The Symptoms of MS

Because lesions are widespread; varying in size and severity, the symptoms of MS and how they are experienced are highly variable. The EDSS, one of the oldest and most widely utilized assessment instruments in MS (Kurtzke, 1983), groups symptoms into

seven main categories which include pyramidal, cerebellar, brainstem, sensory, bowel, and bladder, visual and cerebral (mental) functions. Pyramidal symptoms involve motor dysfunction such as limb weakness, paresis or spasticity. Cerebellar and brainstem dysfunction may manifest as double vision, dizziness, cerebellar ataxia, tremor, spasticity, facial sensory loss and vertigo (Browne, Chandraratna et al., 2014). Symptoms of bowel and bladder dysfunction may involve urinary hesitancy, urgency, retention, incontinence or loss of bowel function (DasGupta & Fowler, 2003). Sensory dysfunction is very frequent in people with MS involving pain or unpleasant feelings of vibration in the limbs, decreased ability to feel touch, position sense, or vibration, and in extreme cases, loss of sensation in the limbs or below the head (Beiske, Pedersen et al., 2004). In terms of visual dysfunction, optic neuritis, defined as blurring of vision in one eye and possible pain or light flashes during eye movement, is a common presenting symptom experienced by people with MS (Brownlee, Hardy et al., 2017). Lastly, symptoms classified as 'cerebral function' affect 43-70% of people with MS (Chiaravalloti & DeLuca, 2008). Symptoms may include problems with memory and even dementia. While the functional system scale within the EDSS includes a wide variety of symptoms, there are several distinct symptoms that are not included in the EDSS, which are extremely common among patients with MS, including fatigue (Bakshi, Shaikh et al., 2000), mental health symptoms (anxiety and depression) (Beiske, Svensson et al., 2008), heat intolerance (White, Wilson et al., 2000), and sexual dysfunction (DasGupta & Fowler, 2003).

1.2.4 Lesions and Neurodegeneration

1.2.4.1 White Matter Lesions

The pathologic hallmark of MS consists of multiple focal areas of plaques as a product of demyelination, axonal damage and oligodendrocyte death within the CNS (Compston et al., 2005). The most common areas for plaques to appear is in white matter, which consists of myelinated axons that form connections between brain cells and are distributed into bundles called tracts (Fields, 2008). Plaques of white matter are commonly found in areas that contain many small vessels, such as around ventricles or outer surfaces of the brainstem or spinal cord (Lassmann, Bruck et al., 2007; Pardini, Sudre et al., 2016). Further, white matter lesions (WMLs), have been described as a product of demyelination and inflammation, involving the action of immune cells infiltrating parenchyma of the CNS due to the disturbance of the blood brain barrier (Compston et al., 2005; Hochmeister, Grundtner et al., 2006). The healthy immune system exhibits self-tolerance and limits attack on the body's own proteins and antigens, yet, in MS, immune cells unpredictably attack one's own myelin antigens (Compston et al., 2005). The inflammatory response involves lymphocytes (predominantly T cells), macrophages and glial cells. In MS, while there are many types of T cells involved in pathology, it is thought that CD4+ (helper) T cells play a role in initiation of lesions while CD8+ (cytotoxic) T cells dominate and multiply at the demyelinated site, far outnumbering CD4+ T cells (Babbe, Roers et al., 2000; Lassmann, 2005). It is believed that in MS, T cells cross the blood brain barrier and initiate the up-regulation of innate molecules of the immune system called microglia, which

are found to be prominent in all actively demyelinating lesions (Lassmann, Bruck et al., 2007; Zrzavy, Hametner et al., 2017).

While MS was thought to be predominantly mediated by T-cells, emerging research using anti-CD20 therapies and their approval in treating relapsing-remitting and primary-progressive MS have highlighted the role of B cells in CNS inflammation (Li, Patterson & Bar-Or, 2018). In two identical phase three trials, Hauser and colleagues (2017) assigned 821 MS participants to receive ocrelizumab (a monoclonal antibody that depletes CD20+ B cells) and 835 MS participants to receive interferon beta-1a (Hauser et al., 2017). It was discovered that ocrelizumab was significantly associated with lower rates of disease activity and progression compared to interferon beta-1a (Hauser et al., 2017). Further, it is suggested that the reduction of CD20+ B cells may prevent their contribution to MS through mechanisms such as proinflammatory-cytokine-mediated activation and antigen presentation (Li, Patterson & Bar-Or, 2018). It is also plausible that reduction of CD20+ B cells may prevent peripheral cellular immunological cascades involved in triggering new relapses (Li, Patterson & Bar-Or, 2018).

Another consequence of the immunologic response is the death of a group of specialized cells within the CNS that produce the myelin sheath, called oligodendrocytes. Oligodendrocyte death occurs at the border of MS lesions; the same areas where high densities of microglia have been discovered (Peterson, Bö et al., 2001). This suggests that microglia may have a role to play in oligodendrocyte death, preventing remyelination of CNS axons. Although microglial cells are recognized for their damaging effects, they also play a dual role in MS as they possess anti-inflammatory properties and release growth

factors which have the ability to support axonal remyelination (Domingues, Portugal et al., 2016); despite this, the underpinnings of this dual role remain unclear (Vogel, Vereyken et al., 2013; Prinz & Priller, 2014; Luo, Jian et al., 2017). Overall, WMLs are areas of inflammation and demyelination scattered throughout the white matter and are highly variable in size and location, ultimately contributing to the array of clinical symptoms experienced by people with MS (Popescu, Pirko et al., 2013).

1.2.4.2 Grey Matter Damage

MS was traditionally considered a disease affecting white matter within the CNS. It has now been established that disruption of nerve cells (cortical grey matter) is an additional component of MS (Pirko, Lucchinetti et al., 2007; Geurts & Barkhof, 2008). The most susceptible regions for grey matter damage within the CNS include the motor cortex, cerebellum and deep grey matter nuclei (Calabrese, Agosta et al., 2009; Gilmore, Donaldson et al., 2009). Four main forms of grey matter damage have been described: Types I, II, III and IV. Type I are leucocortical lesions of the cortex that extend from subcortical white matter (Wu & Alvarez, 2011). Type II are intracortical lesions, found within the cortex while type III are subpial lesions that appear along the pial surface, extending to layers III or IV of the cortex. Lastly, type IV grey matter lesions encompass the width of the cortex, spreading over several gyri and lobes of the cortex (Bø, Vedeler et al., 2003; Calabrese, Filiippi & Gallo, 2010). While it has been noted that grey matter lesions (GMLs) have much less inflammation with fewer associated T-cells, macrophages and microglia than WMLs (Peterson, Bö et al., 2001). However, recent findings from 53

patient cortical biopsy samples suggested that there is an inflammatory profile within GMLs (Lucchinetti, Popescu et al., 2011). In accordance with new research, inflammation has been noted as a key driver of GMLs (Lagumersindez-Denis, Wrzos et al., 2017). Another unique feature of GML is the lack of breakdown of the blood brain barrier (van Horssen, Brink et al., 2007), leading to the concept that T-cells gain entry into the brain via the meninges, choroid plexus or subarachnoid space (Prins, Schul et al., 2015). Although the pathology of GML is becoming clearer, the exact mechanism of GML formation remains unknown. Overall, GMLs are difficult to detect on routine clinical imaging and require more sophisticated ultra-high field 7 Tesla magnetic resonance imaging (MRI). imaging technique (Kilsdonk, Jonkman et al., 2016). With advancing importance of GML contribution to symptomology of MS, there is increasing need for a biomarker of disease progression beyond clinical MRI imaging.

Studies linking WML and GML lesions to clinical symptoms suggest that there is often a mismatch between imaging results and clinical MS profile. For example, while WML size and location could not always explain clinical deficits in people with MS, GML accumulation further explained clinical disability (Calabrese, Agosta et al., 2009; Hulst, Schoonheim et al., 2012; Schoonheim, Popescu et al., 2012). In fact, recent research suggests that GMLs may give rise to motor symptoms of MS. For instance, in 172 people with MS, greater cerebellar grey matter abnormalities (measured by conventional MRI) predicted greater motor impairment (EDSS) (Preziosa, Rocca et al., 2014). In summary, WML and GML cause dysfunction within functional brain networks as well as ascending and descending projections and contribute to a variety of symptoms experienced by people

with MS. There is not always a clear correlation between appearance of WML and GML and clinical symptomology.

1.2.4.3 The Role of Glutamate and GABA in MS

1.2.4.3.1 Glutamate Dysfunction

Neurotransmitters are released at the neuronal synapse allowing signals to transmit from one neuron to another (Zimmerman & Wee, 1984). Excitatory neurotransmitters increase the chance that the postsynaptic neuron will produce an action potential while inhibitory neurotransmitters decrease the probability of neuronal propagation. The neurotransmitter glutamate (excitatory) is the most abundant neurotransmitter in the brain, which allows neurons to work together to carry out complex functions including but not limited to cognition, sensory information, motor coordination (Hassel & Dingledine ,2012). Despite this, glutamate is not a "more is better" molecule. In MS, CNS degeneration including neuronal damage, is partially caused by overactivity of glutamate, better known as excitotoxicity (Stojanovic, Kostic et al., 2014). Research in both animal models and cohorts of MS patients suggest that neuronal damage within the CNS is partially due to higher concentrations of glutamate (Werner, Pitt et al., 2001; Azevedo, Kornak et al., 2014). In animal models of MS, increased levels of glutamate contribute to the production of demyelinated lesions (Werner, Pitt et al., 2001). Similarly, among 343 patients with MS, increased serum glutamate levels predicted an increased rate of axonal deterioration (measured by serum concentrations of *N*-acetylasparate using multivoxel spectroscopy) over a 5-year time period (Azevedo, Kornak et al. 2014). It was further determined that

higher glutamate levels and corresponding *N*-acetylasparate levels were associated with a loss of brain volume, decline in cognitive function, and increased disability score (Azevedo, Kornak et al., 2014). Increased glutamate levels lead to excitotoxicity, resulting in axonal demyelination, synaptic dysfunction and cellular death. Techniques to track glutamate actions may be beneficial in order to detect disease progression and measure the benefits of future disease-modifying therapies.

1.2.4.3.2 GABA Dysfunction

Within the CNS, γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter (Paul, Branton et al., 2014). Low GABA levels have been detected in MS and have been found to correlate with disability (Cawley, Solanky et al., 2015; Nantes, Zhong et al., 2016). In a study by Cawley and colleagues (2015), low levels of GABA in sensorimotor brain regions (quantified by using single voxel MEGA-PRESS magnetic resonance spectroscopy) were reported in 30 people with secondary progressive MS compared to 17 healthy controls (Cawley, Solanky et al., 2015). Moreover, lower levels of GABA significantly correlated with greater physical disability (muscle and grip strength) in MS participants (Cawley, Solanky et al., 2015). Similarly, Nantes and colleagues (2016) found that lowered sensorimotor GABA levels (collected using proton magnetic resonance spectroscopy) were linked to worse motor performance on upper limb motor function using the 9 Hole Peg Test (Nantes, Zhong et al. 2016). In summary, MS-related disability, and likely neuronal modification during injury and repair, are related to altered levels of GABA

neurotransmitter levels. There is a clear need for non-invasive methods to measure glutamate and GABA–mediated activity as a potential indicator of disease progression.

1.3 MEASUREMENTS OF DISEASE SEVERITY IN MS

1.3.1 EDSS and its Usefulness as a Sensitive Outcome Measure

Despite years of criticism by researchers and clinicians, EDSS remains the most standard clinician-reported outcome tool used by neurologists to rank MS disease severity (Meyer-Moock, Feng et al., 2014). The EDSS scoring system is a categorical scale that ranges from no impairment (0) to death (10) in 0.5 increments (Kurtzke, 1983). Within the scale, scores from 0 to 3.5 indicate good functional status with no assistance required; scores between 4.0 to 5.5 suggest impaired walking and people who score between 6.0 and 9.0 require progressively more assistance in activities of daily living.

EDSS has three major weaknesses. First, the EDSS rating system prioritizes impairments in physical functioning, minimizing the contributions of other functional domains such as cognition and sensation. Secondly, people with MS who have unique patterns of symptoms may receive the same EDSS score and potentially receive similar treatment for different MS symptoms. Thirdly, since the EDSS rubric is essentially a categorical scale that has been assigned numerical values, it is relatively insensitive to small changes experienced by the patient and may underestimate progression. For example, researchers explain that EDSS has limited responsiveness to change in disability, which means that even though individuals may notice small changes occurring, the EDSS score may not change (Hobart, Lamping et al., 2001). Marolf and colleagues (1996) investigated

changes in disability scores before and after in-patient rehabilitation and found that 68% of disability scores were unchanged using the clinical disability tool Functional-Independence Measure while 95% of disability scores were unchanged using the EDSS (Marolf, Vaney et al., 1996). Similarly, in more recent research, Rabadi and Vincent (2013) reported that within 76 veterans with MS, the Functional Independence Measure was a more sensitive measure of MS disability than the EDSS (Rabadi & Vincent, 2013). Despite these findings, EDSS continues to remain the most common neurologist-used outcome tool (Meyer-Moock, Feng et al., 2014). As a consequence of poor EDSS responsiveness, clinicians may have been able to intervene and stop these declines in ability, but by the time EDSS score changes, a large change in ability has already occurred. Another well-documented weakness of the EDSS is the poor inter-rater reliability which means that a patient with MS may receive two different EDSS scores from two different neurologists (Meyer-Moock, Feng et al., 2014). More specifically, different ranges of the scale have different levels of inter-rater reliability, with EDSS scores from 1.0 to 3.5 reported to have the greatest inter-rater scoring variability (Goodkin, Cookfair et al., 1992).

Altogether, while EDSS is one of the most popular disease severity tools, it lacks sensitivity has a low ability to detect heterogeneity and poor inter-rater reliability. Thus, it is increasingly recognized that there is a critical need for new measures that are sensitive to small changes in MS symptoms in order to better target treatments. As everyone with MS has their own unique symptoms and pattern of progression it is critical to use a tool which addresses EDSS weaknesses. Imaging and neurophysiological biomarkers provide an opportunity to sensitively measure change within the clinical profiles of MS, perhaps

providing greater granularity than the EDSS and providing a window into the biological underpinnings of disease progression.

1.3.2 Biomarkers of MS

A biomarker in clinical research is an objective tool that indicates a change in underlying disease pathology (Mayeux, 2004). Above all, biomarkers can open the door for personalized medicine, providing the opportunity to track disease progression and provide a personal care plan on an individual level (Polivka, Krakorova et al., 2016). In stroke, both functional MRI or TMS can help measure the integrity of the corticospinal tract (CST) which, in turn, predicts potential for recovery (Braune & Fritz, 1995; Ahonen, Jehkonen et al., 1998; Carter, Patel et al., 2012; Lotze, Beutling et al., 2012). Unfortunately, due to the complexity and unpredictability of MS, finding an accurate and reproducible biomarker has been a notoriously complicated task (Katsavos & Anagnostouli, 2013). Discovery of a useful biomarker of disease severity could be used by clinicians to initiate earlier intervention or to track and observe the effects of drugs and rehabilitative treatments.

1.3.3 MRI as a Biomarker

MRI is notably the most accurate tool for early MS diagnosis and has been recognized as the gold standard method for monitoring MS (Wattjes, Steenwijk et al., 2015). While conventional MRI detects early indication of MS plaques within the CNS, these techniques are confined to structural change and are unable to identify functional

changes within neuronal pathways and networks (Gajofatto, Calabrese et al., 2013). Furthermore, lesion load, collected by conventional MRI (proton density/T2-weighted), has been found to only weakly correlate with clinical disability (Kappos, Moeri et al., 1999; Li, Held et al., 2006; Fisniku, Brex et al., 2008). Li and colleagues (2006) collected MRI data from a database of 1,312 MS patients and determined that lesion load, in fact, correlated with disability (EDSS) in MS patients with EDSS scores up to 4.5 (Li, Held et al., 2006). However, it was found that for scores above 4.5, indicating an increased disability, there was a plateau in the relationship (weak to moderate correlation) with lesion load. More recently, advanced 7-Tesla MRI techniques permit the detection of grey matter pathology and undiscovered cortical lesions (Vigeveno, Wiebenga et al., 2012; Kilsdonk, Jonkman et al., 2016). Therefore, more advanced biomarker techniques may help to explain symptomology. However, MRI is a technique that is resource-heavy as it is costly and time-consuming in order to capture high-quality images (Mills, Mirza et al., 2017). TMS may have the ability to address these weaknesses inherent with MRI as it is more resource-efficient and has the capacity to provide function-related changes within the CST.

1.4 TMS

1.4.1 The Corticospinal Tract

The CST is a white matter tract in the body, known as the principal motor system for controlling voluntary movements (Martin, 2005). The CST begins at the cortex, extends through deep white matter continuing to the brainstem. Once at the brainstem, approximately 75-90% of fibers cross from one side to the other in the medulla which is

known as pyramidal decussation (Martin, 2005). These fibers which descend contra-laterally are called the lateral CST, controlling the movements of the limbs of the opposite side of the body. The anterior CST account for the remaining 10-25%, which does not cross at the levels of the medulla but continues to descend and control the movement of the trunk (Martin, 2005). In particular, damage to the CST leads to impaired motor function (e.g., limb weakness and paresis) as seen in neurological disorders such as stroke (Vargas, Gaudron et al., 2013) and MS (Daams, Steenwijk et al., 2015). Moreover, TMS has the ability to a assess the integrity and excitability (the propensity of a neuron to generate an action potential) of the CST. See Figure 1.1 for CST

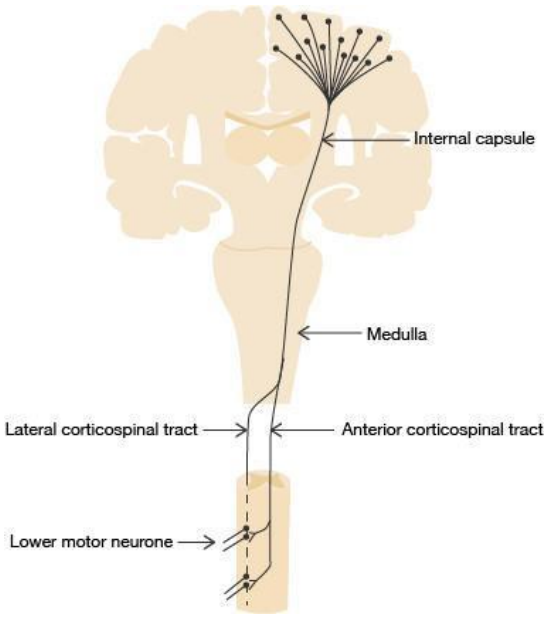

FIGURE 1.1: CORTICOSPINAL TRACT

The corticospinal tract beginning at the motor cortex, running through the internal capsule to the pyramids where the lateral corticospinal tract will descend contralaterally and the anterior corticospinal tract will descend ipsilaterally. Reprinted from Corticospinal System, In https://www.flickr.com/photos/interactive-content/. Copyright 2017 by Chest Heart & Stroke Scotland and The University of Edinburgh. Reprinted with permission.

1.4.2 Using TMS to Investigate the Integrity of the CST

TMS is a non-invasive technique that delivers magnetic stimulation over targeted regions of the brain which can be used for different purposes. For instance, repetitive TMS is a special form of TMS that delivers trains of pulses to the brain which has been approved in some countries for treating depression (Perera et al., 2016). The second key use of TMS is as a biomarker to probe corticospinal excitability and patterns of inhibition and excitation of neuronal networks (Zipser et al., 2018). In several neurological disorders, TMS applied over the motor cortex helps to map the functionality of the CST and understand underlying neuronal network integrity and dysfunction (Caramia, Cicinelli et al., 1991; Caramia, Palmieri et al., 2004; Hallett, 2007; Ni & Chen, 2015). To assess the CST, a coil is held at an optimal position of 45° over the skull corresponding to the region of interest in the motor cortex. The device emits a magnetic pulse (Figure 1.2) (Hallett, 2007) and the current painlessly depolarizes cortical neuronal networks of the motor cortex beneath the scalp and transmits an electrical signal to the corresponding muscle. A muscle contraction induced by the TMS is termed motor evoked potential (MEP) and is quantified by electromyography (EMG). MEPs can be quantified in several different ways which will be discussed. A summary of TMS measures are provided in Table 1.1.

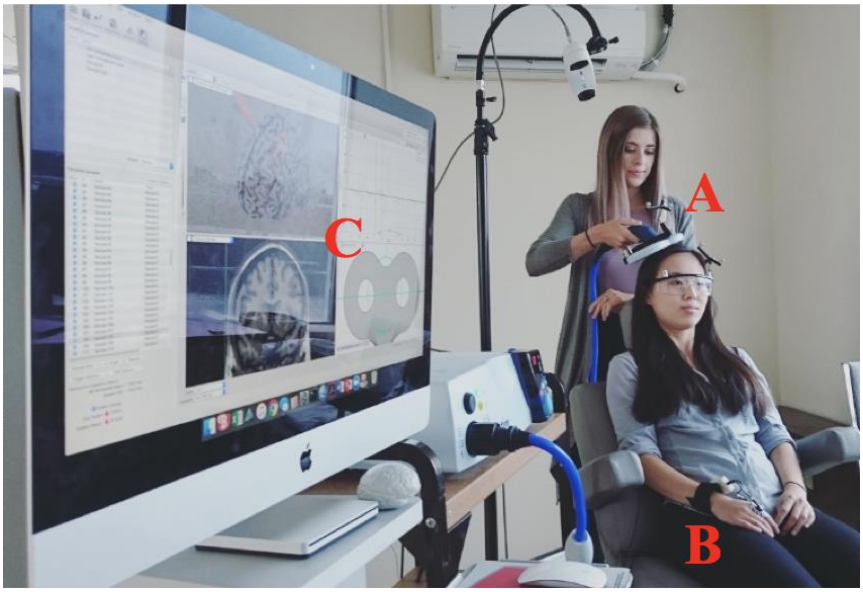

FIGURE 1.2: TRANSCRANIAL MAGNETIC STIMULATION
Participant seated in TMS chair with a coil placed tangentially to the scalp over the motor cortex**.** (A) The TMS coil placed on a 45° angle over the motor cortex (B) The EMG device wrapped around the wrist with electrodes on the skin over the muscle of interest. In our current study, the coil would be placed on the opposite side of the wrist with the electrodes; however, in this figure, assessment of ipsilateral connectivity was being evaluated (C) The TMS Brainsight Neuronavigation software ensures accurate positioning of the TMS coil.

TABLE 1.1: OVERVIEW OF TMS MEASURES

TMS Measure (Single Pulse)	How it is collected	What it provides
Resting Motor Threshold (RMT)	% Maximal stimulator output which evoked 5 out of 10 MEPS of ≥ 50μV during muscle relaxation	Quantification of overall corticospinal excitability
Active Motor Threshold (AMT)	% Maximal stimulator output which evoked 5 out of 10 MEPS of ≥ 20μV during 10-15% of maximal voluntary contraction	Quantification of overall corticospinal excitability during muscle contraction
Cortical Silent Period (CSP)	The average time from the end of the MEP to the return of voluntary EMG activity of 5 out of 10 successful MEPS from AMT	Degree of muscle interruption after muscle contraction: an indicator of brain and spinal inhibition

1.4.3 TMS Measures of the CST

1.4.3.1 Motor Threshold

In order to elicit a MEP in the target muscle, a certain intensity of TMS output is needed, termed the motor threshold; an indicator of corticospinal excitability (Hallett, 2007). To determine motor threshold, the first step is to identify the "hotspot" which is the cortical area most responsible for the TMS-evoked muscle contraction. In order to find the hotspot, TMS is applied to various sites over the primary motor cortex. The site which produces the highest muscular response (MEP peak-to-peak amplitude) in the muscle of interest is chosen as the hotspot. According to criterion of the relative frequency method described by Groppa and colleagues (2012), resting motor threshold (RMT) is collected while the muscle of interest is at rest, determined as the minimum amount of maximum stimulator output (MSO%) of the TMS needed to evoke 5 out of 10 MEPs of $\geq 50\mu V$ (Groppa et al., 2012). Active motor threshold (AMT) is similar to RMT; however, it is collected while an individual contraction the muscle of interest to 10% of their maximum voluntary contraction. AMT is defined as the minimum MSO% needed to evoke 5 out of 10 MEPs of $\geq 200\mu V$ (Groppa et al., 2012). Abnormally high threshold (RMT or AMT) values may indicate dysfunction of the CST stemming from a variety of neuronal factors such as a decrease in excitatory projections from the motor cortex. In other words, in individuals with decreased excitatory projections from the motor cortex, a higher stimulator output from TMS would be needed to elicit a response in the corresponding muscle of interest.

1.4.3.2 Cortical Silent Period

Once a MEP has been produced during voluntary muscle contraction (AMT), there is a period of time (post contraction) when there is a silencing of EMG activity termed the CSP (Triggs, Macdonell et al., 1992). The CSP is obtained from 5 out of 10 of the successful MEPs from AMT collection. The typical method to calculate the CSP is to take the interval (in ms) from the time of the end of the MEP to the return of the background EMG activity (Figure 1.3) (Modugno, Curra et al., 2001). Physiological underpinnings of the CSP remain a highly discussed topic, however it has been accepted that the CSP is the product of both cortical and spinal inhibition (Fuhr, Agostino et al., 1991; Inghilleri, Berardelli et al., 1993; Ziemann, Netz et al., 1993; Ahonen, Jehkonen et al., 1998). Additionally, researchers attribute the production of CSP to the $GABA_B$ inhibitory neuronal system within the CNS (McDonnell, Orekhov et al., 2006; Stetkarova & Kofler, 2013). Recent research by Tremblay and colleagues (2012) investigated the relationship between the CSP and measures of GABA and glutamate in a population of 24 healthy volunteers, measured by proton magnetic spectroscopy and TMS (Tremblay, Beaulé et al., 2012). Interestingly, it was determined that higher glutamate levels were predominantly linked to longer CSP. Concentrations of $GABA_B$ were not correlated with CSP; however, glutamate levels were linked to higher levels of GABA. As a result, Tremblay and colleagues (2012) suggested a possible homeostatic link between both glutamate and $GABA_B$ neurotransmitter systems within the CNS, meaning that, in response to higher glutamate concentration, $GABA_B$ may increase its inhibitory activity to counterbalance excitation.

While the mechanisms of the CSP remain highly discussed, CSP continues to be a measure of interest in MS research when determining the integrity of the CST.

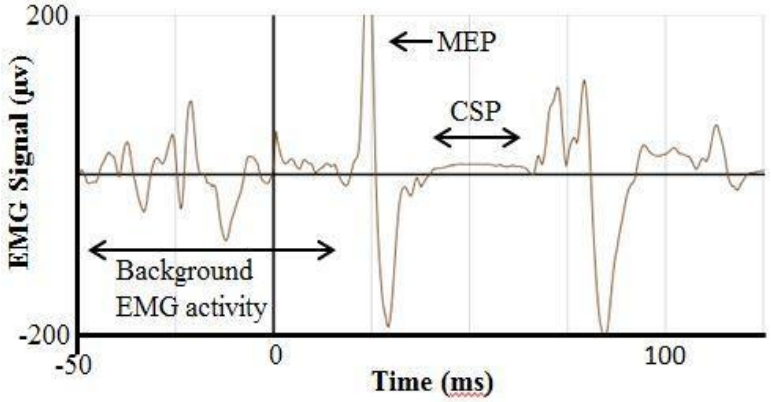

FIGURE 1.3: MOTOR EVOKED POTENTIAL RECORDED BY ELECTROMYOGRAPHY

Example of a motor evoked potential (MEP) from the first dorsal interosseous muscle following TMS over the primary motor cortex. The TMS artifact is indicated at Time 0. The background electromyography (EMG) activity is the activity of the muscle of interest at rest before a MEP of the muscle is elicited. Following the MEP there is a period of EMG suppression, which is termed the cortical silent period (CSP).

1.4.4 TMS Abnormalities in Neurological Disorders

1.4.4.1 Cognitive Associated Disorders

In neurological conditions, abnormal neurophysiological measurements collected from TMS have been associated with motor, cognitive, and sensory symptoms and even with neuropathological abnormalities in the absence of clinical symptoms (Eisen, Bohlega et al., 1989; Heald, Bates et al., 1993; Catano, Houa et al., 1996; Ahonen, Jehkonen et al., 1998; Lorenzano, Dinapoli et al., 2006; Schippling, Schneider et al., 2009; Khedr, Ahmed et al., 2011; Gray, Palmer et al., 2017). For example, among people with Alzheimer's disease, characterized by a decline in memory, reasoning, and executive function, there is prolonged CSP and reduced corticospinal excitability, measured using RMT and AMT compared to controls. Khedr and colleagues (2011) reported prolonged CSP in 45 patients with Alzheimer's disease as compared to 37 healthy controls (Khedr, Ahmed et al., 2011). Additionally, CSP was found to significantly increase with disease severity (mild, moderate, severe) (Khedr, Ahmed et al., 2011). Despite these findings, prolonged CSP may not be a consistent hallmark of Alzheimer's disease since Inghilleri and colleagues (2006) failed to find CSP differences between 20 Alzheimer's patients and 20 healthy controls. The authors suggested that equivocal values could be due to GABA-ergic neurons being preserved in Alzheimer's disease (Inghilleri, Conte et al., 2006). Nonetheless, analysis of the CSP among people with MS who may have cognitive impairment is worthy of investigation due to some shared cognitive symptom characteristics with Alzheimer's disease.

Studies analyzing other TMS measures such as motor threshold in patients with Alzheimer's disease report lower RMT (Di Lazzaro, Oliviero et al., 2004; Khedr, Ahmed et al., 2011) and AMT (Khedr, Ahmed et al., 2011). In contrast, Nardone and colleagues (2008) found no changes in RMT and AMT between 17 Alzheimer's disease patients and 22 healthy controls; however, the patients included in the study had a diagnosis of probable Alzheimer's disease and were described as being in the in milder stages. The authors suggested that impaired cortical excitability may not be apparent at these early stages and recommended testing patients who would be further along in the disease course. (Nardone, Bergmann et al., 2008). It is important to keep in mind that while Alzheimer's disease and MS have similar cognitive symptoms (processing speed, attention, memory), the pathological underpinnings are vastly different. Nevertheless, is possible that people with MS who experience primarily cognitive symptoms may also demonstrate altered CSP and motor thresholds. This phenomenon has yet to be examined.

1.4.4.2 Motor Associated Disorders

Since TMS measures the integrity of the CST, it is not surprising that TMS measures align with severity of symptoms in neurological disorders that affect the motor system such as stroke (Braune & Fritz, 1995; Ahonen, Jehkonen et al., 1998) and Huntington's disease (Modugno, Curra et al., 2001). For over twenty years, TMS has been evaluated as a clinical biomarker in stroke. In 1998, Ahonen and colleagues performed TMS on 29 patients with ischemic stroke and found that the mean CSP was significantly prolonged on the affected side of the brain compared to the un-affected side, supporting

the ability of CSP to detect even small subclinical disturbance in motor function (Ahonen, Jehkonen et al., 1998). Several other studies confirmed the presence of prolonged CSP in the ipsilesional hemisphere (Braune & Fritz 1995; Liepert, Bauder et al., 2000; Gray, Palmer et al., 2017). Not surprisingly, using motor thresholds, studies have shown a higher threshold of the affected hemisphere as compared to the unaffected hemisphere (Heald, Bates et al., 1993, Catano, Houa et al., 1996). These findings demonstrate that TMS is useful to detect abnormal corticospinal excitability by probing the CST and is sensitive to differences in damage between brain hemispheres.

Huntington's disease is a genetic neurological disorder characterized by progressive motor incoordination. In Huntington's research using TMS, two separate studies with sample sizes of 11 and 17, demonstrated significantly prolonged CSP in patients compared to controls (Modugno, Curra et al., 2001; Lorenzano, Dinapoli et al., 2006). Eisen and colleagues (1989) reported shortened CSP in 7 of 9 Huntington's patients as compared to 13 healthy controls (Eisen, Bohlega et al., 1989). Considering that Huntington's disease is characterized by a mixture of motor and non-motor symptoms, measurements of RMT and AMT have been inconclusive. For example, Schippling and colleagues (2009) found that 8 premanifest Huntington's gene carriers and 8 early symptomatic Huntington's patients displayed an increase in both RMT and AMT as compared to 22 healthy controls (Schippling, Schneider et al., 2009). In contrast, Orth and colleagues (2010) found no differences in RMT and AMT between 15 Huntington's carriers compared to 14 controls (Orth, Schippling et al., 2010). Overall, the evidence supports that there may be prolonged CSP and increased RMT and AMT in patients who

have neurological disorders that at least in part, involve the motor system. Therefore, it is plausible that people with MS with primarily motor symptoms would also display similar abnormalities in these TMS measurements.

1.4.4.3 Sensory and Subclinical Associated Disorders

There are limited studies that have evaluated the integrity of the CST using TMS among people who have primarily sensory disorders. This may be due to the fact that sensory dysfunction involves primarily sensory cortex, nuclei, and tracts, rather than the CST. Epilepsy is one disorder in which abnormal brain activity (seizure) can result in unconsciousness, abnormal sensations and bodily movements (Wolf, 2016). The mixed presentation in epilepsy makes it an interesting disorder to evaluate using TMS measures. Reutens and colleagues (1993) found reduced RMT in 20 patients with idiopathic generalized epilepsy as compared to 23 control subjects (Reutens, Berkovic et al., 1993). Conversely, Macdonell and colleagues (2001) found no difference in RMT; though, reported a prolonged CSP in 21 patients compared to 19 healthy controls (Macdonell, King et al., 2001). Some authors suggest that TMS may be able to detect alterations in interconnectivity between the sensory and motor cortices which could be useful to map the neurological underpinnings of sensory disorders (Amadio, Houdayer et al., 2014).

TMS may also reveal pathophysiological abnormalities among people who have pain. In a recent systematic review (Nardone, Versace et al., 2019), the authors reported that people with pain show some TMS abnormalities when using paired–pulse TMS methods (inhibition and facilitation) but not changes in thresholds and CSP. Whether

people with MS having sensory symptoms would have changes in corticospinal excitability detectable using TMS is not known and requires further consideration.

MS is a disease in which patients can often experience long periods of remission. If assessed by their neurologist, they may be considered ‘asymptomatic’ or ‘subclinical’. This group is interesting to examine using TMS because patients may be experiencing changes at the neuropathological level that are not yet apparent to the outside observer. Concerning such subclinical neurological disorders, there has been minimal investigation of CST abnormalities using TMS. MS has a prodromal period that has been reported to be up to five years, in which patients experience vague neurological symptoms (Wijnands, Zhu et al., 2019) that are not detected by current clinical measures. This means that individuals, before being diagnosed with MS, appear asymptomatic, meanwhile, pathophysiological processes are occurring (Högg, Wijnands et al., 2018). These two groups of people with MS (people with preclinical prodromal symptoms or who are in remission) are interesting to study. If better biomarkers could be developed, then intervention could begin more promptly. In terms of using TMS as a potential biomarker, Tataroglu and colleagues (2003) found that people with MS with no clinical symptoms had a prolonged CSP compared to controls (Tataroglu, Genc et al., 2003). With little evidence in the field of asymptomatic neurological disorders, the possibility that people with MS with asymptomatic evaluation may present abnormal TMS measures exists. Further, whether TMS measurements can detect the functional impact of asymptomatic lesions is unknown.

1.4.5 What we Know Thus Far: TMS in MS

1.4.5.1 TMS as a Biomarker in MS

As discussed above, in the field of MS research, TMS has demonstrated abnormalities within the CST in patients with MS. Some of these findings include increased central motor conduction time (Conte, Lenzi et al., 2009, Conte, Lenzi et al., 2009), delayed MEP latency (Kale, Agaoglu et al., 2009), increased motor threshold (Neva, Lakhani et al., 2016) and prolonged CSP (Tataroglu, Genc et al., 2003). Furthermore, researchers have explored TMS as a biomarker of disability and its ability to assess the effects of treatment (Fierro, Salemi et al., 2002, Tataroglu, Genc et al., 2003, Chaves, Kelly et al., 2019). For example, Tataroglu and colleagues (2003) studied 58 patients with MS and found that increased central motor conduction time (the time it takes for the TMS signal to be transmitted through the motor pathway to the muscle of interest) predicted increased disability using the EDSS (Tataroglu, Genc et al., 2003). In other research, Chaves and colleagues (2019) found that in 82 patients with MS, prolonged CSP was indicative of low fitness measured by a graded maximal exercise test (Chaves, Kelly et al., 2019). Additionally, Fierro and colleagues (2002) assessed the change in motor threshold, central motor conduction time and CSP in response to two treatment doses of methylprednisolone in 9 patients with relapsing-remitting MS (Fierro, Salemi et al., 2002). They detected an improvement (reduction) in CSP with both high and low treatment doses and an improvement in motor threshold and central motor conduction time with the highest dose of treatment. Overall, research suggests that TMS could be a promising tool to map

symptom severity and test the benefits of drug and rehabilitative treatments on motor performance.

1.4.5.2 The Importance of TMS as a Biomarker at an Individual Level in MS

In order for a biomarker such as TMS to be advantageous, it should strongly correlate with severity of symptoms in a cohort and at an individual level over time (Woo, Chang et al., 2017). To our knowledge, studies which have investigated TMS as a biomarker tend to group all patients together and have yet to separate patients by their main symptoms which essentially disregards the heterogeneity of MS. Whether TMS could be used to distinguish clinical profiles of MS (motor, cognitive, sensory or asymptomatic presentation), and whether changes in TMS measures would be associated with symptoms of disease severity, is worthy of investigation (Ferreri, Pauri et al., 2003, Ni & Chen, 2015). Historically, TMS has been most useful in diseases where there is dysfunction of the CST (Cortes, Black-Schaffer et al., 2012). Since TMS primarily assesses the CST, it is plausible that TMS may only provide information on brain integrity among people who have motor symptoms; failing to detect underlying abnormalities among people who have non-motor MS symptoms. This thesis aimed to test the usefulness of TMS as a biomarker in groups of people with MS who had differing clinical profiles.

As an overarching goal, we undertook this study as a first step to understand if TMS could be a useful biomarker in MS. We aimed to determine if TMS measures could distinguish clinical profiles of MS and if these parameters would align with disease severity. We first examined a cohort of people with MS, recruited from an MS clinic who

presented with a wide range of EDSS scores. Using the functional profiles within the EDSS scoring system, the patients were grouped into clinical profiles (motor, sensory, cognitive and asymptomatic). TMS was performed on each brain hemisphere and RMT, AMT and CSP data were gathered. Secondly, we aimed to identify if these TMS parameters could predict symptom severity (walking speed and cognition) within each clinical profile, with better sensitivity than the EDSS.

1.5 SUMMARY

MS is an unpredictable neurodegenerative disease involving the autoimmune attack of myelin within the CNS. The EDSS is the most common tool used to provide people with MS a measure of their disease severity; however, because it is an observer-rated categorical scale, it has been reported to lack sensitivity, has limited capacity to detect heterogeneity and low inter-rater reliability. The need to develop an objective biomarker, more sensitive than the current EDSS, is critical and TMS may have the potential to fill this gap. With accumulating evidence for abnormal TMS measures in neurological disorders, it is possible that TMS measures, specifically RMT, AMT, and CSP, may have the ability to distinguish clinical profiles of MS and may further be able to predict symptom disease severity. The first objective of this research was to investigate whether TMS measures could differentiate between four clinical profiles of MS; motor, sensory, cognitive and asymptomatic groups. The second objective was to determine whether TMS measures could predict symptom severity measures with better sensitivity than the current disease severity measure, EDSS. If TMS proves to be a useful biomarker in the field of MS, its use could facilitate

personalized medicine, non-invasively, and relatively inexpensively, tracking the progression of the disease and treatment responses on an individual level with greater sensitivity than the EDSS.

Chapter 2: Methods and Results

2.1 METHODS

2.1.1 Participants

Persons with MS (n=38) were recruited as part of a longitudinal provincial MS database coordinated by the Health Research Innovative Team in MS. Inclusion criteria included 1) able to walk at least indoors with or without aid; 2) diagnosed with MS (of any type) by a MS neurologist using McDonald criteria (McDonald, Compston et al., 2001); 3) 18 years or older; 4) able to participate in TMS assessment as per standardized TMS screening form (Rossi, Hallett et al., 2009) and 5) at least 3 months relapse-free. Demographics collected included age, sex, MS type and disease duration (years) (Table 2.1).

2.1.2 EDSS Classification

The MS neurologist provided the EDSS score based on clinical observations of the patient in combination with completing the functional system scale which included seven domains; pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual and cerebral. Patients were scored on each functional system scale domain ranging from 0- 5 or 6 (higher scores indicate greater impairment). Four main groups of participants were created based on their clinical deficits. Categories included; sensory symptoms (visual, brainstem and sensory functions) (n=11), motor symptoms (bowel, bladder, pyramidal and cerebellar functions) (n=12), cognitive symptoms (cerebral functions) (n=8) and asymptomatic MS

(n=7). Each participant was assigned to one of four clinical groups based on the category in which they had the highest score (EDSS functional system score).

2.1.3 Procedure

After obtaining consent and gathering demographic information (age, sex, and level of education), participants completed the Montreal Cognitive Assessment (MoCA), the Symbol Digit Modality Test (SDMT) and the Multiple Sclerosis Impact Scale (MSIS-29) (all data collection forms can be accessed in Appendix A). Participants were then asked to walk on an instrumented walkway (ProtoKinetic Walkway), followed by TMS.

2.1.3.1 Cognitive Assessments

The MoCA is a cognitive screening test, developed by Nasreddine and colleagues in 2005, which assesses eight aspects of cognition including memory, visuospatial abilities, and executive function (Nasreddine et al., 2005). Scores range from 0-30. Scores below 26 indicate mild to moderate cognitive impairment. MoCA has been demonstrated as a reliable and valid cognitive screening tool in the MS population (Dagenais, Rouleau et al., 2013). The SDMT assesses cognitive function through a simple 90-second substitution task where participants substitute numbers for given geometric figures using a reference key. It has been validated as a measure of cognitive processing speed among people with MS with a value below the cut-off of 40 indicating cognitive impairment (Drake, Weinstock-Guttman et al., 2010). Higher scores are indicative of faster cognitive processing speed.

2.1.3.2 Walking Assessment

Participants walked on a 4’ X 14’ instrumented ProtoKinetic walkway in order to record an individual’s footfalls (ProtoKinetics Inc, Havertown PA, USA). The instrumented walkway records and calculates pressure and spatiotemporal gait characteristics through a grid system. The grid system of the ProtoKinetic walkway is designed with load cells; thus, when participants walk on the walkway, information about the location and number of load cells activated, as well as the force on the load cells is transmitted to the computer system (PKMAS software). Variables provided include force of foot on the ground, step length, stride length, stride width, and walking velocity.

In MS research, the instrumented walkway has been demonstrated as a valuable tool when evaluating the impact of cognitive tasks on gait parameters (Kirkland, Wallack et al., 2015). In other MS research, the ProtoKinetic walkway was used to illustrate the use of bipedal hopping to predict subtle impairments in lower limb neuromuscular performance (Kirkland, Downer et al., 2017). In our study, participants were asked, using a standardized script, to walk twice across the walkway at a comfortable self-selected walking pace. The participants’ self-selected walking velocity (cm/s) was then calculated by dividing the sum of the stride length measurements by the sum of the stride time measurements (provided by the PKMAS software).

2.1.3.3 Subjective Impact of MS

The MSIS-29 consists of 29 questions that ask about the impact of specific MS symptoms on everyday life. It is scored according to a 5-point Likert scale ranging from 1

(no impact) to 5 (extremely impacted by MS). Nine questions address the psychological impact of MS (score range of 5-45) and 20 questions address the physical impact of MS (score range from 20-100) with higher scores indicating a greater perceived impact of MS on the individuals' psychological and physical state (Hobart, Lamping et al., 2001). Some of the questions in the MSIS-29 include, "In the past two weeks, how much have you been bothered by problems with your balance?" and "In the past two weeks, how much have you been bothered by feeling mentally fatigued?". The validity and reliability of both the physical and psychological subsections of the MSIS-29 have been confirmed (Hobart, Lamping et al., 2001).

2.1.3.4 TMS

The participant's brain excitability in each hemisphere was measured by eliciting monophasic TMS pulses, using a BiStim 200^2 stimulator connected to a figure-of-eight coil with an outer diameter of 70mm (Magstim, Co. Whitland UK). To measure electromyography (EMG) activity and collect the MEPs, the skin was prepared (Gilmore & Meyers, 1983) and foam surface electrodes (Kendall 200 Covidien, Mansfield, MA) were placed on the belly of the first dorsal interosseous muscle. The reference electrode and ground electrode were placed on the interphalangeal joint of the index finger and the styloid process, respectively. A neuronavigation device (Brainsight Rogue Research Inc, Montreal, QC, Canada) with a built-in EMG system was used for the collection of MEPs. The EMG system uses a 2500V/V amplification and 12-bit resolution analog to digital converter, it collects with a sampling rate of 3kHz, 4.5mVpp of the input range, gain of

600V/V with a bandwidth of 16-550Hz. A brain template from the Montreal Neurological Institute was integrated into the Brainsight software as a 3-dimensional stereotaxic image in order to accurately target the region of the motor cortex corresponding to the first dorsal interosseous muscle (Collins, Neelin et al., 1994).

Participants were seated upright, and the coil was held tangentially to the scalp at an angle of 45° from the midline. First, suprathreshold stimulations were fired at different sites over the primary motor cortex. The area with the highest response in the first dorsal interosseous muscle (MEPs peak-to-peak amplitude) was chosen as the *hotspot*. Secondly, motor thresholds were determined as the minimum amount of intensity of the TMS output which evoked 5 out of 10 MEPs of $\geq 50\mu V$ during muscle relaxation, known as RMT and $\geq 200\mu V$ during 10-15% of first dorsal interosseous maximal voluntary contraction, known as AMT (Rossini, Barker et al., 1994; Goss, Hoffman et al., 2012). Motor threshold values were reported as percentages of the maximum stimulator output. All MEPs were elicited bilaterally, that is, in each hand/brain hemisphere. The CSP was collected from the 5 out of 10 successful MEPs of the AMT and averaged over the total CSP's obtained. The CSP duration was defined as the time from the end of the MEP to the return of voluntary EMG activity as reported in previous studies (Nakashima, Wang et al., 1995; Modugno, Curra et al., 2001; Tataroglu, Genc et al., 2003).

Since MS lesions are often spread throughout the CNS affecting both cerebral hemispheres, we collected TMS in both hands and labeled the hands as 'stronger' or 'weaker' based on hand grip and pinch strength measured using a dynamometer (Lafayette Instruments, Lafayette, IN, and B&L Engineering, Santa Ana, CA, respectively).

Participants performed the hand grip and pinch strength task twice and the average score was reported. In MS patients, the side of the body is thought to be the side that is most affected (Fritz, Keller et al., 2017), thus, a participant's weaker side was determined based on the hand with the lowest combined grip and pinch strength measures (grip + pinch).

2.1.4 Statistical Methods

Values of CSP, RMT and AMT between the more affected and less affected hemispheres were compared using paired-sample t-tests within the sample as a whole. Next, an ANOVA Bonferroni Post Hoc test was used to assess the differences in TMS measures between clinical groups. $P<0.05$ was considered significant. Effect sizes were expressed as partial eta squared ($\eta 2$) where $\eta 2$ of 0.01 was considered a small effect, 0.06 a moderate effect, and 0.14 a large effect (Cohen, 1992). Simple linear regression analyses were conducted in order to determine whether TMS measures predicted cognitive (MoCA, SDMT, MSIS-29 Psychological), or physical (walking velocity, MSIS Physical) outcome severity within the clinical profiles. Subsequently, stepwise linear regression was used to determine which TMS measures best predicted symptom severity. Each model was evaluated by the model R and r_2 value. Data were analyzed using IBM SPSS version 23.

2.2 RESULTS

2.2.1 Participants

Participants on average were 48.1 (± 9.76) years of age. There were 35 individuals with relapsing-remitting MS and 3 with secondary progressive MS. A one-way ANOVA

was used to compare patients demographic information including age, EDSS, walking velocity, MoCA, SMT, MSIS Physical and MSIS Psychological. When comparing disability scores (EDSS), those in the motor group had a higher disability score than those in the sensory and asymptomatic groups (Table 2.1). Additionally, those in the cognitive group had a greater perceived impact of MS on their psychological state compared to those in the asymptomatic group (Table 2.1).

TABLE 2.1 PARTICIPANT DEMOGRAPHIC INFORMATION

Clinical Profile	**Motor**	**Sensory**	**Cognitive**	**Asymptomatic**
Subjects	12	11	8	7
Age (mean ± SD)	51.8 ± 9.1	44.3 ± 11.1	50.3 ± 8.5	42.3 ± 9.8
Sex				
Female/Male	9/3	4/7	6/1	3/4
Type of MS				
Secondary Progressive	2	0	1	0
Relapsing-Remitting	10	11	7	7
EDSS (mean ± SD)	3.4 (± 0.6)	1.9 (± 0.3)**a**	2.2 (± 0.7)	0**b**
Walking velocity (mean ± SD)	92.9cm/s (± 25.3)	112.4cm/s (± 19.2)	98.8cm/s (± 25.3)	107.1cm/s (± 14.7)
MoCA (mean ± SD)	25.5 (± 3.6)	27.1 (± 2.3)	26.5 (± 4.8)	25.6 (± 3.9)
SDMT (mean ± SD)	42.4 (± 12.8)	51.8 (± 4.9)	50.0 (±3.1)	47.9 (± 12.9)
MSIS Physical (mean ± SD)	44.7 (± 17.8)	32.4 (± 13.4)	47.9 (± 20.8)	28.9 (± 10.2)
MSIS Psychological (mean ± SD)	18.5 (± 7.7)	14.5 (± 4.4)	24.3 (± 8.6)	17.8 (± 7.4)**c**

EDSS: Expanded Disability Status Scale
MoCA: Montreal Cognitive Assessment
SDMT: Symbol Digit Modality Test
MSIS: Multiple Sclerosis Impact Scale
± Denotes the standard error value
a Denotes a statistical significance from the motor group $p<0.05$
b Denotes a statistical significance from the motor group $p<0.001$
c Denotes a statistical significance from the cognitive group $p<0.05$

2.2.2 Differences in TMS measures between brain hemispheres

There was a difference of hemisphere on RMT ($t_{(35)} = -3.387$, $p < 0.001$,), AMT ($t_{(36)} = -4.654$, $p < 0.001$) and CSP ($t_{(35)} = -2.175$, $p < 0.001$). As expected, the RMT was higher in the hemisphere corresponding to the weaker hand (45.83% ± 12.01) compared to that of the stronger hand (41.24% ± 10.37) in all clinical groups. The AMT was also higher in the hemisphere corresponding to the weaker hand (40.00% ± 13.24) than the stronger hand (34.05% ± 8.81). Additionally, the CSP was longer in the hemisphere corresponding to the weaker hand (33.49ms ± 18.12) than the stronger hand (26.72ms ± 12.74) (Figure 2.1).

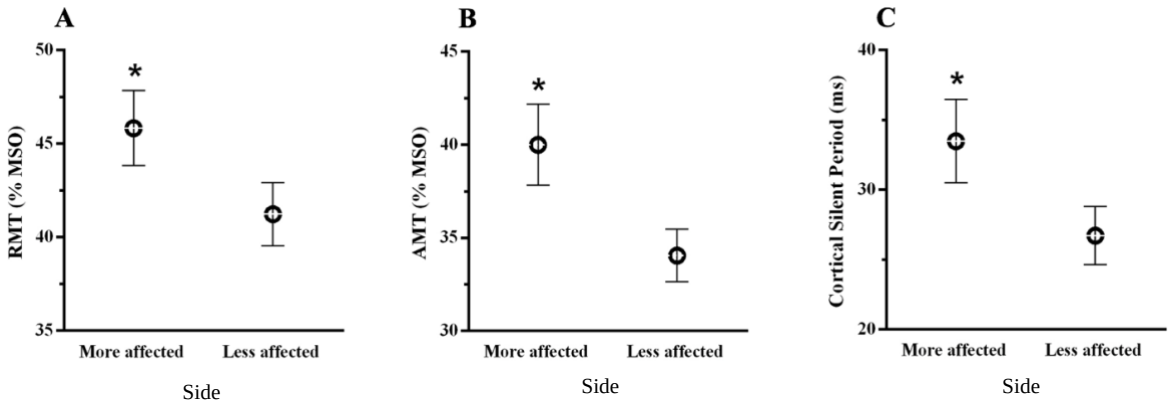

FIGURE 2.1: TMS DETECTED HEMISPHERIC DIFFERENCES IN MS PARTICIPANTS

(A) MS participants show a higher mean resting motor threshold (RMT) in the hemisphere corresponding to the weaker hand (more affected side) than the stronger hand (less affected side) (B) MS participants show a higher mean active motor threshold (AMT) in the hemisphere corresponding to the weaker hand (more affected side) than the stronger hand (less affected side) (C) MS participants show a higher mean cortical silent period (CSP) in the hemisphere corresponding to the weaker hand than the stronger hand. * ($p<0.01$), error bars are SEM

2.2.3 Differences in TMS measures between clinical groups

There was an interaction effect of clinical group and hemisphere on CSP ($F_{(1,32)}$ = 4.45, p = 0.010, n_{p2} = 0.29), however; there was no interaction for RMT ($F_{(1,32)}$ = 0.50, p = 0.695, n_{p2} = 0.05) and AMT ($F_{(1,32)}$ = 1.58, p = 0.214, n_{p2} = 0.13). Both of these non-significant interactions approach moderate and large effective sizes. This is suggestive that these sample sizes may not be large enough to detect a statistical significance and should be considered when interpreting these results.

In the hemisphere corresponding to the stronger hand, participants with motor impairments had a significantly longer CSP ([$F_{(3,33)}$ = 3.87, p = 0.018] 35.65ms ± 16.64) than those with sensory impairments (20.52ms ± 5.07). The CSP of the hemisphere corresponding to the stronger hand was not different between the remaining clinical groups (Figure 2.2). In the hemisphere corresponding to the weaker hand, participants with motor impairments had significantly longer CSP (51.65ms ± 20.40 [$F_{(3,33)}$ = 8.965, p < 0.001]) than those with sensory impairments (24.14ms ± 5.15), cognitive impairments (29.40ms ± 14.71) and those who were asymptomatic (25.33ms ± 9.76) (Figure 2.2).

As there were no differences in RMT and AMT between brain hemispheres, the hemisphere values were averaged for subsequent analyses. RMT for the motor group (51.04% ± 12.73 [$F_{(3,37)}$ = 38.60, p < 0.001) was significantly higher compared to that of the sensory group (34.77% ± 4.35) and those who were asymptomatic (39.86% ± 1.99). The RMT of the cognitive group (47.81% ± 6.85) was significantly higher than the sensory group (Figure 2.3).

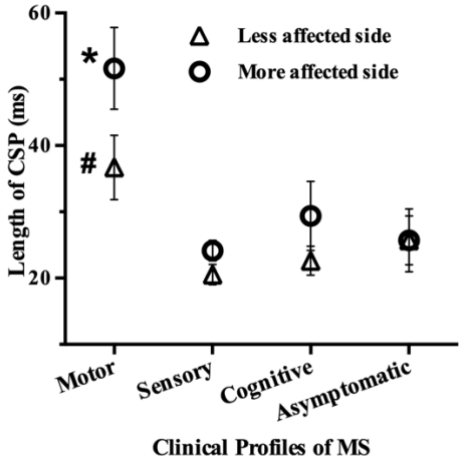

FIGURE 2.2: THE DIFFERENCE IN CSP BETWEEN CLINICAL MS PROFILES
The CSP (cortical silent period) of the hemisphere corresponding to the stronger hand (less affected side) was longer in individuals with motor impairments than those with sensory impairments. The CSP of the hemisphere corresponding to the weaker hand (more affected side) was longer in individuals with motor impairments than those with sensory impairments, cognitive impairments and those who are asymptomatic. # indicates a statistically significant difference from the sensory group ($p<0.01$). * indicates a statistically significant from all clinical groups ($p<0.01$). Error bars represent standard error of the mean.

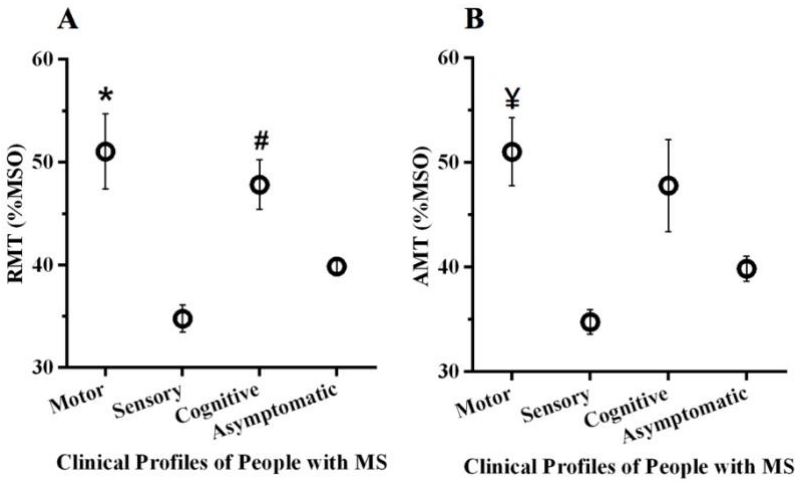

FIGURE 2.3: THE DIFFERENCE IN RMT AND AMT BETWEEN CLINICAL MS PROFILES

(A) The mean RMT (resting motor threshold) was higher in individuals with motor impairments than those with sensory impairments and those who are asymptomatic. The RMT of the cognitive group was significantly higher than the sensory group. (B) The mean AMT (active motor threshold) of the motor group was significantly higher than those in the sensory, cognitive and asymptomatic group. * indicates a statistically significant difference from the sensory and asymptomatic groups. # indicates a statistically significant difference from the sensory group ($p<0.01$). ¥ indicates a statistically significant difference from the sensory group ($p<0.01$)

In terms of AMT values, only the AMT of the motor group (43.95% ± 11.23 [$F_{(3,34)} = 5.79$ $p = 0.003$]) was significantly higher than sensory group (29.64% ± 3.89). However, there were no significant differences between the motor group and the asymptomatic (32.86% ± 3.18) and cognitive groups (40.06% ± 12.48). There were also no significant differences between the three non-motor groups (Figure 2.3).

2.2.4 Relationship between symptom severity and TMS Measures

Since the TMS results suggested that there were more robust changes in the hemisphere corresponding to the weaker hand and previous studies in stroke and MS have confirmed that TMS changes primarily in that hemisphere (Beaulieu & Milot, 2018); we chose to complete simple linear regression on values collected from that side. We found that CSP and AMT most consistently predicted physical and cognitive symptom severity for the motor and cognitive groups but not for the sensory and asymptomatic groups (Table 2.2 and 2.3). For example, in the motor group, CSP predicted 64% of variance in walking velocity and 61% and 91% of variance for the subjective physical and psychological impact scores of MS (MSIS-29) respectively. CSP provided no predictive value of the severity of physical or cognitive symptoms in the sensory or asymptomatic clinical groups. Overall, CSP provided stronger predictive value and was more consistent than AMT.

TABLE 2.2 RELATIONSHIP BETWEEN SYMPTOM SEVERITY MEASURES AND CSP OF THE WEAKER SIDE FOR CLINICAL MS PROFILES

Clinical Indicators of MS	F statistic	p-Value	R_2
Motor Group			
Cognitive Variables			
• MoCA	1.559	0.243	0.053
• SDMT	1.593	0.239	0.150
• MSIS Psychological	13.965	0.005	0.608*
Motor Variables			
• Mean Velocity	15.879	0.003	0.638*
• MSIS Physical	87.372	0.000006	0.907*
Sensory Group			
Cognitive Variables			
• MoCA	0.002	0.965	0.000
• SDMT	1.079	0.329	0.009
• MSIS Psychological	0.675	0.433	0.070
Motor Variables			
• Mean Velocity	5.062	0.051	0.360
• MSIS Physical	0.385	0.550	0.041
Cognitive Group			
Cognitive Variables			
• MoCA	6.499	0.044	0.520*
• SDMT	18.608	0.005	0.716*
• MSIS Psychological	0.841	0.394	0.123
Motor Variables			
• Mean Velocity	17.939	0.008	0.738*
• MSIS Physical	1.384	0.284	0.052
Asymptomatic Group			
Cognitive Variables			
• MoCA	0.393	0.558	0.073
• SDMT	0.034	0.665	0.040
• MSIS Psychological	0.559	0.488	0.101

Motor Variables • Mean Velocity • MSIS Physical	 0.211 0.962	 0.003 0.372	 0.040 0.161

*Statistical significance ($p<0.05$)
CSP- Cortical Silent Period
MoCA- Montreal Cognitive Assessment
SDMT- Symbol Digit Modality Test
MSIS- Multiple Sclerosis Impact Scale

TABLE 2.3: RELATIONSHIPS BETWEEN SYMPTOM SEVERITY MEASURES AND MOTOR THRESHOLDS OF THE WEAKER SIDE FOR CLINICAL MS PROFILES

	RMT			AMT		
Clinical Indicators of MS	**F statistic**	**p-Value**	**R^2**	**F statistic**	**p-Value**	**R^2**
Motor Group						
Cognitive Variables						
• MoCA	6.849	0.026	0.347*	7.006	0.024	0.412*
• SDMT	1.819	0.207	0.154	2.636	0.136	0.209
• MSIS Psychological	2.253	0.164	0.184	5.022	0.049	0.334*
Motor Variables						
• Mean Velocity	2.840	0.123	0.221	9.479	0.012	0.487*
• MSIS Physical	5.579	0.040	0.358*	23.553	0.001	0.702*
Sensory Group						
Cognitive Variables						
• MoCA	0.817	0.392	0.093	0.001	0.971	0.0002
• SDMT	3.138	0.114	0.282	2.486	0.154	0.237
• MSIS Psychological	2.253	0.164	0.184	2.178	0.174	0.195
Motor Variables						
• Mean Velocity	0.058	0.815	0.006	2.137	0.178	0.192
• MSIS Physical	0.640	0.444	0.066	0.506	0.495	0.053
Cognitive Group						
Cognitive Variables						
• MoCA	9.350	0.022	38.721*	38.721	0.001	0.866*
• SDMT	1.590	0.254	4.074	4.074	0.090	0.404
• MSIS Psychological	0.977	0.361	0.689	0.689	0.438	0.103
Motor Variables						
	1.089	0.345	5.969	5.969	0.058	0.544

• Mean Velocity • MSIS Physical	5.628	0.055	11.473	11.473	0.015	0.657*
Asymptomatic Group						
Cognitive Variables						
• MoCA	0.151	0.714	0.171	0.773	0.419	0.134
• SDMT	1.422	0.287	0.221	0.005	0.948	0.001
• MSIS Psychological	0.734	0.431	0.128	0.998	0.364	0.166
Motor Variables						
• Mean Velocity	0.495	0.513	0.090	0.757	0.424	0.131
• MSIS Physical	0.719	0.435	0.126	1.287	0.308	0.205

*Statistical significance ($p<0.05$)
AMT- Active Motor Threshold
RMT- Resting Motor Threshold
MoCA- Montreal Cognitive Assessment
SDMT- Symbol Digit Modality Test
MSIS- Multiple Sclerosis Impact Scale

2.2.5 Comparison of TMS and EDSS to Predict Symptom Severity in MS

To determine whether TMS measures predicted symptom severity compared to the EDSS, all TMS measures and EDSS were included as predictors of physical and cognitive symptoms for the four clinical groups. Since previous simple linear regressions determined that CSP and AMT were able to predict several symptom severity measures only for the motor and cognitive groups but not for the sensory and asymptomatic groups, the clinical groups were combined into two subgroups (motor/cognitive and sensory/asymptomatic) for step-wise linear regression analysis.

A stepwise regression was calculated to predict symptom severity measures based on EDSS and TMS measures. The EDSS was entered into the model first (Dependent Variable=EDSS + Error) to model 2 (Dependent Variable= EDSS + CSP + AMT + RMT + Error). When assessing predictors of walking velocity, EDSS was not a significant predictor. After entering significant TMS predictors as well as EDSS into the model, for the motor and cognitive group, CSP (not RMT, AMT or EDSS) was the only predictor of walking velocity and accounted for 59% of the variance ($F_{(1,16)} = 22.82$, $p < 0.001$) (Figure 2.4). For every 1ms increase in CSP the walking velocity decreased by 0.95cm/s. Figure 2.4 illustrates walking speeds and CSP in two representative EMG outputs of participants with the same EDSS. There were no TMS predictors of walking speed identified for the sensory and asymptomatic combined group (data not shown)

When assessing predictors of the subjective physical impact score, EDSS was not a significant predictor when added to the model by itself. After adding CSP, RMT and AMT to the model, the AMT was the only predictor of subjective physical impact of MS

score, accounting for 64% of its variance ($F_{(1,17)} = 30.63$, $p < 0.001$) in the motor/cognitive combined group (Figure 2.4). The subjective physical impact score of MS increased by 1.31 points for every 1% increase in AMT (maximal stimulator output percentage). In addition, the EDSS model was not predictive of cognitive impairment (MoCA). When TMS measures were added in the second model, AMT was found to be the only predictor of MoCA, accounting for 60% of its variance ($F_{(1,17)} = 30.63$, $p < 0.001$). The MoCA score decreased by 0.24 points for every 1% increase in AMT (maximal stimulator output percentage) (Figure. 2.4 and Table 2.4). There were no TMS predictors of MSIS-29 (physical or psychological) identified for the sensory and asymptomatic group (data not shown).

TABLE 2.4 SUMMARY OF STEPWISE REGRESSION ANALYSES FOR PREDICTORS OF SYMPTOM SEVERITY FOR MOTOR AND COGNITIVE MS GROUPS

Motor and Cognitive Group

Clinical Indicator	**Model Components**	**p**	**R_2**	**β**
Mean Velocity	CSP	0.000206	0.588	-.950
MSIS Physical	AMT	0.000036	0.643	1.306
MoCA	AMT	0.000103	0.598	-0.244
SDMT	-	-	-	-
MSIS Psychological	-	-	-	-

AMT- Active Motor Threshold
CSP- Cortical Silent Period
MoCA- Montreal Cognitive Assessment
SDMT- Symbol Digit Modality Test
MSIS- Multiple Sclerosis Impact Scale

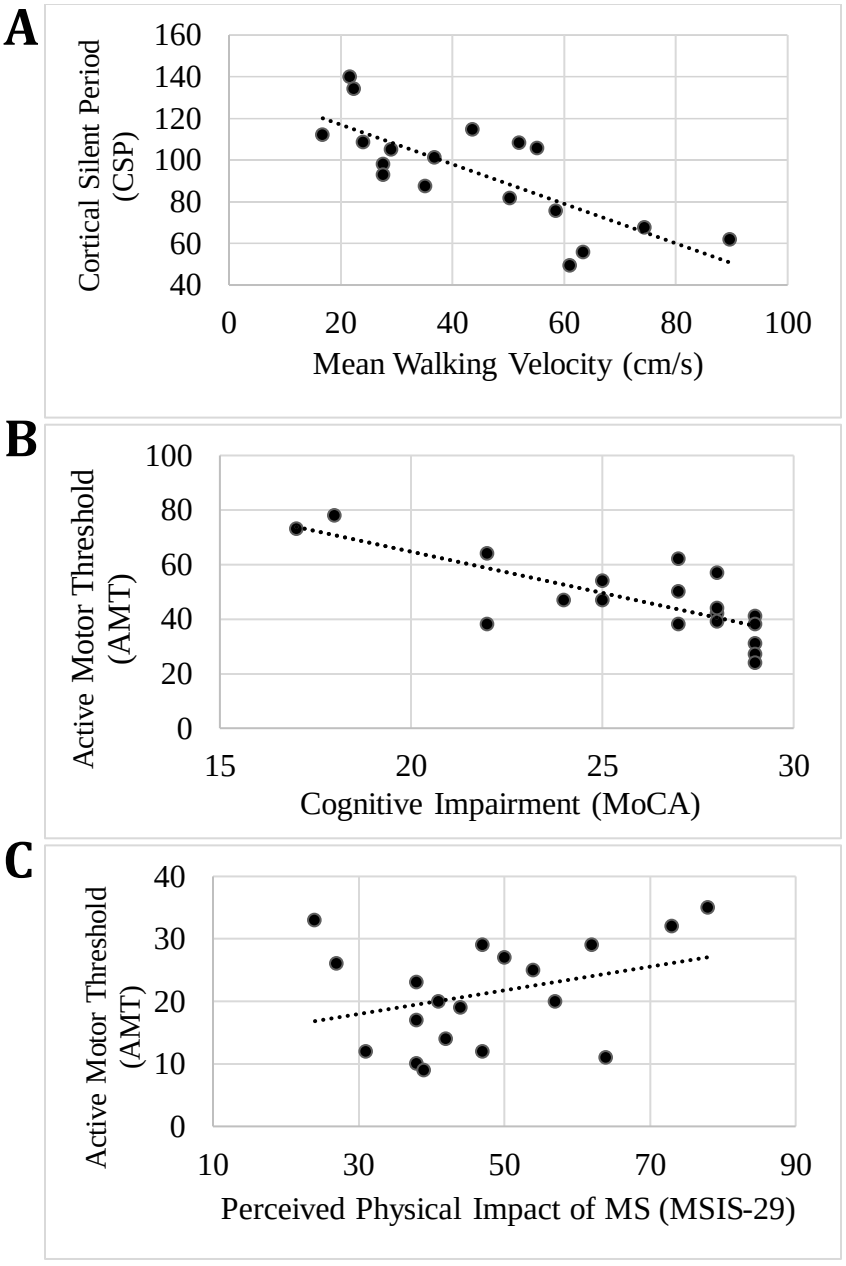

FIGURE 2.4: THE RELATIONSHIP BETWEEN TMS MEASURES AND SYMPTOMS OF DISEASE SEVERITY FOR PEOPLE WITH MS WITH MOTOR AND COGNITIVE PROFILES

A) Cortical silent period (CSP) significantly predicts participant's mean walking velocity ($p<0.001$). B) Active motor threshold (AMT) significantly predicts cognitive impairment (Montreal Cognitive Assessment (MoCA)) ($p<0.001$). C) Active motor threshold (AMT) significantly predicts participant's score of perceived physical impact of MS (MSIS-29) ($p<0.001$).

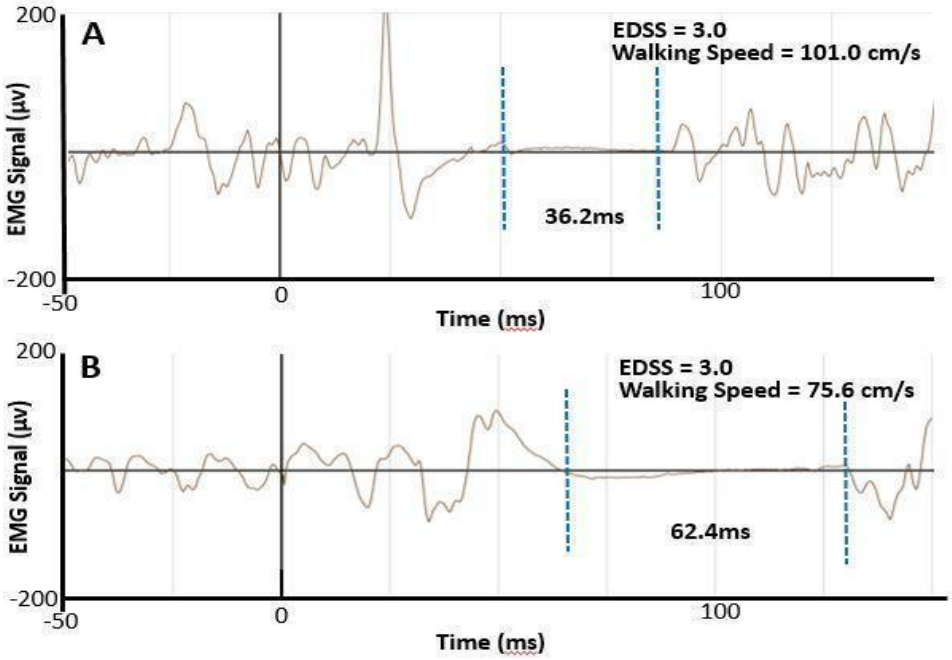

FIG 2.5: REPRESENTATIVE CSP OF TWO PARTICIPANTS WITH THE SAME EDSS SCORE

A) 62 year-old female with primary motor symptoms, a walking speed of 101.0cm/s and a CSP (cortical silent period) of 36.2ms B) 40 year old female with primary cognitive symptoms, a walking speed of 75.6cm/s and a CSP of 63.4ms. EDSS: Expanded Disability Status Scale

Figure 2.5 illustrates how patients in the motor and cognitive profile groups with the same EDSS score had substantially different CSP and AMT values. For example, although scoring the same on the EDSS (Score of 2), one patient had an AMT value of 25% maximum stimulator output and the other with a value of 42% maximum stimulator output.

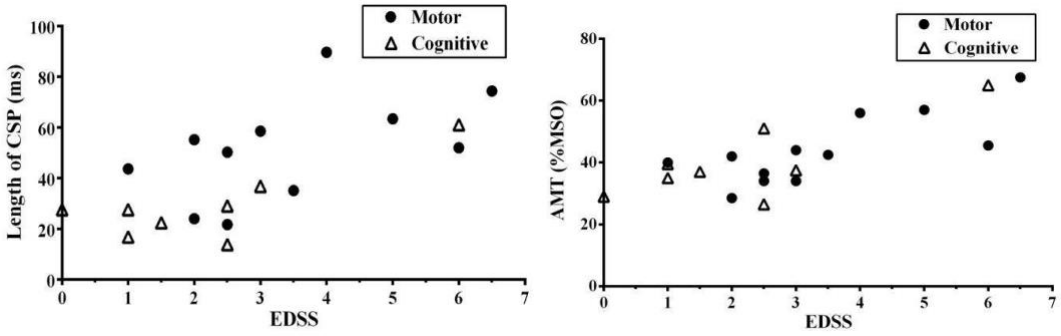

FIG 2.6: CSP AND AMT DETECT CHANGES IN PEOPLE WITH MS WITH THE SAME EDSS

The relationship between the length of the CSP (cortical silent period), the AMT (active motor threshold) and the expanded status disability scale (EDSS) scores for individuals with MS who displayed motor symptoms and cognitive symptoms. %MSO: Percentage of maximum stimulator output.

Chapter 3: Discussion

3.1 OVERVIEW

The research outlined in this thesis examined the use of TMS measures as a biomarker of symptom severity in MS. There were two main objectives of this thesis. The first objective was to investigate whether TMS measures could differentiate four clinical profiles of MS; motor, sensory, cognitive and asymptomatic groups. Among 38 people with MS, we found that the TMS measure, AMT was significantly higher and the TMS measure, CSP was significantly prolonged in the hemisphere corresponding to the weaker hand of the motor group, compared to the other clinical profiles of MS. This finding suggested that TMS may be more useful as a biomarker among people who have primarily motor symptoms. The second objective was to determine whether TMS measures could predict symptom severity measures with better sensitivity than the current disease severity measure, EDSS. All four clinical groups were included in this second analysis for exploratory purposes. TMS measures were found to predict symptom severity measures for both the motor and cognitive groups of MS; thus, these groups were combined for further analysis. In the motor and cognitive group (n=20), CSP was the strongest predictor of walking speed with a prolonged CSP indicating a slower walking speed. CSP is an indicator of the degree of motor cortical inhibition with higher levels of corticospinal inhibition being found to correlate with reduced capacity for neuroplasticity (Jurado-Parras, Delgado-García et al., 2016). Additionally, in the motor and cognitive group, AMT was the strongest predictor of cognitive impairment measured by the MoCA and perceived physical impact of MS measured by the MSIS-29. A higher

AMT predicted a lower score on the MoCA and greater perceived physical impact of MS using the patient-reported MSIS-29. Overall, EDSS was not predictive of symptom severity measures for the motor and cognitive group. Among participants in the sensory and asymptomatic clinical groups, none of the TMS measures or EDSS predicted symptom severity (motor or cognitive performance or subjective health).

In this chapter, two major findings will be highlighted including CSP as a predictor of walking symptom severity (motor and cognitive group) and AMT as a predictor of cognitive symptom severity (motor and cognitive group). Secondly, we will discuss the implications for individual profiles of MS (motor, cognitive, sensory and asymptomatic). Lastly, we will explore how this study could be used to guide the development of future clinical measurement tools to advance the field of MS rehabilitation research.

3.2 TMS AS A BIOMARKER OF SYMPTOM SEVERITY

3.2.1 Walking Ability is Best Predicted by CSP in People with Motor and Cognitive MS Profiles

Walking is an important clinical marker of disease progression in MS. Our findings demonstrated that specific TMS measure, CSP, could be a biomarker of walking ability, and by extension, disability progression. The average mean walking speed of healthy adults up to the age of 59 is approximately 140cm/s (Bohannon & Andrews, 2011) and decreases over time to approximately 95cm/s in individuals over the age of 80 (Pirker & Katzenschlager, 2017). In our study, the walking speeds of individuals with

motor and cognitive symptoms were 92.9cm/s and 98.8cm/s respectively. While these groups were on average 50 years of age, their walking speed was comparable to a healthy individual at least 30 years older (Pirker & Katzenschlager, 2017). Furthermore, we showed that for every 1ms increase in CSP, walking velocity decreased by 0.95cm/s in the motor and cognitive subgroups. The value of CSP as a potential biomarker is not exclusive to this study and has been suggested to be an important predictor of motor function through work in the field of other neurological disorders, such as stroke (Gray, Palmer et al., 2017). For instance, Gray and colleagues (2017) reported a relationship between prolonged CSP and increased motor impairment (Wolf Motor Function Test task completion time) among 13 people with stroke. In a study examining the effects of wrist extensor muscle training on CSP in people with chronic stroke, Sun et al reported that CSP significantly decreased after training (Sun, Ledwell, Boyd & Zehr, 2018). Additionally, among people with MS, shorter CSP correlated with less cortical damage measured using MRI (Nantes, Zhong et al., 2016). Overall, our study results align with those that indicate the importance of CSP as a possible biomarker of motor function.

3.2.2 CSP as a Biomarker of Walking Ability in a Clinical Setting

In MS, problems with walking are one of the most common and burdensome deficits (LaRocca, 2011). MS studies report that difficulties with walking are linked to fall risk (Matsuda, Shumway-Cook et al., 2012) and an increased need for healthcare usage which contributes to economic burden and decreased quality of life (Pike, Jones et al., 2012). In our study, it was interesting to detect that while CSP was a significant

predictor of walking speed for the motor and cognitive group, EDSS was not predictive at all. Visual inspection of gait (as assessed in EDSS) is not as sensitive compared to measurements using sophisticated methods such as kinetics such as that used within an instrumented walkway. For instance, Goodkin and colleagues (1992), found that the EDSS was insensitive to disease progression in the lower ranges, from 1.0-3.5, when walking deficits are likely more subtle (Goodkin, Cookfair et al., 1992). Furthermore, more recent research by Galea and colleagues (2017) showed that EDSS was insensitive to gait and balance changes over a 12-month period in 38 people with MS with an EDSS score ≤ 3 (Galea, Cofré Lizama, Butzkueven & Kilpatrick, 2017). Early identification of deterioration is important in order for clinicians to quickly adapt the treatment regime. Our results support that TMS is useful in detecting small differences in walking quality. TMS out-performed EDSS suggesting that by the time individuals with MS begin to display lower EDSS scores, they likely have accumulated substantial disability. In this study, CSP predicted walking disability in a cross-sectional cohort. It would be important to examine CSP longitudinally in order to determine whether this TMS measure changes over time at an individual level. Earlier detection of walking decline could potentially help develop and monitor a personal plan to prevent risks of falls, injuries, further decline and in turn, improve quality of life.

3.2.3 AMT is Predictive of Cognitive Impairment for People with Motor and Cognitive Profiles of MS

In our study, we discovered that higher AMT thresholds were predictive of lower MoCA scores (a cognitive screening tool) for the motor and cognitive groups, however, AMT was not predictive of the SDMT, another cognitive test of processing speed. This result differs from our previous work by Chaves and colleagues (2019), who found that in a cohort of 82 patients with MS, lower AMT in the hemisphere corresponding to the patient's weaker side (determined by pinch and grip strength) was associated with greater cognitive impairment measured using the SDMT (Chaves, Kelly et al., 2019). The two tools, MoCA and SDMT, measure different constructs and they have different administration and scoring properties. MoCA includes 11 items (eg. visuoconstructional ability, recall) and provides an indication of overall global cognitive ability whereas SDMT has only one task that tracks specifically processing speed. Notably, our cohort scored relatively high on the SDMT, with an average score well above the cut off of 40, suggesting only mild processing speed challenges. The MoCA scores were, on average, at or near in the impaired range (<26). Our sample size was also small (n=20) compared to the cohort examined by Chaves and colleagues (2019) and may not have been large enough. However, the links between AMT and cognitive functioning using two measurement methods in two different studies suggest that TMS does indeed correlate with cognitive screening tests, which is surprising considering that cognition does not typically involve the CST. It is possible that the integrity of the CST is indicative of overall corticospinal tract integrity or that TMS is actually measuring the motor aspects

of the cognitive test (drawing a figure or writing an answer). Future research should use cognitive tests that do not require a motor component in order to decipher whether TMS is associated with impairments that are cognitive rather than motor.

3.2.4 Is AMT a Potential Biomarker of Cognitive Impairment in MS in a Clinical Setting?

Statistics show that approximately 40-65% of people with MS, experience cognitive symptoms to varying extents (Jongen, Ter Horst et al., 2012), yet, these declines are often overlooked and have been labelled the 'forgotten disability' (Rahn, Slusher et al., 2012). Cognitive decline reduces quality of life, related to activities of daily living and negatively impacts social involvement (Baumstarck-Barrau, Simeoni et al., 2011). Although measurement of cognitive impairment in MS has improved with the inclusion of the SDMT in the MS Functional Composite Measure (Brenton et al., 2019), the development of treatments targeting cognitive impairment in MS is only in its preliminary stages (Amato, Zipoli et al., 2006). For instance, Chan and colleagues (2017) published a phase II study reported that high-dose of the medication, simvastatin, was associated with an improvement in the Frontal Assessment Battery (a measure of cognitive executive function) compared to placebo-treated groups (Chan, Binks et al., 2017). Using TMS as a biomarker in a study such as this one would help uncover how exactly such a treatment works to enhance cognition. Since cognitive impairment has been related to thalamic and basal ganglia atrophy, which occurs later in MS (Batista, Zivadinov et al., 2012), TMS may be able to detect changes in these deep structures that

have important contributions to motor outputs. Researchers investigating TMS in Parkinson's disease, proposed that TMS has the ability to detect disruption between thalamocortical connections, resulting in a disinhibition of the motor cortex and thus, indicated by a decrease in motor cortical inhibition (Ridding et al., 1995; Seiss & Praamstra, 2004). The results of the research in this thesis supports that the TMS measure, AMT, could be useful in a clinical setting, not only to track cognitive changes but to test the effectiveness of new pharmaceutical and cognitive rehabilitation treatments in those with motor and cognitive profiles of MS. In summary, the discovery of a tool that tracks cognitive dysfunction in MS is imperative because maintaining cognitive reserve is a way to combat disease progression (Sumowski, Rocca et al., 2013). Future studies should investigate whether AMT accurately tracks treatment-induced changes in cognition which could potentially prompt quicker and more personalized therapy.

3.3 TAKING A STEP BACK: TMS IS LESS USEFUL FOR SENSORY AND ASYMPTOMATIC PROFILES OF MS

Indeed, MS is a very complex neurological disorder, with heterogeneity of lesion location and different patterns of neurodegeneration. Such heterogeneity means that some patients have problems with walking, others with cognition, and some with autonomic problems or any combination of these. It is quite plausible that no single biomarker or test will be able to track neurophysiological underpinnings of MS symptom severity. A suite of biomarkers of disability may be necessary depending on an individual's symptom profile. From our results, it appears that TMS was useful for people with MS who had

primarily motor and cognitive symptom profiles and was not as useful for those with sensory and asymptomatic profiles. It is important to consider that patients in this study were categorized by their "primary" symptom and actually experienced mixed deficits. In future, examining the relationship between TMS measures and scores of sensory impairments may help elucidate a relationship, if one exists.

3.3.1 TMS May Help Identify Cerebellar Impairment

3.3.1.1 TMS Predicts Symptom Severity for People with Cerebellar Symptoms of MS

In our sample of people with motor symptoms (n=12), six people had primarily pyramidal involvement while six experienced a combination of both pyramidal and cerebellar symptoms determined by the neurologist's assessment of the Kurtzke functional system score. We found that the TMS biomarker CSP was sensitive to changes in walking ability regardless of whether motor impairments were due to paralysis (pyramidal) or incoordination (cerebellar). In accordance with our findings, Tataroglu and colleagues (2003) reported significantly prolonged CSP in 12 of 15 people with MS with cerebellar symptoms compared to patients with MS without cerebellar symptoms (Tataroglu, Genc et al., 2003). On the basis of this finding, the researchers suggested that impairments could be attributed to the disruption in the circuitry loop from the cerebellum to the motor cortex, known as the cerebello-thalamo-cerebral loop. Although the participants in the motor group in the study outlined in this thesis had mixed pyramidal and cerebellar findings, distinguishing between the two types of impairment

could help inform and track treatments that are targeted for one or the other. Future research should confirm the usefulness of TMS as a potential recovery (or progression) biomarker for those with cerebellar symptoms. As a first step, it would be important to determine whether TMS measures could detect subtle changes in balance and coordination such as center of pressure sway or finger/foot tapping tests.

3.3.2 Do TMS Parameters Predict Disease Severity Among People with Exclusive Cognitive Profiles of MS?

To recapitulate our findings, for those with MS with primary cognitive symptoms, CSP was the strongest significant predictor of walking velocity and AMT was the strongest significant predictor of cognitive impairment (MoCA) and perceived physical impact of MS (MSIS-29). It is quite probable that in our study, TMS detected widespread cortical dysfunction rather than cognitive impairment per se. We observed that most patients experienced more than one type of symptom concurrently. For instance, in our cognitive profile group, 5 of 8 people displayed varying levels of underlying pyramidal impairment. Other groups have reported that cognitive and physical dysfunction are closely linked. Ruano and colleagues (2017) investigated determinants of cognitive impairment in 1040 patients with MS and discovered a significant association between the increasing degree of cognitive impairment (Brief Repeatable Battery, Stroop Test) and greater physical impairment (EDSS) (Ruano, Portaccio et al., 2017). Interestingly, disease subtype (relapsing-remitting or progressive) and duration did not predict cognition. Although challenging, future research should attempt to recruit people who

have fewer overlapping symptoms in order to better understand the use of TMS as a predictor of symptom severity for those with cognitive impairment.

3.3.2.1 TMS May be Detecting Subtle CST Abnormalities in Cognitive MS Profiles

Although the majority of our subjects had mixed symptoms, three experienced exclusively cognitive impairment. When examining these three subjects in more detail, longer CSP was associated with slower walking speed, similar to the rest of the group. For example, the CSPs of these three individuals were 16.78ms, 29.09ms and 61.08ms, and their walking speeds were 112cm/s, 104.86cm/s, and 49.21cm/s respectively (Figure 3.1). Of interest, the third patient's walking speed was only 30% of typical walking speed (120cm/s). It is possible that the neurologist did not report or detect slow walking speed in this patient. A major limitation of the study was that we were reliant on the neurologist's ratings in order to classify patients into symptom profile groups. Nonetheless, the neurological exam is the cornerstone and main outcome measures in many clinical trials of MS treatments. (Uitdehaag, 2014) The fact that the TMS measure, CSP, was a better predictor overall that the neurologist-reported EDSS suggests that CSP detected subtle abnormalities within the CST that were not detectable when assessed by the neurologist. Similarly, Kale and colleagues (2009) found that in 51 patients with no pyramidal symptoms (noted by the patient's neurologic evaluation), TMS abnormalities were still detected. For instance, 67% of these patients displayed amplitude abnormality (Kale, Agaoglu et al., 2009). Thus, aligning with our results, Kale and colleagues (2009)

suggested that TMS detected possible underlying subclinical involvement within the CST. Future studies should investigate the use of MRI in addition to this TMS protocol, which would allow for the visual detection of potential underlying lesions within the CST and matching brain structure with brain function.

3.3.3 TMS is Not a Suitable Biomarker for Sensory and Asymptomatic MS Profiles

Some people with MS experience pain, numbness and tingling as their primary symptoms while others have no detectable symptoms at the time of testing. We categorized these clinical profiles into sensory and asymptomatic groups, respectively. Our data revealed that CSP and AMT were not useful predictors of the symptom severity (walking speed, MoCA, MSIS-29 (Physical)) measures for those with sensory symptoms and asymptomatic profiles of MS likely because they may have minimal CST damage. Reasonably, TMS is a measure of the integrity of the CST and thus may not predict symptom severity that is due to damage of other networks outside the motor system. The inability for TMS to predict symptom severity within these profiles highlights the need to explore other outcome measures that evaluate areas of the brain involved in sensory and asymptomatic MS such as MRI, functional MRI and diffusion tensor imaging.

3.3.3.1 The Next Generation of Biomarkers: Imaging of Both Brain Structure and Function

As discussed, TMS primarily assesses the function of the CST. Other tools such as MRI, on the other hand, provide a better assessment of CNS structure. In fact, the two

modalities, TMS and MRI, could be considered complementary to one another. Although MRI is considered the gold standard for monitoring MS, studies have provided evidence pointing to its weak correlation with clinical disability (Li, Held et al., 2006). Li and colleagues (2006) gathered a subsample of 1,312 MS patients from 11 randomized control trials to examine the relationship between conventional MRI (proton density/T2 weighted burden of disease) and clinical disability using the EDSS (Li, Held et al., 2006). The results indicated a significant but weak to moderate association with degree of disability; despite this, this study took place in 2006; hence, MRI techniques were not as advanced. Since this time, the implementation of the 7 Tesla MRI now has the ability to uncover grey matter pathology and previously undiscovered cortical lesions (Vigeveno, Wiebenga et al., 2012; Kilsdonk, Jonkman et al., 2016). It is important to mention the increasing use of multi-modal imaging techniques with MRI (using multiple methods at the same time) in MS research. With the use of multimodal imaging, Tewearie and colleagues (2014) were able to identify that thalamic atrophy (measured by MRI), was associated with disruption of cortical functional networks in MS (measured by magnetoencephalography), which was additionally related to worse cognitive and physical disability (Tewarie et al., 2014). Thus, utilization of advanced techniques and multi-modal neuroimaging will allow for the interplay between structure and function in the assessment of disease severity and will aid in the search for personalized biomarkers within symptom profiles of MS.

Diffusion tensor imaging is an advanced MRI-based neuroimaging technique that detects movement of water molecules within the brain thereby providing a measure of the

integrity of the myelin sheaths and cell membranes (Pokryszko-Dragan, Banaszek et al., 2018). This detailed evaluation of tissue microarchitecture with the use of tissue water diffusion rates localizes even the smallest lesions within white matter tracts (Soares, Marques et al., 2013). Recent studies have demonstrated the clinical relevance of diffusion tensor imaging for those with subtle CNS damage in MS. Gratsias and colleagues (2015) studied 84 patients with MS and found that diffusion tensor imaging detected subtle white matter damage in the early stages of MS (average disease duration of 5.6 years) and further determined that greater degeneration in white matter significantly correlated with greater clinical disability (EDSS) (Gratsias, Kapsalaki et al., 2015). Therefore, diffusion tensor imaging may have the ability to detect very subtle and minuscule changes in white matter tracts for those with asymptomatic MS who lack clinical symptoms yet have accumulating lesion load. Diffusion tensor imaging may also be a useful biomarker of disability among people with primarily sensory MS symptoms. Pokryszko-Dragan and colleagues (2018) studied 50 patients with relapsing-remitting MS and reported that diffusion tensor imaging parameters detected areas of damage within the thalamus (Pokryszko-Dragan, Banaszek et al., 2018); an important deep subcortical structure containing major sensory nuclei (Baron, Binder et al., 2010). The search for optimal brain imaging techniques that provide accurate and sensitive measurement of disease progression among those with sensory and asymptomatic profiles of MS is ongoing.

3.4 OTHER TMS PARAMETERS AS POTENTIAL BIOMARKERS IN MS

Outside of the TMS measures discussed in this thesis, there are other TMS parameters that are used in MS research to provide information regarding activity and brain function including axonal, excitatory or inhibitory synaptic excitability in distinct neuronal networks (Tataroglu, Genc et al., 2003; Sahota, Prabhakar et al., 2005; Conte, Lenzi et al., 2009; Nantes, Zhong et al., 2016). All of these TMS measures have one commonality; the ability to identify the integrity of the corticospinal tract. In addition to the AMT and CSP, abnormalities in other TMS parameters such as central motor conduction time, short-interval intracortical inhibition and long-interval intracortical inhibition have been identified as predictors of clinical disability in MS (Tataroglu, Genc et al., 2003; Conte, Lenzi et al., 2009; Vucic, Burke et al., 2012). These additional TMS parameters have been found to correlate with clinical disability (EDSS), and therefore, are being considered as potential biomarkers to monitor disability (Sahota, Prabhakar et al., 2005; Vucic, Burke et al., 2012; Nantes, Zhong et al., 2016).

3.4.1 Central Motor Conduction Time

Central motor conduction time is the length of time it takes for the TMS signal to be transmitted through the motor pathway to the muscle of interest (Rossini, Barker et al., 1994). Studies have reported significantly longer central motor conduction time among people with MS compared to healthy controls (Tataroglu, Genc et al., 2003; Conte, Lenzi et al., 2009). Additionally, Sahota and colleagues (2005) showed that, in 30 patients with MS with acute relapse or progressive disease, central motor conduction time improved in

patients who displayed both pyramidal and cerebellar clinical improvements (Sahota, Prabhakar et al., 2005). While our research only involved investigations of CSP, RMT and AMT, there is research which supports central motor conduction time as a potential biomarker of disability. Whether this measure would be able to track disease progression among those who experience non-motor symptoms (cognitive or sensory) is worthy of further investigation.

3.4.2 Measures of Intracortical Inhibition

Short and long-interval intracortical inhibition parameters are other noteworthy TMS measures that are collected using a TMS technique called paired-pulse at different interstimulus intervals (Zipser et al., 2018). This technique involves priming the brain with a subthreshold TMS pulse that can either suppress or potentiate the subsequent stimulus depending on the interstimulus interval chosen. It is known that short intracortical inhibition takes place at 20ms intervals and long intracortical inhibition at 200ms intervals (Ni & Chen, 2015). In particular, short-interval inhibition has been found to represent a period of motor cortex inhibition mediated by intracortical interneural circuits of inhibition by GABA receptors and has been reported as reduced or non-existent in neurological disorders such as Alzheimer's (Di Lazzaro, Oliviero et al., 2004) and MS (Vucic, Burke et al., 2012). These measures may be relevant to MS research because CNS degeneration is partially attributed to excitotoxicity, caused by overactivity of the CNS neurotransmitter glutamate and insufficient inhibitory inputs to counterbalance inhibitory signals from GABA, an inhibitory neurotransmitter (Lazo-

Gomez, Velázquez et al., 2019). Both short and long-interval intracortical inhibition parameters provide insight into this inhibitory transmission within the CNS, however, we will direct our attention to short-interval intracortical inhibition; a measure that is showing a growing body of evidence demonstrating its efficacy as a biomarker of disability in MS.

3.4.2.1 Short-Interval Intracortical Inhibition

In MS research, short-interval intracortical inhibition has been identified as an important measure that correlates with disability (Vucic, Burke et al., 2012; Nantes, Zhong et al., 2016). Recent research demonstrated that short-interval intracortical inhibition was significantly decreased in patients with secondary progressive MS (n=15) as compared to those with relapsing-remitting MS (n=25) and healthy controls (n=66). Also, decreased short-interval intracortical inhibition correlated with greater disability (measured by the EDSS) for those with secondary progressive MS (Vucic, Burke et al., 2012). In another study, Nantes and colleagues (2016) assessed short-interval intracortical inhibition in 36 patients with MS and detected abnormally low short-interval intracortical inhibition in people with relapsing-remitting MS (n=22) and progressive MS (n=14) as compared to healthy controls (n=18) (Nantes, Zhong et al., 2016). Nantes and colleagues (2016) proposed that short-interval intracortical inhibition detects cortical damage, as seen in those with secondary progressive MS and may also be sensitive to white matter tract damage as seen in relapsing-remitting MS. Accordingly, these studies illustrate that short-interval intracortical inhibition detects degeneration within the brain,

linked to clinical disability. It is important to remember that the measure used in these studies, EDSS, is a subjective categorical rating scale that is notoriously insensitive to subtle symptom worsening (Hohol, Orav et al., 1995). Whether intracortical inhibition could predict measures of symptom severity such as walking speed, cognitive changes or sensory symptoms is not known. In addition to the classically measured CSP and AMT, future studies should include other TMS parameters such as central motor conduction time and short-interval intracortical inhibition to ensure potentially meaningful variables for monitoring MS disability.

3.5 FUTURE DIRECTIONS AND IMPLICATIONS

3.5.1 TMS: A Future Component of the 'No Evidence of Disease Activity' Paradigm?

The term 'No Evidence of Disease Activity' or NEDA has been recently coined in order to set a new standard for MS disease-modifying therapies (Giovannoni, Turner et al., 2015). In order to confirm that a treatment provides complete protection against disease progression, three NEDA criteria have to be met: (1) there are no relapses, (2) there is no disability progression and (3) there is no MRI activity indicating inflammation and lesions (Havrdova, Galetta et al., 2009, Giovannoni, Cook et al., 2011). Recent consensus supports adding a fourth criteria; no evidence of brain atrophy (Guevara, Garrido et al., 2019). Researchers noted that although this paradigm provides optimal treatment monitoring outcomes, NEDA will continue to be modified in order to evolve with advancements in technology (Giovannoni, Turner et al., 2015). High resolution MRI

provides definitive evidence of whether a patient is experiencing new lesions or atrophy (structural change), but it is limited by its inability to identify changes in brain function. The "functional" measure is currently relying on the clinical exam (EDSS change); however, our findings illustrate that TMS measures CSP and AMT can predict disease severity measures of MS (walking speed, perceived physical impact of MS and cognitive impairment) for those with cognitive and motor symptoms, with more precision than the standard EDSS. TMS provides a window on how lesions and atrophy are affecting brain network integrity; important information that can be used as metrics in order to test the effectiveness of medication and treatment therapies in MS. Having said this, our results support that the TMS measures that we investigated did not have the capability to predict disease severity for sensory and asymptomatic profiles of MS. Future research should build upon existing and emerging TMS measures which could be incorporated into NEDA criteria. Overall, as a non-invasive method to measure corticospinal tract integrity and brain function, TMS has the potential to contribute missing functional information and strengthen the NEDA criteria.

3.5.2 The Use of TMS in a Clinical Setting

3.5.2.1 TMS Addressing Personalized Medicine

The foundation of personalized medicine in MS is to fully understand one's current level of disability. Evidence suggests that treatments geared towards specific impairments work better than generalized programs (Rannisto, Rosti-Otajärvi et al., 2015). Nevertheless, personal medicine in MS is made challenging due to the vast heterogeneity

and unpredictability of the disease because of various combinations of lesion location and burden within the CNS (Gafson, Craner et al., 2017). In addition, different levels of brain reserve and the ability for compensation between people make lesions of the same area display different effects on function between individuals. As previously discussed, limitations of EDSS have been brought to light concerning sensitivity, heterogeneity, and inter-rater reliability. Thus, a new biomarker for disability in MS is crucial in order to establish the foundation for personalized medicine; where the frontier of research lies.

It is important to note that while we display preliminary evidence of the benefits of TMS sensitivity to symptom severity measures as compared to EDSS, clinical translation from EDSS to TMS comes with many challenges. EDSS has many benefits, being that it is easy to administer, cheap and very time efficient. On the other hand, TMS requires expensive equipment, a TMS technician, and interpretation. Future research would be necessary to validate the clinical value of TMS as a biomarker of disability over time, as compared to the EDSS. While these challenges are evident, the emphasis of personalized medicine in the field of MS is a necessity. If TMS is proven to be a sensitive biomarker of disability, allowing assessment of the effectiveness of new medication and rehabilitation treatment strategies, then the weight of personalized medicine achieved from TMS may outweigh the challenges that come with it.

3.6 LIMITATIONS

There are some limitations to this study that could influence the present results. Although our study is novel in terms of separating people with MS into main categories of

MS symptoms from the neurologist reports, these classifications were not always concrete. An evaluation of each participant's neurological report was completed; however, it can be challenging to determine a singular defining clinical profile due to the heterogeneity of MS. Thus, classifying people with MS by their main clinical deficit had a level of subjectivity. Another limitation was that participants drug-modifying therapy was not collected or analyzed in this research. Some drug modifying therapies impact glutamate levels in the brain such as Fingolimod, which can protect neurons from glutamatergic excitotoxic damage (Landi et al., 2015). In this case, these drug-modifying therapies may have an effect on TMS measures and should be taken into consideration in future TMS research. In our study, the participant's weaker side was determined based on the hand with the lowest combined grip and pinch strength measures (grip + pinch). It would be preferable for prospective research to incorporate participants MRI scan in order to detect patients weaker/stronger side providing more accuracy to the side with more inflammation and degeneration.

There were some notable limitations involving our interpretation of TMS parameters. We aimed to compare our TMS measures to other studies, yet, there are several different protocols for the collection of these measures that limited this comparison. For example, our participants used 10% maximum contraction during AMT collection while other studies have used 50% maximum contraction (Haug & Kukowski, 1994; Tataroglu, Genc et al., 2003). Moreover, even differences in the type of coil used can produce different TMS results due to different cortical elements being activated (Taylor, Allen et al., 1997). For instance, it has been shown that the CSP is shorter with the figure-of-eight coil as

opposed to the round coil due to different cortical elements being activated, making it difficult to compare our results to clinical studies using a round coil (Oozumi, Ito et al., 1992). Additionally, the lack of healthy controls in this study limited our interpretation of the results. In other words, although we know that the motor group displayed prolonged CSP as compared to other clinical MS profiles, we are unsure if the CSP would have been longer or shorter than CSP of healthy controls.

Lastly, it is important to acknowledge the sample size of this clinical population. When performing linear and stepwise regressions, there was a range from 7 participants to 5 predictors (tables 2.2/2.3: asymptomatic group), to 20 participants and 4 predictors (motor-cognitive group in step-wise analysis). Thus, these regressions results do pose a serious risk of type 1 errors. Nonetheless, the data reported in this study could be a useful foundation for creating a larger scale study. To summarize, given the various TMS procedures, the implication of a standardized TMS protocol for MS research should be warranted to increase reproducibility. Additionally, studies should incorporate healthy controls for optimal comparison between studies to allow for greater opportunity to elucidate the capability of TMS as a biomarker of disability in MS.

3.7 CONCLUSION

To our knowledge, we are the first research group to take into consideration the heterogeneity of MS by separating participants into the four main categories of motor, cognitive, sensory and asymptomatic profiles when using TMS. First, we investigated the ability of TMS to distinguish people with MS by main clinical profiles of MS including

motor, cognitive, sensory and asymptomatic profiles. Secondly, we determined if TMS measures predicted clinical measures of disease severity, beyond the EDSS. We found that two TMS measures CSP and AMT were useful for people with MS with motor and cognitive symptoms. Additionally, in the motor and cognitive group, CSP was the best predictor of walking speed, while AMT was the best predictor cognitive impairment and perceived physical impact of MS. Further, we suggest that that TMS may be predictive for symptom severity measures in those with cognitive symptoms of MS due to possible detection of subtle abnormalities within the CST.

Findings from this study covered the first steps necessary to evaluate the competence of TMS as a sensitive biomarker of disability in comparison to the EDSS, which has its recognized weaknesses. The next necessary step involves the development of a protocol to test the validity and reliability of TMS within these clinical profiles of MS. With further investigation that addresses our limitations, TMS may be found to be a valuable biomarker that can be used to track sensitive changes in disease progression and improvement in MS for people with motor and cognitive symptoms.

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