DISSERTATION

ASSOCIATIONS BETWEEN NEUROANATOMY AND NEUROPHYSIOLOGY WITH TURNING PERFORMANCE IN PEOPLE WITH MULTIPLE SCLEROSIS

Submitted by

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ABSTRACT

ASSOCIATIONS BETWEEN NEUROANATOMY AND NEUROPHYSIOLOGY WITH TURNING PERFORMANCE IN PEOPLE WITH MULTIPLE SCLEROSIS

Neurodegenerative diseases such as multiple sclerosis (MS) are associated with decreased mobility and a variety of changes affecting neural structure and function. Due to the cortical influence on various aspects of mobility, it is likely that these neural adaptations negatively affect mobility, and therefore, increase the potential for falls. Additionally, the progression of MS has been associated with cortical grey matter atrophy, and adaptions to neurophysiological activity. While previous research has demonstrated associations between levels of inhibition and a variety of turning characteristics in neurotypical young and older adults, it remains unclear if associations exist between cortical structure and function for dynamic lower limb control for daily tasks such as turning. Therefore, purpose of this project was to understand how sensorimotor cortical thickness and corticospinal excitation and inhibition contribute to turning performance in both people with MS (PwMS) and age-matched neurotypical control (HC) participants.

Participants were asked to conduct a series of 360˚ in-place turns at two self-selected speeds and 180˚ turns during a self-selected pace two-minute walk test. Quantification of turning was assessed using wireless inertial sensors placed on each foot, around the waist, on the sternum, and on the forehead. Grey matter (GM) thickness of the sensorimotor cortex (i.e., pre-, para-, and postcentral gyri) was

measured via magnetic resonance imaging (MRI) and processed using FreeSurfer 6.0.0 (http://surfer.nmr.mgh.harvard.edu/, Harvard University, Boston, MA, USA). To measure corticospinal excitation and inhibition single-pulse transcranial magnetic stimulation (TMS) was performed. The leg region of both motor cortices was identified by acquiring the resting motor threshold (RMT) of the tibialis anterior. To assess neurophysiology, participants sustained an isometric contraction in dorsiflexion at 15% of their maximal voluntary contraction for three-minutes. Simultaneously, a TMS stimulation was delivered at 120% of RMT every 7-10 seconds. This procedure was conducted for both cortical hemispheres.

A total of forty-nine individuals (23 HC, 26 MS) participated in the study. PwMS demonstrated reduced turning performance for a variety of 360˚ turning variables, although only one variable was significant between groups for the 180˚ turns. GM thickness revealed significant cortical thinning of the pre- and paracentral gyri in the MS group, while the postcentral gyrus did not demonstrate between group differences. For TMS measures, PwMS demonstrated reductions in excitation and inhibitory capacity compared to neurotypical controls. All significant correlations were primarily observed in the MS group and demonstrated lateralization, such that they were limited to the left hemisphere. The current results showed that both cortical thickness and inhibitory activity were associated with turning performance in PwMS, but not in the HC group.

The associations between inhibitory activity and turning performance were stronger than the associations between cortical thickness and turning performance. These results may indicate that inhibitory activity is more associated with dynamic lower limb movements compared to GM thickness. Furthermore, these results suggest that

PwMS may rely on different neural resources to perform dynamic movements typically associated with fall risk.

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CHAPTER 1 – INTRODUCTION AND EXPERIMENTAL AIMS

INTRODUCTION

Multiple sclerosis is a chronic, immune-mediated, demyelinating neurological disease of the central nervous system (CNS). Among young adults, multiple sclerosis (MS) is the major cause of neurological impairment leading to irreversible long-term disability throughout the disease course (Brownlee, Hardy et al. 2017). Individuals diagnosed with MS present with a heterogeneous amalgamation of symptoms which often include changes in sensation, balance, bladder control, cognition, mobility, and vision (Brownlee, Hardy et al. 2017). While symptoms vary among individuals with the disease, there are also several MS phenotypes determined by initial symptomology and severity. Clinical phenotypes either include relapsing-remitting or primary progressive form of the disease (Lublin, Reingold et al. 2014). Relapsing-remitting (RRMS) is the most common form of the disease affecting 85-90% of people with MS (PwMS) (Brownlee, Hardy et al. 2017). This phenotype is characterized by relapses, which are episodic symptomatic flair-ups lasting longer than 24 hours not associated with secondary infection or fever (Lublin, Reingold et al. 2014). Relapses are followed by periods of remission which are variable and often characterized by some degree of symptomatic relief lasting an indeterminate amount of time (Lublin, Reingold et al. 2014). RRMS is a female-dominated disease, affecting women three times as often as men, and typically affects individuals in their second or third decade of life (Kalincik, Vivek et al. 2013). Roughly 36% of patients initially diagnosed with RRMS are subsequently diagnosed with secondary progressive MS (SPMS), which is characterized by gradual disability between relapses, although

sometimes occurring in the absence of a definite relapse entirely (Brownlee, Hardy et al. 2017, Coret, Pérez-Miralles et al. 2018). While the conversion to SPMS is likely extended due to current disease modifying drugs, the age at initial diagnosis and subsequent accumulation of disability appears to be independent of current therapies (Coret, Pérez-Miralles et al. 2018). Lastly, primary progressive MS (PPMS) has a diagnostic rate of 10 – 15% and is characterized by subtle yet progressive neurological impairment and subsequent disability without episodes of relapse or remission, and further exhibits no sex bias (Brownlee, Hardy et al. 2017).

Due to the heterogeneity of symptoms, clinical diagnosis is often difficult without a specialized neurologist. Conventionally, the diagnosis was solely based on clinical observation; however, diagnostic criteria in combination with advancements in technology have augmented early detection and diagnostic aptitude (Brownlee, Hardy et al. 2017). As such, accurate diagnosis is now achieved through the combination of symptom type, onset, and lesion location determined via magnetic resonance imaging (MRI). Specifically, for a diagnosis of RRMS, lesions must be located in two of four sites – the periventricular, juxtacortical, infratentorial, and/or the spinal cord (Polman, Reingold et al. 2011). This is in conjunction with having clinical presentations including but not limited to, acute optic neuritis, trigeminal neuralgia, cerebellar ataxia, sensory symptoms of the CNS, or asymmetric limb weakness (Polman, Reingold et al. 2011). Together these clinical symptoms and localized lesions provide moderately definite diagnostic criteria which have vastly improved early detection and disease modifying interventions.

PREVALENCE OF MULTIPLE SCLEROSIS

Recent diagnostic improvements have led to a greater capacity for quantifying global occurrences of MS. In the 1970's it was projected that MS affected roughly 58 per 100,000 individuals in the United States (Baum and Rothschild 1981). Over time this rate has been drastically revised suggesting 300,000 to 400,000 individuals in the United States live with the disease; however, these numbers are largely based on relatively old data (Evans, Beland et al. 2013, Feigin, Abajobir et al. 2017). In an attempt to capture more specific and reliable rates, a recently published hallmark study conducted the most compressive estimation analysis to date (Wallin, Culpepper et al. 2019). This study assessed a total of 125 million medical records from a variety of public and private insurance sources (i.e., government and private insurance agencies) (Wallin, Culpepper et al. 2019). As of 2019, it was estimated that 362.6 per 100,000 people were diagnosed with MS, totaling roughly 913,925 individuals in the United States live with MS (Wallin, Culpepper et al. 2019). These improved rates make MS comparable to other neurological diseases such as Parkinson's disease (PD), which was estimated to affect roughly 930,000 individuals in the United States in 2018 (Marras, Beck et al. 2018). Interestingly, while the prevalence numbers are similar between the two neurological diseases, the National Institutes of Health (NIH) allotted $109 million for MS and $218 million for PD government sponsored research funding for the 2021 fiscal year (https://report.nih.gov/categorical_spending.aspx).

ETIOLOGY OF MULTIPLE SCLEROSIS

ENVIRONMENTAL

While diagnostic capacity has improved and prevalence has been amended, the etiology of MS remains largely unknown. Science suggests a number of factors influence

the likelihood of eventual MS diagnosis such as environmental, genetic, and pathogenic influences (Noseworthy, Lucchinetti et al. 2000, Ascherio and Munger 2007, Alonso and Hernán 2008, Consortium 2013). It has been well documented that the occurrence of MS follows a global latitudinal pattern, where individuals residing further from the equator in both the northern and southern hemispheres exhibit a higher incidence of MS (Alonso and Hernán 2008). Specifically, it has been reported that for increasing increments of 10 degrees in latitude the incidence of MS rises by 30% in women and 50% in men (Alonso and Hernán 2008). Some speculate that the increased incidence at higher latitudes may be associated with factors including vitamin D, sun exposure, and the rates of various infections (Ascherio and Munger 2007, Ascherio and Munger 2007). A number of studies have reported an inverse association between levels of vitamin D and the risk of developing MS (Ascherio and Munger 2007). Vitamin D is synthesized through sun exposure, and as individuals decrease their exposure either due to their location (i.e., living at higher latitudes with longer dark periods of the year) or lifestyle adverse impacts may increase their risk of eventual MS diagnosis (Munger, Levin et al. 2006, Ascherio and Munger 2007). These findings likely suggest that environmental factors participate in the etiology of MS. While MS is not solely observed at greater latitudes this suggests that genetic and pathogenic determinates are also important for uncovering the etiology of the disease. For instance, some evidence supports the notion that MS may be triggered by various pathogens to individuals with a genetic predisposition (Noseworthy, Lucchinetti et al. 2000, Kurtzke 2005, Marrodan, Alessandro et al. 2019).

INFECTIOUS FACTORS AND GENETIC SUSCEPTIBILITY

It is generally accepted that autoimmune diseases, like MS, result from complex interactions between genetic susceptibility and infectious factors naturally occurring in the environment (Marrodan, Alessandro et al. 2019). Specific to MS, several studies have implicated the presence of infectious diseases during childhood and young adulthood as strong determinants of eventual diagnosis (Correale and Gaitán 2015). This association is thought to occur due to a pathogen or microbial infection inducing an inappropriate autoimmune response. Such that the autoimmune response becomes detrimental likely due to an underlying condition or genetic predisposition.

Researchers have described a number of potential viral and bacterial infectious triggers in MS (Marrodan, Alessandro et al. 2019). While a number of viral triggers have been postulated, the Epstein-Barr virus has been identified as having the strongest link to MS (Correale and Gaitán 2015). The Epstein-Barr virus has an infectious rate of nearly 90% in people during their first decade of life, however, those who contract the infection later in life are known to have a greater risk of developing MS (Thacker, Mirzaei et al. 2006). Conversely, studies have shown that those individuals who are not infected by the Epstein-Barr virus have a lower risk of developing MS (Pakpoor, Disanto et al. 2013). The human herpesvirus 6 is another pathogen that has been implicated as a risk factor for MS, but has the virus is often dormant for extended periods of time it is likely more associated with MS related exacerbations or relapses (Marrodan, Alessandro et al. 2019). Lastly, the group of human endogenous retroviruses have also been considered for their role in MS (Leibovitch, Lin et al. 2019). These viruses are part of human DNA and make us roughly 8% of the human genome (Marrodan, Alessandro et al. 2019). Under normal physiological conditions, they are often inactive due to deactivating mutations, however,

they may become activated due to certain pathological conditions and subsequently produce viral transcripts and proteins (Marrodan, Alessandro et al. 2019). One such protein Syncytin-1 has increased expression in MS and has been associated with actively demyelinating lesions (van Horssen, van der Pol et al. 2016).

In addition to viral triggers, bacterial triggers have also been implicated in the etiology of MS. The *Helicobacter pylori* bacteria was shown to be less abundant in various cohorts of PwMS compared to neurotypical controls (Marrodan, Alessandro et al. 2019), suggesting that *Helicobacter pylori* may serve a protective role in MS (Cook, Crooks et al. 2015). Another bacteria *Chlamydia pneumoniae* is a common gram-negative bacterium that often affects the respiratory tract in humans. Roughly 40% - 70% of adults have antibodies against this organism due to adolescent inoculation (Marrodan, Alessandro et al. 2019). While the current role of this bacterium in MS is inconclusive, studies show that PwMS have greater proportions of positive *Chlamydia pneumonia* cultures compared to neurotypical controls (Marrodan, Alessandro et al. 2019). While the evidence of a causal role is lacking, it has been postulated that *Chlamydia pneumonia* could be associated with an inflammatory response (Marrodan, Alessandro et al. 2019). Together these results suggest that the etiology of MS is multifactorial, stemming from various environmental influences that affect individuals who are genetically predisposed to the disease.

A recent study conducted by the International Multiple Sclerosis Genetics Consortium evaluated five potential loci in a large data set of 20,000 individuals (Consortium 2013). The five single nucleotide polymorphisms assessed included: rs228614 (MANBA), rs630923 (CXCR5), rs2744148 (SOX8), rs180515 (RPS6KB1), and

rs6062314 (ZBTB46) for their associations with MS risk (Consortium 2013). The study concluded that each of these five loci were consistently and significantly associated with MS and subsequently surpassed the threshold for genome-wide corrected significance achieving a p-value less than 5×10^{-8} (Consortium 2013). While these loci are associated with MS, the biochemical and pathophysiological mechanisms underlying these associations for genetic susceptibility are still under investigation.

DISEASE PROGRESSION AND SEVERITY ASSESSMENTS

While etiology remains fleeting, MS is a chronic and progressive disease affecting the CNS. MS results in a gradual decline of neurologic function, although without a comprehensive understanding as to the underpinnings of decline, a number of instruments have been developed to track functional impairments and describe clinical severity. The most common instrument used to describe disease severity is the Expanded Disability Status Scale (EDSS) (Kurtzke 1983). The EDSS is a graded clinician administered evaluation, meant to assess various functional systems of the CNS. As such, the EDSS is used to not only track disease progression, but also the effectiveness of therapeutic interventions, and clinical trials. The EDSS consists of an ordinal rating scale ranging from zero (normal neurological exam) to 10 (death due to MS). The scale increases in increments of 0.5, scores less than 3.5 are solely based on neurological examination (i.e., bladder control, visual impairment, cognition, etc.) while scores greater than 4.0 begin to measure the degree to which individuals are physically handicapped. Scores ranging from 4.0 - 6.0 are largely based on ambulatory ability, for scores greater than 6 individuals are typically unable to ambulate independently and require an assistive device such as a cane, walker, or wheelchair. Another widely administered instrument is

the Multiple Sclerosis Functional Composite (MSFC) which was also developed to assess disease progression (Rudick, Antel et al. 1997, Cutter, Baier et al. 1999). The MSFC is a multidimensional assessment tool combining a three-part scale for evaluating the degree of motor and cognitive impairment. The assessment includes the evaluation of upper and lower limb motor function and concentration/attention cognitive function. Instead of an ordinal scoring scale, scores are tabulated as z-scores meant to determine a particular range of dysfunction.

While those are the two primary instruments for tracking disease progression, a number of other instruments have been developed to target more specific components of impairment. Since ambulatory ability is heavily affected as a result of MS, and a component of disease progression, additional instruments have been developed to measure the progression of gait dysfunction. Two common measures include The Ambulation Index and the 12-Item MS Walking Scale. The Ambulation Index records the time and degree of assistance needed to walk 25 feet on a scale of zero (fully functional, asymptomatic) to ten (bedridden) (Hauser, Dawson et al. 1983). The 12-Item MS Walking Scale measures how MS has impacted an individual's walking ability by asking and twelve circumstantial based questions during the prior two weeks on a scale of 1 (not at all) to 5 (extremely) scale (Hobart, Riazi et al. 2003).

NEUROANATOMICAL AND NEUROPHYSIOLOGICAL IMPAIRMENTS OF THE CENTRAL NERVOUS SYSTEM IN MULTIPLE SCLEROSIS

NEUROANATOMICAL IMPAIRMENTS

Within the CNS, MS is characterized by the presence of temporally and spatially distributed multifocal inflammatory demyelinated plaques (McDonald, Compston et al.

2001). The pathological features of the disease include the breakdown of the blood-brain barrier, multifocal inflammation, demyelination, oligodendrocyte loss, reactive gliosis, axonal damage, and varying degrees of grey matter atrophy (Dutta and Trapp 2011). This damage can lead to irreparable consequences which range from transient dysfunction to irreversible impairment, and notably, often observed early in the disease course (Dutta and Trapp 2011). Many of these pathological features are sensitive to MRI which has been used diagnostically and longitudinally to track disease progression and lesion development. While a number of MRI analysis techniques (i.e., functional MRI (fMRI) and diffusion tensor imaging (DTI)) have been implemented to study MS, structural MRI analysis is befitting for documenting both global and regional neuronal tissue adaptations, such as atrophy (e.g., grey matter and white matter) and lesion characteristics.

Progressive brain atrophy is considered irreversible brain damage and a well-known feature of MS, affecting both grey and white matter (Miller, Barkhof et al. 2002). While MS is often characterized as a white matter disease, grey matter atrophy typically precedes white matter atrophy and can be observed in the earliest stages of the disease (Chard, Griffin et al. 2002, Miller, Barkhof et al. 2002, Chard, Griffin et al. 2004, Dalton, Chard et al. 2004, Chard and Miller 2009). In particular, a study conducted by Calabrese, et al. (2007) discovered that people prior to their MS diagnosis (the following year) demonstrated reduced cortical thickness (i.e., grey matter atrophy) (Calabrese, Atzori et al. 2007). While grey matter atrophy has been detected in the earliest stages of the disease, lesion detection remains important for clinical diagnosis. In individuals with RRMS and a disease duration of roughly 3 years patients presented with grey and white matter atrophy, with grey matter atrophy sometimes demonstrating an association with

lesion load (Chard, Griffin et al. 2002). However, a follow-up investigation of the same cohort two years later discovered further grey matter atrophy, no additional white matter atrophy, and the association between grey matter atrophy and lesion load was no longer significant (Tiberio, Chard et al. 2005). Moreover, it has been shown that grey matter atrophy is more strongly associated with motor and cognitive dysfunction than lesion load (De Stefano, Matthews et al. 2003, Sailer, Fischl et al. 2003, Dalton, Chard et al. 2004).

GENERAL NEUROANATOMICAL REGIONS OF SUSCEPTIBILITY

Likely a product of analysis capabilities, early investigations primarily assessed global atrophy, demonstrating significant differences between PwMS and neurotypical controls. Since those investigations more advanced analyses have been incorporated to uncover regional predilections as a result of having MS. Regional susceptibilities of atrophy have been documented in MS, such that rates of grey matter atrophy are not uniform across the brain (Eshaghi, Marinescu et al. 2018). Particular areas of the cortex appear to be more vulnerable to atrophy as a result of the disease, for instance, cortical regions such as the frontal lobes (superior and medial frontal gyrus and sulcus), frontobasal (frontal poles), cingulate cortex, temporal gyri and sulci, and limbic system all demonstrated significant atrophy in both hemispheres (Sailer, Fischl et al. 2003, Eshaghi, Marinescu et al. 2018). Although patterns of atrophy have also been observed, suggesting non-uniformity and hemispheric susceptibility (Lansley, Mataix-Cols et al. 2013, Steenwijk, Geurts et al. 2016). Subcortical regions are also highly susceptible to atrophy, often showing early indications of atrophy in structures such as the thalamus, brainstem, and basal ganglia (Eshaghi, Prados et al. 2018). Though the disease as a

whole seems to target particular regions of the brain, various regional atrophy patterns have been associated with disease phenotype, severity, and duration.

DISEASE PHENOTYPES AND GREY MATTER ATROPHY

As described, there are a number of specific MS phenotypes including clinically isolated syndrome (CIS), relapsing remitting (RRMS), secondary progressive (SPMS), and primary progressive (PPMS). Among each phenotype, similar regions experience grey matter atrophy albeit to different extents. These results are probably best detailed from the MAGNISM multi-center study, which analyzed 1,424 MS participants of varying phenotypes along with the inclusion of neurotypical controls (Eshaghi, Prados et al. 2018). In this study, the deep grey matter regions (e.g., thalamus, putamen, globus pallidus, caudate, and amygdala) as a whole, patients with CIS demonstrated the least amount of atrophy with increasing amounts observed in PPMS, RRMS and, finally SPMS presenting with the greatest level of atrophy (Eshaghi, Marinescu et al. 2018). Interestingly, this phenotypic atrophic trend was seen in a variety of other regions including the whole brain, temporal lobe, cerebellum, parietal lobe, frontal lobe, and the brain stem (Eshaghi, Marinescu et al. 2018). These results demonstrate that varying degrees of grey matter atrophy affects nearly all regions of the brain. However, when assessing the longitudinal effects of the disease in specific structures and phenotypes, particular structures demonstrate larger annual rates of atrophy. For reference, in a neurotypical population the structures which demonstrate the greatest annual loss (≤ 1% annually) of grey matter include the putamen, caudate, and parahippocampal gyrus (Eshaghi, Prados et al. 2018). However, the annual loss of grey matter for the remaining structures is typically less than 0.5% annually (Eshaghi, Prados et al. 2018). In CIS annual

atrophy for the structure's demonstrating the greatest loss remains between 1-2% (Eshaghi, Prados et al. 2018). Those structures demonstrating the greatest loss include the caudate, pallidum, putamen, and the temporal poles (Eshaghi, Prados et al. 2018). The remaining structures all demonstrate a loss of around 0.5% annually (Eshaghi, Prados et al. 2018). In RRMS the trends of atrophy in deep grey matter nuclei are similar, although atrophy of the putamen reaches 2% annual atrophy (Eshaghi, Prados et al. 2018). Those with SPMS demonstrate the greatest structural atrophy across all phenotypes with the majority of structures demonstrating greater than 1% annual atrophy, with the caudate and putamen exhibiting even greater rates at around 2% annual atrophy (Eshaghi, Prados et al. 2018). Finally, in PPMS the atrophic trends are similar to RRMS, although the pallidum and putamen show increased rates of atrophy (Eshaghi, Prados et al. 2018). Interestingly, the results from this study did not include parietal grey matter atrophy in their post-hoc analysis due to no significant longitudinal change for any phenotype. Conceivably, this could be due to a substantial multiple comparison correction and/or the range (i.e., EDSS 0-7) and low median (i.e., median 2.0) degree of severity analyzed in the RRMS cohort (Eshaghi, Prados et al. 2018). While the parietal cortex was not included in the prior results, the structures that lie within that region often do demonstrate significant amounts of atrophy in MS (De Stefano, Matthews et al. 2003, Calabrese, Atzori et al. 2007, Lansley, Mataix-Cols et al. 2013, Narayana, Govindarajan et al. 2013, Steenwijk, Geurts et al. 2016, Bergsland, Horakova et al. 2018, Azevedo, Cen et al. 2019, Rocca, Valsasina et al. 2021).

In a follow-up analysis from the same MAGNISM study group, the authors performed an event-based model to determine the sequence of atrophic occurrence

rather than the rate of observed atrophy (Eshaghi, Marinescu et al. 2018). The results from this analysis were an attempt to understand the sequential nature of atrophy using the same large cohort of MS participants. At baseline, 24 regions of the brain demonstrated smaller grey matter volumes in the collective MS cohort compared to the neurotypical group (Eshaghi, Marinescu et al. 2018). Interestingly, the authors describe baseline differences in the posterior cortices, temporal lobes, brainstem, and precentral cortices. To note, this particular analysis was not longitudinal, rather a statistical analysis to describe atrophy progression. In estimating the sequence of atrophy the authors described sequential similarities for patients with RRMS, PPMS, SPMS, and CIS forms of the disease. For instances in RRMS, the first regions demonstrating atrophy were the posterior cingulate cortex and precuneus, followed by the middle cingulate cortex, brainstem, and thalamus. The last regions to become atrophic were the pallidum and medial precentral gyrus (which somatotopically represents the trunk and leg region of the motor cortex) (Eshaghi, Marinescu et al. 2018). In PPMS the first regions that demonstrated atrophy were the thalamus, cuneus, precuneus, and pallidum. Followed by the brainstem, precentral gyrus, and posterior cingulate cortex with the frontal operculum and middle temporal gyrus being the last to show atrophy. These sequential changes were associated with disease duration, where for every 4.76 years of the disease another set of structures exhibited atrophy (Eshaghi, Marinescu et al. 2018). While these results encompass the largest cohort study to be conducted in MS, they are comparable to other studies that have documented similar regions of cortical atrophy for volumetric and thickness metrics (Sailer, Fischl et al. 2003, Tiberio, Chard et al. 2005, Roosendaal,

Bendfeldt et al. 2011, Narayana, Govindarajan et al. 2013, Nygaard, Walhovd et al. 2015, Steenwijk, Geurts et al. 2016, Rocca, Battaglini et al. 2017).

While atrophy appears to be sequential as a result of disease duration and phenotype, there also appear to be specific patterns of cortical atrophy in MS. A recent study conducted by Steenwijk, et al. (2016) introduced a rather novel method to identify cortical thickness patterns in people with longstanding MS (Steenwijk, Geurts et al. 2016). In particular, the authors identified 10 distinct atrophic patterns. Briefly, the 10 patterns were: Pattern 1 - atrophy of the bilateral superior frontal gyrus, and medial side into the paracentral gyrus, Pattern 2 - atrophy of the bilateral supramarginal cortex, extending into the inferior division of the postcentral gyrus, and atrophy of the left middle temporal gyrus, Pattern 3 - relative preservation of the bilateral insula, Pattern 4 - atrophy of the bilateral temporal pole and entorhinal cortex, Pattern 5 - atrophy of the bilateral posterior cingulate, extending into isthmus cingulate and lingual cortex, Pattern 6 - atrophy of the sensorimotor cortex, Pattern 7 - atrophy of the bilateral parahippocampal gyrus, Pattern 8 - relative preservation of the bilateral frontal pole extending into the medial orbitofrontal gyrus, Pattern 9 - relative preservation of the right anterior cingulate, and Pattern 10 - relative preservation of the left anterior cingulate (Steenwijk, Geurts et al. 2016). Two of the patterns (Pattern 4 – bilateral temporal poles, and Pattern 5 – bilateral posterior cingulate cortex) demonstrated the greatest cortical atrophy in PwMS which supports the findings from the MAGNISM multi-center study (Eshaghi, Marinescu et al. 2018). Additionally, these results support atrophy occurring in regions associated with the default mode network and the limbic system which play a particular role in attention, emotion, and memory (Sailer, Fischl et al. 2003, Narayana, Govindarajan et al. 2013, Eshaghi,

Bodini et al. 2014). Moreover, these atrophic patterns appear to be clinically relevant, such that Pattern 6 (sensorimotor atrophy) has been associated with increased physical dysfunction, while Patterns 4 and 5 (posterior cingulate cortex, bilateral temporal poles, respectively) have been associated with cognitive dysfunction, specifically information processing speed (Steenwijk, Geurts et al. 2016). Which in prior studies the posterior cingulate cortex, bilateral temporal poles have been implicated in playing an important role in cognitive dysfunction (Hulst, Gehring et al. 2014, Rocca, Battaglini et al. 2017). Collectively, atrophy appears to be sequential and non-random, affecting each MS phenotype differently but together resulting in deleterious changes to brain structure. While disease duration and phenotype negatively affect brain structure, disease severity similarly appears to be associated with certain atrophic regions associated with the sensorimotor cortex (Narayana, Govindarajan et al. 2013).

CORTICAL ATROPHY AND DISEASE SEVERITY

While cortical and subcortical atrophy is common in PwMS, a number of studies have taken these findings a step further to assess the relationships between cortical atrophy and disease severity. It has been shown that people with mild disability as measured by the EDSS (≤ 3.0) demonstrate reduced cortical thickness bilaterally in the distal superior temporal gyrus and sulcus (Sailer, Fischl et al. 2003). Additionally, in people with more moderate disability (EDSS 3.5 – 5.5) similar thinning is overserved, with additional atrophy in the parietal and frontal medial gyri (Sailer, Fischl et al. 2003). Lastly, those with severe disability (≥ 6.0) demonstrate similar regions of thinning to the moderately disabled individuals with the addition of the pre-motor and motor cortices presenting with significant atrophy (Sailer, Fischl et al. 2003). Since that initial study, other

investigators have demonstrated similar results. For instance, in a cohort of 250 RRMS patients with a median EDSS of 2.0 associations between EDSS and cortical atrophy of the pre-central gyrus, superior temporal gyrus, paracentral gyrus, and the middle temporal gyrus, were identified (Narayana, Govindarajan et al. 2013). While this study did not analyze the relationships between subsets of EDSS scores, they still confirm particular regions that are associated with clinical disease severity. Moreover, similar results have been discovered in other studies demonstrating strong associations between grey matter atrophy in the sensorimotor cortices and PwMS having higher EDSS scores (Bodini, Khaleeli et al. 2009, Prinster, Quarantelli et al. 2010, Lansley, Mataix-Cols et al. 2013). Since the EDSS is highly associated with motor function and ambulation particularly, these motor related regions demonstrating significant associations help to elucidate the structural underpinnings of motor dysfunction in MS.

NEUROPHYSIOLOGICAL IMPAIRMENTS IN MS

Since the 1980s non-invasive brain stimulation (NIBS) techniques have offered various methods for the assessment of neurophysiology, neural transmission, and neural plasticity of the motor systems. The most common NIBS technique applied for the assessment of the motor system is single pulse transcranial magnetic stimulation (spTMS), which has been used in research to examine neurophysiological underpinnings of motor impairment (Sahota, Prabhakar et al. 2005). The primary components of neurophysiology assessed through spTMS are cortical excitation via measures of glutamatergic activity and inhibition via gamma-aminobutyric acid (GABA) (Groppa, Oliviero et al. 2012). Importantly, in neurotypical adult brains, the balance of excitation and inhibition ensures proper neural functioning, although in MS this balance appears to

be disrupted as a product of pathology (Sale, Berardi et al. 2014, Chaves, Devasahayam et al. 2020)

In MS, the role of glutamate is not completely understood, although glutamatergic homeostasis is believed to be an important consideration for disease status and progression (Ayache, Creange et al. 2014). Imbalanced glutamatergic homeostasis can be the result of many factors including increased production, activated immune cells, reduced transport, and impaired metabolism of glutamate (Obrenovitch, Urenjak et al. 2000, Pitt, Werner et al. 2000). As a result, it is believed that these imbalances lead to excess release of extracellular glutamate and subsequently an excitotoxic environment, which results in deleterious effects on neural tissues (Pitt, Werner et al. 2000). While glutamatergic levels naturally fluctuate, relapses, lesion formation, and disease progression in MS are associated with these periods of increased extracellular glutamatergic release (Vallejo-Illarramendi, Domercq et al. 2006, Ayache, Creange et al. 2014, Ayache, Creange et al. 2015, Chaves, Wallack et al. 2019). While TMS does not offer the opportunity to directly measure extracellular levels of glutamate, it does provide an approach to investigate glutamatergic synaptic activity. Given the toxic role, glutamate is thought to have on MS, the TMS literature has primarily focused on the assessment of cortical excitability rather than inhibitory function. Many studies have reported excitability differences between neurotypical controls and PwMS utilizing spTMS to measures a number of variables thought to represent glutamatergic activity. Specifically, research has reported increases in motor threshold intensity (Cruz-Martinez, Gonzalez-Orodea et al. 2000, Jorgensen, Nielsen et al. 2005, Gagliardo, Galli et al. 2007), reductions in motor evoked potential (MEP) amplitude (Cruz-Martinez, Gonzalez-Orodea et al. 2000,

Jorgensen, Nielsen et al. 2005, Gagliardo, Galli et al. 2007), reductions in MEP area (Schubert, Wohlfarth et al. 1998, Gagliardo, Galli et al. 2007), and increases in MEP latency duration (Jones, Streletz et al. 1991, Kukowski 1993). All of which are measures used to assess intracortical excitability of the motor cortex.

Motor thresholds are thought to reflect the excitability of the cortico-cortico and thalamo-cortical axons which reside in the deep layers of the motor cortex (Ziemann 2004). Motor thresholds are determined while a muscle is either at rest (resting motor threshold (RMT)) or during an active contraction (active motor threshold (AMT)). Both measures have been used in TMS studies as they are requisite to other common TMS measures. While the majority of studies have demonstrated higher motor threshold values in MS (Jorgensen, Nielsen et al. 2005, Gagliardo, Galli et al. 2007), a number of studies have reported no motor threshold differences between PwMS and neurotypical controls (Liepert, Mingers et al. 2005, Vucic, Burke et al. 2012, Di Sapio, Bertolotto et al. 2014). While studies have reported inconsistent results the RMT has demonstrated differences between neurotypical controls and PwMS having mobility disability and PwMS with no disability, while AMT has only demonstrated differences between disabled and non-disabled PwMS (Gagliardo, Galli et al. 2007).

MEP amplitude is one of many MEP quantifiable measures and is thought to reflect the density of cortico-motor neuronal projections from the motor cortex (Ziemann 2004). MEPs are the product of a single pulse producing an action potential that descends the corticospinal tract resulting in brief muscular depolarization. MEP amplitude is determined by measuring the amplitude of the electromyography (EMG) spike commonly reported in microvolts (mV). Some researchers have normalized their MEP measures to a compound

muscle action potential (CMAP) by eliciting a supramaximal electrical stimulation (within the popliteal fossa or on the peroneal nerve for the lower limbs). The majority of studies reporting MEP amplitude observe reductions in PwMS compared to neurotypical control participants (Kukowski 1993, Cruz-Martinez, Gonzalez-Orodea et al. 2000, Schmierer, Niehaus et al. 2000, Tataroglu, Genc et al. 2003, Jorgensen, Nielsen et al. 2005, Sahota, Prabhakar et al. 2005, Gagliardo, Galli et al. 2007). Given that MEP amplitude is thought to reflect cortico-motor projections it is not surprising that this measure has been associated with disease severity via the EDSS, where those with reduced amplitude demonstrated greater disease severity (Cruz-Martinez, Gonzalez-Orodea et al. 2000, Tataroglu, Genc et al. 2003). Additionally, MEP amplitude has been associated with pyramidal and cerebellar functional scores of the EDSS, where those with reduced MEP amplitude demonstrate poorer functional scores (Tataroglu, Genc et al. 2003). Behaviorally, gait ataxia in PwMS has been associated with reduced MEP amplitude, where those with more severe ataxia demonstrate reduced MEP amplitudes (Cruz-Martinez, Gonzalez-Orodea et al. 2000). While the majority of studies demonstrate reduced amplitudes, a study conducted by Thickbroom, Sacco et al. (2008) reported no differences for the leg muscles between PwMS and neurotypical controls at rest; however, did observe increased MEP amplitude in an exercised foot after a fatiguing foot tapping exercise (Thickbroom, Sacco et al. 2008). For the neurotypical controls in the same study, however, the increased amplitude was observed in both legs, not just the exercised leg. These results suggest that PwMS can increase corticomotor excitability similar to neurotypical controls, although not to the same degree (Thickbroom, Sacco et al. 2008). Importantly, stimulation targeted the vertex, therefore these results could be a

product of stimulation overflow. Such that the electromagnetic current activated the leg region of the motor cortex in both hemispheres.

MEP area is measured via the rectified EMG response and commonly computed from the first negative EMG deflection to when the EMG trace return to baseline (Soto, Valls-Solé et al. 2006). PwMS demonstrate reduced MEP area compared to neurotypical controls which has been associated with aspects of lower limb function, mobility, and muscle weakness (Schubert, Wohlfarth et al. 1998, Gagliardo, Galli et al. 2007, Di Sapio, Bertolotto et al. 2014). Additionally, PwMS who display walking limitations demonstrate reduced MEP area compared to PwMS without walking limitations (Schubert, Wohlfarth et al. 1998, Gagliardo, Galli et al. 2007).

MEP latency is defined as the duration from stimulation to the first EMG deflection of the target muscle, representing the neural transmission time. In PwMS, MEP latency to the lower limbs is typically delayed compared to neurotypical controls (Tataroglu, Genc et al. 2003, Thickbroom, Sacco et al. 2008). For instance, one study reported 42.5% longer latencies in the lower limbs compared to neurotypical controls (Sahota, Prabhakar et al. 2005). To date, no studies have directly associated lower limb MEP latency to lower limb function or behavior. Although, one study integrated the upper and lower limb MEP latency measures and demonstrated significant associations between latency and the cumulative EDSS score and the sensory score of the EDSS. Such that those with longer latencies demonstrated greater severity (Tataroglu, Genc et al. 2003).

Lastly, MEP duration is measured from the first EMG deflection to the return of baseline EMG. To our knowledge, only two studies have measured MEP duration of the lower limbs in PwMS. Both have reported longer MEP durations in PwMS compared to

neurotypical controls (Kukowski 1993, Schubert, Wohlfarth et al. 1998). Further, no associations have been made between MEP duration and lower limb functional measures; however, Schubert, Wohlfarth et al. (1998) reported no differences in MEP duration before and after a bout of walking in PwMS (Schubert, Wohlfarth et al. 1998).

Intracortical inhibition results from the binding of the neurotransmitter GABA to either an ionotropic $GABA_A$ or metabotropic $GABA_B$ receptor, triggering either short- or long-lasting (respectively) effects of inhibition (Mott and Lewis 1994). Inhibition and GABAergic activity have been associated with aspects of memory and cognition, but also motor function in a variety of neurotypical and atypical populations including PwMS (Mott and Lewis 1994, Stagg, Bachtiar et al. 2011, Sale, Berardi et al. 2014, Cawley, Solanky et al. 2015, Cassady, Gagnon et al. 2019, Swanson and Fling 2019). While levels of inhibitory activity have been measured in the upper and lower limbs in PwMS the results remain inconclusive. Studies have reported increased GABAergic activity (Manson, Palace et al. 2006, Manson, Wegner et al. 2008, Bhattacharyya, Phillips et al. 2013, Cawley, Solanky et al. 2015, Nantes, Zhong et al. 2016), no differences in activity (Thickbroom, Sacco et al. 2006, Nantes, Zhong et al. 2016, Neva, Lakhani et al. 2016), and reduced levels of GABAergic activity in PwMS compared to neurotypical controls (Santarnecchi, Rossi et al. 2015). Additionally, inhibitory differences appear to be associated with various factors such as level of neurological impairment (Nantes, Zhong et al. 2016), phenotype (Tataroglu, Genc et al. 2003), and relapse activity (Caramia, Palmieri et al. 2004), such that those with greater impairment and more progressive phenotypes often present with greater inhibitory activity. While inhibitory activity has been

assessed in MS, studies focusing their assessment to the lower limbs remains largely unexplored.

The most commonly reported measure of intracortical inhibition is termed the cortical silent period (cSP) which is a measure of $GABA_B$ receptor mediated inhibitory synaptic activity (Fuhr, Agostino et al. 1991, Cantello, Gianelli et al. 1992, Siebner, Dressnandt et al. 1998, Werhahn, Kunesch et al. 1999). To date, one study has directly reported cSP measures from the tibialis anterior (TA) in PwMS (Tataroglu, Genc et al. 2003). Although one additional study reported TA related cSP measures for PwMS in their supplemental material they developed a scoring algorithm through a complex quantification approach using an amalgamation of excitatory and inhibitory TMS measures which were then used to associate with various functional scores of the EDSS (Kiylioglu, Parlaz et al. 2015). As such, the Tataroglu, Genc et al. (2003) study remains the only study directly reporting cSP durations from the TA in PwMS. Specifically, the study assessed three clinical phenotypes of MS (a subclinical group, RRMS, and PPMS) all of which demonstrated significantly longer silent period durations compared to neurotypical controls (Tataroglu, Genc et al. 2003). The authors also reported no associations between total EDSS and silent period duration ($r = 0.12$, $p \geq 0.05$); however, a significant (albeit weak) association ($r = 0.32$, $p \leq 0.05$) was reported between the Kurtzke Cerebellar sub-score of the EDSS and silent period duration (Tataroglu, Genc et al. 2003). Important to note, these associations were combined silent period durations from all phenotypes and further combined the silent period durations from both upper and lower limbs limiting the interpretation of the results.

FALLS IN MULTIPLE SCLEROSIS

Falls in PwMS are common, with nearly 56% experiencing a fall during any three-month period (Nilsagard, Gunn et al. 2015, Comber, Sosnoff et al. 2018). Of the individuals who do fall, being a male and having an EDSS of 4.0-6.0 report falling the most (Nilsagard, Gunn et al. 2015). Additionally, falls occur most often indoors between the hours of 6:00 am-6:00 pm and importantly of those who experience a fall nearly 50% incur an injury as a result, limiting their mobility and negatively affecting their quality of life (Peterson, Cho et al. 2008, Sosnoff, Socie et al. 2011, Comber, Coote et al. 2017). Associated with quality of life, those individuals with a history of falling often report an increased fear of falling and tend to reduce their physical activity and social participation/engagement, which subsequently increases the likelihood of future falls (Peterson, Cho et al. 2007, Peterson, Cho et al. 2008, Nilsagard, Gunn et al. 2015). A relatively recent meta-analysis identified eighteen risk factors associated with falls in PwMS (Gunn, Newell et al. 2013). However, given the variation of analyses, reporting of methods, and sheer lack of studies evaluating risk factors, the review focused on four primary factors associated with the highest incidence of falls in PwMS: the use of mobility aids (i.e., walker or cane), imbalance, cognitive dysfunction, and a diagnosis of the progressive MS phenotype (Gunn, Newell et al. 2013). Furthermore, the authors discussed an additional five likely risk factors: spasticity, fear of falling, gait, disease severity, and incontinence concerns (Cameron and Nilsagård 2013). Having a broad understanding as to the influences associated with fall risk is imperative for clinicians to target lifestyle adjustments and ameliorate motor/cognitive impairments associated with increased fall risk.

MOBILITY IMPAIRMENTS IN MULTIPLE SCLEROSIS

LINEAR AND NON-LINEAR GAIT IMPAIRMENTS IN MULTIPLE SCLEROSIS

Impaired ambulation is a hallmark of MS and an important predictor for the occurrence of falls and disease severity. As such a variety of factors are thought to influence gait dysfunction in MS including muscle weakness (Rudroff, Kindred et al. 2014), spasticity (Balantrapu, Sosnoff et al. 2014), changes in brain morphology (Peterson and Fling 2018), corticospinal tract integrity (Reich, Zackowski et al. 2008), somatosensory conduction and gaiting (Arpin, Gehringer et al. 2017), and cognitive function (Hamilton, Rochester et al. 2009).

Similar to other diseases, PwMS demonstrate reductions in both quantity and quality of gait. Quantity measures of walking such as median strides per bout of walking and number of strides per hour are both reduced in PwMS (Shah, McNames et al. 2020). Furthermore, those particular measures are also able to discriminate between PwMS and neurotypical controls (Shah, McNames et al. 2020). Moreover, conventional spatiotemporal measures of linear gait are impaired in PwMS. For instance, gait speed, stride length, and cadence are often reduced in PwMS compared to neurotypical controls (Spain, St George et al. 2012, Shah, McNames et al. 2020). Additionally, the collective components which make up the gait cycle are altered, such that PwMS spend greater percentages of time in double support and reduced percentages of time in the swing phase of a gait cycle (Novotna, Sobisek et al. 2016, Comber, Coote et al. 2017, Cameron and Nilsagard 2018). Variability of spatiotemporal characteristics of gait, often measured via the coefficient of variation (CoV) are also altered and increased in PwMS. Such that their gait patterns demonstrate inconsistency during a bout of continuous walking (Crenshaw, Royer et al. 2006, Kalron 2016).

While the majority of gait related research has focused on linear walking, studies have shown that turning is a ubiquitous component of walking (Leach, Mellone et al. 2018). Specifically, it has been recorded that up to 45% of all steps taken throughout the day are related to changes in direction (Glaister, Bernatz et al. 2007). While this research largely remains under investigation in MS, some studies have documented differences in turning dynamics (Adusumilli, Lancia et al. 2018, Soke, Guclu-Gunduz et al. 2019, Shah, McNames et al. 2020). As such, initial reports have documented increased turn durations and reduced turn velocities during both 180˚ and 360˚ turns (Spain, St George et al. 2012, Soke, Guclu-Gunduz et al. 2019). Suggesting that both linear and non-linear walking dynamics are impaired in PwMS, and likely influence the high rates of observed falls and subsequent injuries.

POSTURAL INSTABILITY IN MULTIPLE SCLEROSIS

Postural stability and control require complex cortical sensorimotor integration (Horak 2006). PwMS exhibit reduced postural stability compared to neurotypical controls, which is likely associated with reduced proprioceptive signaling and slowed efferent responses, resulting in an inability to correct for postural errors in a time-effective manner (Cameron, Horak et al. 2008, Cattaneo, Ferrarin et al. 2012, Huisinga, St George et al. 2014). As such, postural control is not only affected during normal stance conditions (i.e., standing on a firm surface with eyes open), but also during more difficult stance conditions (i.e., standing on a compliant surface with eyes closed) in PwMS. For instance, PwMS demonstrate greater postural instability in stance conditions where the reliance is primarily controlled via the somatosensory or vestibular systems (Comber, Sosnoff et al. 2018). In addition, PwMS demonstrate decreased ability to effectively manage

compensatory responses to external perturbations (Peterson, Huisinga et al. 2016), and difficulty controlling center of pressure velocities, lengths and displacements compared to neurotypical controls (Sosnoff, Socie et al. 2011). Furthermore, individuals with MS have significantly altered trunk sway (Spain, St. George et al. 2012), center of mass (CoM) sway (Kanekar and Aruin 2013), CoM sway jerk (Spain, St. George et al. 2012), and decreased time to boundary (Van Emmerik, Remelius et al. 2010, Cattaneo, Ferrarin et al. 2012, Whittier, Richmond et al. 2020) measures compared to neurotypical controls. Together, a variety of postural stability measures are impaired in MS, likely leading to a higher incidence of falls in this population.

NEUROANATOMICAL AND NEUROPHYSIOLOGICAL ASSOCIATIONS TO MOBILITY IN MULTIPLE SCLEROSIS

The neural mechanisms associated with mobility impairment are crucial for understanding the deleterious adaptations occurring in PwMS. This topic of investigation has primarily focused on disability associated with natural aging and diseases such as Parkinson's disease (Peterson and Horak 2016, Swanson and Fling 2018, Swanson and Fling 2019) although given the updated prevalence rates more attention has been placed on PwMS (Peterson and Fling 2018).

Virtually all regions of the brain exhibit grey matter atrophy in MS (Eshaghi, Marinescu et al. 2018). The progressive loss of grey matter in neurotypical populations is typically a slow process, in MS the rate of loss is substantially accelerated and associated with disease related components such as phenotype, severity, and duration (Nourbakhsh, Azevedo et al. 2016, Eshaghi, Marinescu et al. 2018). Given that disease severity is heavily weighted on ambulation, a number of studies have shown that both global and

region grey matter atrophy relates to ambulatory ability in PwMS. Specifically, global grey matter volume has been associated with clinical gait measures including the Timed 25 Foot Walk Test, the 6 Minute Walk Test, and the Timed Up and Go (Motl, Hubbard et al. 2015, Nourbakhsh, Azevedo et al. 2016, Jakimovski, Weinstock-Guttman et al. 2018). Regional, deep grey matter (i.e., thalamus, caudate, pallidum, putamen), brainstem, and cerebellar atrophy have also been associated with the same clinical gait measures and additionally the Ambulation Index (Edwards, Gong et al. 1999, Jasperse, Vrenken et al. 2007, Liptak, Berger et al. 2008, Shiee, Bazin et al. 2012, Motl, Hubbard et al. 2015, Grothe, Lotze et al. 2017, Jakimovski, Weinstock-Guttman et al. 2018). While these specific gait measures have been shown to be both valid and clinically relevant for PwMS, they are often broad characterizations of general mobility (Cattaneo, Regola et al. 2006, Nilsagard, Lundholm et al. 2007, Goldman, Marrie et al. 2008, Rosti-Otajärvi, Hämäläinen et al. 2008, Polman and Rudick 2010, Kieseier and Pozzilli 2012). In a recent study conducted by Liparoti et al., (2019), the researchers attempted to understand the effects of structural damage on more specific kinematic and kinetic gait parameters in PwMS. Their findings discovered impairments to gait velocity, stance time, double support, and variability of swing time compared to neurotypical controls (Liparoti, Della Corte et al. 2019). However, their global grey matter atrophy measures neglected to find significant differences between groups, furthermore, grey matter atrophy was not associated with any of the kinetic or kinematic gait measures for either group (Liparoti, Della Corte et al. 2019). While to our knowledge the Liparoti et al., (2019) study is the first to employ gold standard gait analysis and associations to grey matter atrophy, the lack of correlation may be a product of associating global grey matter atrophy instead of assessing motor specific

regional atrophy. Additionally, their cohort of RRMS participants collectively had an EDSS ≤ 2.0 which may account for their lack of atrophic differences and subsequent associations. In a study conducted by Prosperini, et al. (2013), they assessed the associations between cerebellar grey matter atrophy and postural stability in PwMS and neurotypical controls (Prosperini, Sbardella et al. 2013). Their results showed that grey matter atrophy of somatosensory areas of the cerebellum (i.e., the anterior lobules (IV, V, VI) and lobule VIII) were associated with reduced posturometric values (i.e., increased center of pressure path length) (Prosperini, Sbardella et al. 2013). While structural alterations are prevalent in MS the associations are often made to broad measures of ambulation and functional adaptations instead of associating specific atrophy in motor related regions and objective movement characteristics.

Currently, there is a debate as to whether the neural adaptations observed in PwMS are adaptive such that they are counteracting and compensating for the observed structural and functional changes or maladaptive and merely the product of functional and structural degradation leading to behavioral dysfunction (Peterson and Fling 2018). Uncovering the truth behind both of these theories is ultimately quite challenging and remains opaque. However, it is conceivable that neural adaptions are both adaptive and maladaptive responses depending on the particular type and location of apparent adaptation. For instance, functional MRI analysis has discovered increased activation of the contralateral sensorimotor cortex and non-motor specific regions may be adaptive. Specifically, a couple of studies conducted by Rocca and colleagues demonstrated increased activity in the contralateral supplementary motor area, putamen, and ipsilateral cerebellum in PwMS was associated with less pronounced motor impairments, while

those with greater impairment demonstrated reduced activity in the same regions (Rocca, Colombo et al. 2005, Rocca, Ceccarelli et al. 2010). Moreover, people with RRMS and an EDSS of zero exhibited the greatest increases of brain activity in the contralateral motor cortices compared to those with EDSS scores greater than zero (Rocca, Colombo et al. 2005, Rocca, Ceccarelli et al. 2010). Similar results have been demonstrated although in ipsilateral non-motor specific brain regions, such that greater activity was observed in PwMS having fewer motor symptoms in the inferior temporal gyrus, central opercular cortex, and lingual gyrus (Giorgio, Portaccio et al. 2010). While the results from the Giorgio, et al. (2010) study demonstrated non-motor ipsilateral activity differences, ipsilateral activity of the motor cortices were not different. Conversely, a study conducted by Reddy, et al. (2002) documented increased activity of the ipsilateral pre-motor and primary motor cortex in PwMS having greater upper extremity motor impairment (via finger tapping) (Reddy, Narayanan et al. 2002). Additionally, when controlling for lesion load and subdividing MS participants based on better or worse motor symptoms, those with larger impairments demonstrated greater bilateral motor cortex activity (Reddy, Narayanan et al. 2002). While greater activity has been associated with better motor performance within MS cohorts, greater activity has also been observed in PwMS compared to neurotypical controls (Giorgio, Portaccio et al. 2010). While ipsilateral non-motor related regions displayed greater activity in less motor impaired PwMS, bilateral motor specific regions (precentral gyrus and postcentral gyrus) showed greater activity in mildly impaired (EDSS < 2) PwMS compared to neurotypical controls (Giorgio, Portaccio et al. 2010). Regarding these specific fMRI findings, interpreting whether these neural adaptations are adaptive or maladaptive is challenging. As described, there are data to

suggest increased brain activity may be adaptive in PwMS, however, there are also data suggesting increased brain activity is maladaptive. The notion that increased brain activity is maladaptive to motor impairment is accentuated when comparing motor specific activity between neurotypical controls and PwMS, but also within MS cohorts (Reddy, Narayanan et al. 2002, Giorgio, Portaccio et al. 2010).

While fMRI measures brain activity via the blood oxygen level dependent (BOLD) signal, other neuroimaging techniques such as TMS have been employed to assess neurophysiological adaptations associated with motor impairments in PwMS. Different from fMRI which effectively measures the amount of oxygenated blood relative to an activated brain region, TMS offers the ability to investigate biochemical activity by inducing localized neuronal depolarization which has been aligned with specific neurophysiological receptor activity. As such, the notions alluding to greater activity being adaptive or maladaptive have gained interest in recent years through the use of TMS and the assessment of neurophysiology linking to poorer motor performance.

TMS is uniquely adept at assessing neurophysiological excitability mediated by glutamate and inhibition mediated by gamma-aminobutyric acid (GABA) receptor activity. While TMS offers an opportunity to assess relative levels of glutamate and GABA, it also allows for the assessment of cortical activity, networks, and structures particularly accompanying the primary motor cortices, corpus callosum, and corticospinal tract, which participate in the mediation of voluntary movements (Hallett 2000, Chen, Cros et al. 2008). Since the first studies in the 1980s utilizing TMS, the technique has become widely implemented in a variety of populations to study the associations between neurophysiology and movement. Specifically, in healthy aging populations inhibitory

(GABAergic) synaptic activity has been implicated in the control and coordination of linear gait and non-linear turning (Swanson and Fling 2018, Swanson and Fling 2019). However, the use of TMS in PwMS has largely been used to assess upper extremity movements and broad associations with the disease severity (Nantes, Zhong et al. 2016, Nantes, Zhong et al. 2016, Neva, Lakhani et al. 2016). Specifically, in MS reduced excitability via MEP characteristics and greater inhibitory activity via the cortical and ipsilateral silent period (cSP and iSP, respectively) have been observed (Tataroglu, Genc et al. 2003, Nantes, Zhong et al. 2016, Neva, Lakhani et al. 2016). These imbalances have been associated with the EDSS such that decreased excitability (i.e., smaller MEPs) and increased inhibition (longer cSP and iSP) correlate with greater disease severity (Neva, Lakhani et al. 2016). Additionally, greater inhibition has been associated with more severe upper extremity disability and coordination (Nantes, Zhong et al. 2016). While studies associating excitation and inhibition to motor impairments and disease severity have been completed in MS, they have almost entirely been done assessing the upper extremities. While the majority of TMS studies in MS have focused their assessments on the upper extremities there have been a few studies assessing the lower limbs. However, nearly all of them have only assessed measures of excitation. Largely these studies have found associations between reduced excitability and walking limitations and greater disease severity, although specific measures of gait impairments have yet to be assessed (Schubert, Wohlfarth et al. 1998, Gagliardo, Galli et al. 2007). Regarding inhibition, there has been a single study that assessed the cSP duration in the TA muscle in three MS phenotypes (Tataroglu, Genc et al. 2003). As described prior, this study discovered that those with more severe forms of the disease (i.e., PPMS) displayed greater levels of

inhibitory activity (Tataroglu, Genc et al. 2003). Moreover, the authors reported a significant association between greater inhibitory activity and worse cerebellar sub-scores of the EDSS (Tataroglu, Genc et al. 2003). While our understanding regarding mobility and neurophysiology has expanded over the last decade, the implementation of TMS specific protocols to assess the neurophysiological underpinnings of mobility in MS remains largely uncovered.

In general, the evaluation of neuroanatomical structure and neurophysiological function for assessing mechanisms of mobility have improved over the last decade. Moreover, researchers have begun to implement those techniques to elucidate the neural underpinnings of mobility impairments in MS. Research has established that grey matter atrophy is evident in MS and progressively affects a variety of cortical and subcortical structures throughout the course of the disease, such that global and regional atrophy is associated with worsening disease severity and broad measures of ambulatory ability. While less research has been conducted, there are clearly neurophysiological adaptations which accompany MS and demonstrate deleterious associations with components of movement. However, studies have yet to merge the assessment of neuroanatomical structure and neurophysiological function to elucidate the neural underpinnings of specific measures of mobility impairment in MS. Furthermore, the ability to objectively quantify mobility has greatly improved over the last decade and can be implemented without the need for specific gait laboratories and expensive equipment. However, to date, no studies have employed specific objective characteristics of mobility to measures of neural structure or function in MS. Moreover, no studies have assessed

specific turn related measures to regional cortical grey matter atrophy or excitatory and inhibitory neurophysiological activity.

CHAPTER 2 – TURNING DIFFERENCES BETWEEN NEUROTYPICAL CONTROLS AND PEOPLE WITH MULTIPLE SCLEROSIS

INTRODUCTION

The effects of multiple sclerosis (MS) are multifaceted, resulting in various deleterious adaptations throughout the course of the disease. A major adaptation and hallmark of the disease is progressive mobility dysfunction (Cameron and Nilsagard 2018). This results in nearly 56% of people with MS (PwMS) experiencing a fall during any three-month period (Nilsagard, Gunn et al. 2015, Comber, Sosnoff et al. 2018). Moreover, of those who fall, nearly 50% endure an injurous fall further limiting their mobility and negatively affecting their quality of life (Peterson, Cho et al. 2008, Sosnoff, Socie et al. 2011, Comber, Coote et al. 2017). The occurrence of falls is closely associated with disease severity which is commonly measured using the Expanded Disability Status Scale (EDSS) which incrementally (half steps) classifies an individual's level of disability on a scale from 0-10 (Kurtzke 1983). Levels between 0 – 3.5 indicated non-mobility disability (i.e., bladder control complications, visual impairment, cognition deficits, etc.), from 4 on, the level of disability is largely classified by the degree of ambulatory disfunction. As such, once mobility is affected, individuals typically experience a nonlinear association between falls and disability level. For instance, EDSS scores between 4.0 (when quantifiable mobility limitations are first acknowledged) – 6.0 (when a walking aid is required) experience the greatest incidence of falls (Nilsagard, Gunn et al. 2015). Interestingly, PwMS who do experience a fall often report falling indoors rather than outdoors and tend to fall in the morning and afternoon hours rather than the evening

or nighttime hours (Nilsagard, Gunn et al. 2015). These falls are not only a major concern to the medical system but more so to the welfare of the individuals themselves.

Aside from environmental factors, personal fall risk factors include a perceived or objective observation of imbalance and gait dysfunction which often influences all aspects of mobility (Cattaneo, De Nuzzo et al. 2002, Soyuer, Mirza et al. 2006, Nilsagård, Lundholm et al. 2009, Kasser, Jacobs et al. 2011, Gunn, Newell et al. 2013, Nilsagard, Gunn et al. 2015). One such study reports that over 26% of falls occur indoors while standing, walking, and/or turning (Mazumder, Murchison et al. 2014). While that percentage may seem trivial, standing, walking, and/or turning represent the greatest chance for mobility related falls (Mazumder, Murchison et al. 2014). Of those three mobility related components, it is likely that the transitionary movement of turning (i.e., changing directions) makes up a significant portion of the fall. According to the healthy aging literature, it has been recognized that turning is ubiquitous, accounting for roughly 45% of all steps taken throughout the day (Glaister, Bernatz et al. 2007, Mancini, Schlueter et al. 2016, Leach, Mellone et al. 2018). Additionally, as living arrangements adapt and people spend more time indoors and in more confined spaces the number of turns performed throughout the day increases (Weaver, Robinovitch et al. 2016, Mancini, Weiss et al. 2018). While turns are common and increasingly more so due to living arrangements, turns represent a precarious and sometimes dangerous movement when compared to static standing or linear walking. For instance, in neurotypical older adults a fall while turning increases the risk for a hip fracture by a factor of eight compared to a fall that occurs during a bout of linear walking (Cumming and Klineberg 1994, Feldman and Robinovitch 2007). Additionally, it has been reported that females (who make up the

majority of those diagnosed with MS) are predisposed to reduced bone mineral density, thus further increasing the risk of a hip fracture resulting from a fall while turning (Hearn and Silber 2010, Harbo, Gold et al. 2013). Given the inherent susceptibility for fractures due to reduced bone density, and the fact that falls are common during transitionary movements in PwMS, it is important to identify the specific kinematic adaptations that influence reduced turning performance for individuals with MS.

To date the vast majority of gait research in all populations including MS has focused on linear walking and static balance; however, turning is ever-present in nearly all aspects of daily movement. Turning is defined as a whole-body change in trajectory and requires a considerable amount of movement planning, strength, balance, and lower limb coordination to be performed successfully. During a turn, it is essential for both legs to be independently controlled both spatially and temporally, such that one leg may take a quick large step while the other leg takes a slower short or even backward step to change direction. A recently published study demonstrated both coordination and spatiotemporal mobility metrics demonstrate differences between neurotypical controls and PwMS. Specifically, Richmond, et al. (2020) demonstrated significantly reduced gait kinematic performance (i.e., gait speed (m/s), stride length (m), and phase coordination index (˚)) in PwMS (Richmond, Swanson et al. 2020). Additionally, studies have documented longer 180˚ turn durations and slower turn velocities in PwMS for turns captured during the Timed Up and Go (TUG) test (Spain, St George et al. 2012, Lorefice, Coghe et al. 2017, Adusumilli, Lancia et al. 2018). Moreover, one study has shown a relationship between 360˚ turn duration and higher fall rates in PwMS (Soke, Guclu-Gunduz et al. 2019). Lastly, a recent study examining seven consecutive days of real-

world gait kinematic collection reported significant differences in the average turn angle between PwMS and neurotypical controls such that, PwMS demonstrated reduced average turn angles (Shah, McNames et al. 2020).

Collectively, these results suggest that PwMS display reduced spatiotemporal turning kinematics; however, no studies have specifically been designed to carefully assess various but specific types of turns. For instance, no studies have specifically assessed spatiotemporal kinematics of turning in-place (360˚) or turning while walking (180˚) using body-worn sensors. Therefore, the purpose of this study was to exclusively examine spatiotemporal characteristics of turning in-place at two different self-selected speeds (i.e., fast and natural) and while walking at a natural pace in PwMS and neurotypical controls using inertial wireless sensors. We hypothesized that for 360˚ in-place fast turns, that turn duration (s) would be significantly longer, while turn angle (˚), and peak turn velocity (˚/s) would be significantly reduced for PwMS. For consecutive and alternating 360˚ turns performed at a self-selected pace we hypothesized that turn duration (s) would be longer, while turn angle (˚), peak turn velocity (˚/s), and number of turns would be significantly reduced for PwMS. Lastly, for 180˚ turns performed while walking, we hypothesized that PwMS would demonstrate significantly longer turn duration (s) and a greater number of steps to complete a turn, while turn angle (˚), and peak turn velocity (˚/s) would be significantly reduced in PwMS compared to neurotypical control participants.

METHODS

PARTICIPANTS

A total of forty-nine individuals participated in the study; 23 neurotypical adults (15 females; age range, 23 – 76 years; mean age 46.8 ± 15.9 years) and 26 individuals with relapsing remitting multiple sclerosis (RRMS) (19 females; age range 29 – 70 years; mean age 48.2 ± 12 years; mean EDSS 3.5; range 0 – 4). Enrollment flow chart and complete demographics for each group are displayed in Figure 2.1 and Table 2.1, respectively. All participants were able to walk and stand for a minimum of 10 minutes without the use of an assistive device. The neurotypical control participants were free from any clinically diagnosed neurological disease which would impact their mobility. This study was approved by the Colorado State University Institutional Review Board, and all participants provided written informed consent prior to participating.

TURNING COLLECTION

Participants came into the laboratory for two testing sessions which occurred on separate days, although no more than 10 days apart. Testing sessions included an instrumented assessment of 360˚ (turns in-place) and 180˚ (turns while walking) turns while wearing wireless inertial sensors. Participants wore three wireless inertial sensors placed on each foot, around the waist at the level of L5 (Swanson and Fling 2019). All sensors were attached to the body using elastic Velcro straps to ensure sensors were snug enough to limit unwanted sensor movement without being cumbersome or uncomfortable to the participant.

Two distinct types of in-place 360˚ turns were measured, although they were both characterized as a 360˚ turn clockwise with an immediate 360˚ turn counterclockwise. One type of 360˚ turn consisted of three separate 360˚ turning trials at participant's self-selected fast pace. The second type of 360˚ turn trial consisted of 1-minute of continuous

but alternating 360˚ turns at participant's self-selected natural pace. Prior to the trials, a foot template was placed between the feet (then removed) to ensure consistent foot placement for all trials and participants. Additionally, participants were asked to step through the turns and instructed not to spin on their toes, heels, or conduct a military style turn. These turns were conducted in an open laboratory space, barefoot on linoleum flooring with a research assistant was present as a spotter in case of balance loss. These turns were conceived to simulate a turn occurring in a tight space such as a kitchen or bathroom.

Turns while walking were characterized as 180˚ turns, which individuals completed while performing a two-minute self-selected pace walk test. Prior to the trial, a foot template was placed (then removed) to ensure consistent foot placement for all trials and participants. Participants were asked to walk back and forth down a hall of 30 meters in length. The walkway was marked with high visibility tape at each end to cue participants on where to turn around. Participants were asked to turn around 'naturally', to mimic forgetting something in a room they had just come from.

ANALYSIS

DATA ANALYSIS

Both the 360˚ and 180˚ turns were collected using previously validated turning algorithms via Opal wireless inertial sensors (128Hz), and Mobility Lab software (Version 2) (Opal Sensors, APDM Inc., Portland, OR) (El-Gohary, Pearson et al. 2014). Data from the Opal sensors were automatically and wirelessly streamed to a laptop computer which automatically quantified turning metrics (El-Gohary, Pearson et al. 2014, Horak, King et al. 2015). The primary turning metrics for 360˚ in-place fast turns included, turn duration

(s), turn angle (˚/s), and peak turn velocity (˚/s). The primary turning metrics for 1-minute 360˚ in-place self-selected paced turns included turn duration (s), turn angle (˚/s), peak turn velocity (˚/s), and number of turns (#) completed. Lastly, the primary metrics for 180˚ turns while walking included turn duration (s), turn angle (˚/s), peak turn velocity (˚/s), and number of steps (#) in the turn.

STATISTICAL ANALYSIS

All statistical analysis was conducted in JMP Pro 15 with an alpha level set at 0.05. To test for normality a Shapiro-Wilk test was conducted for all turning measures (i.e., 360 in-place fast pace, 1 minute 360˚ in-place self-selected, and 180˚ turns while walking) and variables, which demonstrated a normal distribution for all data and variables.

To assess for differences between the three, 360˚ fast pace turn trials a repeated measures analysis of variance (RMANOVA) was performed. No significant differences were observed and therefore, all variables were averaged together. Between-group differences for age were examined via a one-way ANOVA while between-group sex differences were examined via a chi-square test. To determine between-group differences for all other demographic measures and turning variables independent t-tests were performed. All data are presented as mean ± SD unless noted otherwise.

RESULTS

Regarding the current chapter and the following two chapters, 91 individuals were assessed for eligibility. After the enrolment process, 61 individuals were consented with 49 completing all components (i.e., turning, MRI, and TMS) of the study, and thus included in the final analysis (see Figure 2.1 for a flow diagram showing allocation, excluded participants, and dropouts). In total 23 neurotypical healthy control participants and 26

individuals with RRMS were included in the final analysis. Characteristics of study participants are presented in Table 2.1. No significant differences were observed for age ($F_{(1,48)} = 0.13$, $p = 0.72$) or sex ($\chi^2_{(2)} = 0.36$, $p=0.55$) between cohorts. Additionally, weight, height, and BMI were not significantly different between groups. Both the neurotypical controls and PwMS demonstrated similar levels of weekly physical activity. The MS group reported a greater number of falls 6 months prior to participation. Specifically, three participants reported having one fall and four reported experiencing between 2 and 4 falls. The neurotypical control cohort reported no falls 6 months prior to participation.

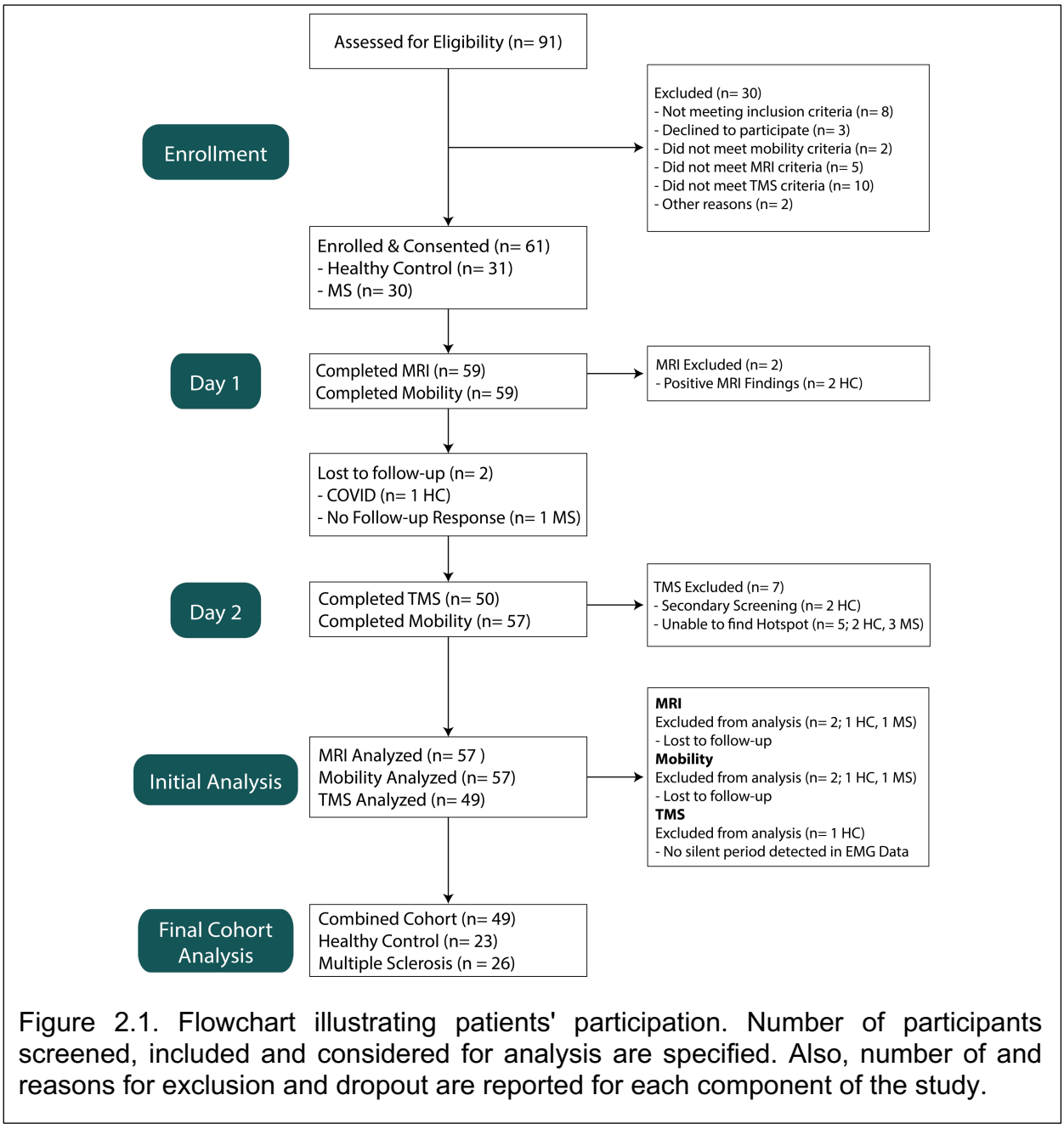

Figure 2.1. Flowchart illustrating patients' participation. Number of participants screened, included and considered for analysis are specified. Also, number of and reasons for exclusion and dropout are reported for each component of the study.

Table 2.1. Detailed Demographics and Participant Characteristics

	Control		MS		
	Mean	SD	Mean	SD	p-value
Gender	15F | 8M		19F | 7M		
Age (yrs.)	46.76	15.93	48.19	11.95	0.72
Height (in.)	66.87	2.99	65.25	2.88	0.06
Weight (lbs.)	158.48	29.09	150.77	20.61	0.29

BMI	24.79	3.35	24.99	3.84	0.84
Disease Duration (yrs.)	-	-	11.69	10.72	
EDSS (mean, range)	-	-	3.5	0-4	
Falls in the Last Month (#)	0	0	0.07	0.27	0.18
Falls Last 6 Months (#)	0	0	0.5	0.99	**0.02**
Exercise (% Yes)	96		96		
Exercise per Week (hrs.)	4.9	2.58	5.3	3.99	0.67
Type of Exercise					
Aerobic	9		14		
Anaerobic	1				
Aerobic + Anaerobic	12		10		
Other			1		
Education					
Highschool	0		1		
Associates	0		4		
BA/BS	10		8		
Masters	10		9		
Doctorate	3		4		
Questionnaires					
Becks Depression Inventory	2.43	3.16	7.77	7.2	**0.002**
Short Form-36					
General Health	83.26	11.24	59.04	22.89	**<0.0001**
Physical Function	96.28	5.06	75.96	23.02	**0.0001**
Energy/Fatigue	70.22	14.18	51.54	18.64	**0.0003**
Roles Limitations	94.56	18.39	60.58	38.84	**0.0004**
Social Functioning	92.93	14.51	74.52	21.65	**0.0012**
Pain	90.43	11.22	73.46	21.97	**0.0017**
Emotional Problems	91.3	20.64	61.54	42.89	**0.004**
Emotional Wellbeing	83.3	11.61	75.23	12.85	**0.026**
Change in Health	56.52	13.52	60.58	21.42	0.439
Modified Fatigue Impact Scale - Total Score (0-84)	-	-	31.85	14.67	
MS Walking Scale-12 (%)	-	-	26.12	24.9	
MS Walking Scale-12 Total Score	-	-	24.54	11.95	

Notes: BMI – Body Mass Index; EDSS – Expanded Disability Status Scale; BA/BS – Bachelor of Arts or Science, respectively.

360˚ IN-PLACE FAST TURNS

In-place 360˚ fast pace turns demonstrated significant differences between groups for both turn duration and peak turn velocity, while turn angle did not demonstrate significance between groups (Table 2.2). Specifically, PwMS demonstrated significantly longer turn durations ($t_{(47)}$ = 3.822, p = 0.0004) (Figure 2.2 – A) and slower peak turn velocities ($t_{(47)}$ = -3.556, p = 0.0009) (Figure 2.2 – B). While turn angle was not significantly different between groups ($t_{(47)}$ = -1.531, p = 0.13).

Table 2.2. Between group differences for 360˚ in-place fast turn variables measured using wireless inertial sensors.

	Control		MS		
Turn Variables	Mean	SD	Mean	SD	p-value
Turn Duration (s)	1.85	0.30	2.44	0.69	<0.001
Turn Velocity (˚/s)	368.61	65.98	293.30	80.36	<0.001
Turn Angle (˚)	384.60	11.72	378.57	15.36	0.13

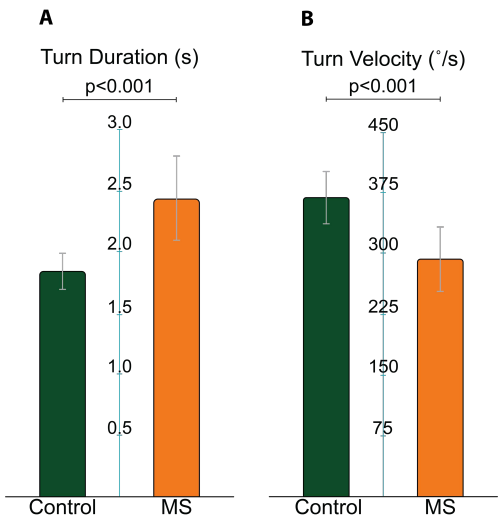

Figure 2.2. Turn duration (A) and turn velocity (B) demonstrated significant differences between neurotypical controls and PwMS.

360˚ IN-PLACE 1-MINUTE CONTINUOUS TURNS

Continuous but alternating, self-selected 360˚ in-place turns demonstrated significant differences for all turn variables (Table 2.3). Specifically, PwMS demonstrated significantly longer turn durations ($t_{(47)}$ = 2.376, p = 0.022) (Figure 2.3 – A), slower peak turn velocities ($t_{(47)}$ = -2.262, p = 0.028) (Figure 2.3 – B), reduced turn angles ($t_{(47)}$ = -2.198, p = 0.033) (Figure 2.3 – C), and fewer number of turns completed ($t_{(47)}$ = -2.210, p = 0.032) (Figure 2.3 – D)compared to neurotypical controls.

Table 2.3. Between group differences for 360˚ in-place 1-minute continuous turn variables measured using wireless inertial sensors.

	Control		MS		
Turn Variables	Mean	SD	Mean	SD	p-value
Turn Duration (s)	2.87	0.46	3.36	0.90	0.02
Turn Velocity (˚/s)	232.42	38.43	203.21	50.25	0.03
Turn Angle (˚)	391.05	15.25	377.53	25.77	0.03
Turns Completed (#)	19.43	3.26	16.96	4.40	0.03

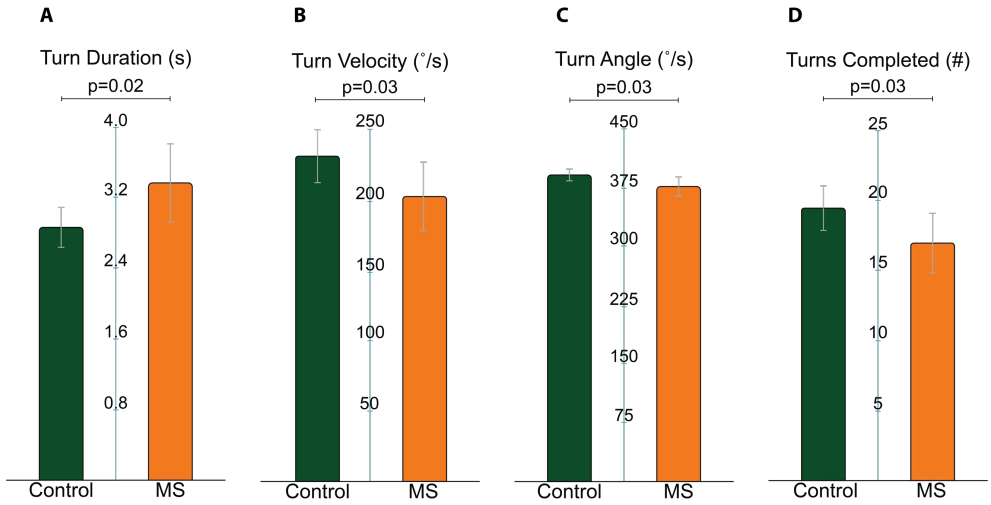

Figure 2.3. Turn duration (A), turn velocity (B), turn angle (C), and turns completed (D) demonstrated significant differences between neurotypical controls and PwMS.

180˚ SELF-SELECTED PACE TURNS WHILE WALKING

Turns completed during the self-selected pace two-minute walk test did not demonstrate significant differences between groups for turn duration, velocity, or angle (p ≥ 0.15)

(Table 2.4). Although the number of steps in the turn nearly met significance ($t_{(47)} = 2.008$, $p = 0.0504$) (Figure 2.4).

Table 2.4. 180˚ in-place self-selected pace turn variables measured with inertial sensor for neurotypical controls and PwMS.

	Control		MS		
	Mean	SD	Mean	SD	p-value
Turn Duration (s)	2.04	0.31	2.18	0.37	0.15
Turn Velocity (˚/s)	212.14	29.38	206.16	38.40	0.55
Turn Angle (˚)	184.44	7.93	183.48	7.91	0.67
Steps in Turn (#)	3.57	0.67	3.96	0.67	0.0504

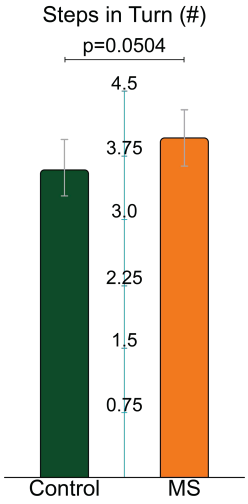

Figure 2.4. Steps in the turn demonstrated near significance between neurotypical controls and PwMS.

DISCUSSION

The primary objectives for this study were to examine spatiotemporal characteristics of turning in-place and while walking in PwMS and neurotypical control participants using wireless inertial sensors and validated algorithms. In partial agreement with our hypothesis, our results highlighted significant differences for 360˚ in-place turning measures. Conversely, no significance was observed between groups for any turn

variables measured during the 180˚ turns performed while walking, rejecting our hypothesis.

For the 360˚ in-place fast pace turns, our results demonstrated significant differences for both turn duration and peak turn velocity between groups. Specifically, PwMS took longer to complete 360˚ in-place turns at their self-selected fast pace and did so at a reduced peak velocity compared to their neurotypical counterparts. Similar to the results from the fast pace 360˚ turns, the one minute of consecutive but alternating (i.e., clockwise with an immediate counterclockwise turn) turns demonstrated significance between groups. Specifically, turn duration, peak turn velocity, turn angle, and number of turns all demonstrated significance between groups. Of the turns completed, PwMS demonstrated turns that were longer in duration and slower in speed. Additionally, PwMS showed significantly reduced turn angles, subsequently leading to fewer completed turns over the duration of the trial. The results from the two 360˚ turn measures may indicate that PwMS, regardless of increased speed, maintain reduced turning performance compared to neurotypical controls. Thus, our results suggest that PwMS when asked to perform turns at their self-selected fast pace are either unable or unwilling to increase their speed to the same degree as their healthy control counterparts. These results parallel prior studies that have documented increased turn duration in PwMS, which has been shown to be associated with increased disability and fall risk (Spain, St George et al. 2012, Pau, Porta et al. 2017, Adusumilli, Lancia et al. 2018, Soke, Guclu-Gunduz et al. 2019). While some MS participants reported experiencing falls within the prior six months of participation, we did not run any associations between number of falls and turn duration given that the majority of MS participants reported experiencing no falls.

Turn angle for the 1-minute 360˚ self-selected pace turns were reduced in PwMS, such that their turn angles on average were closer to the prescribed 360˚ mark compared to the neurotypical controls who averaged a turn angle of 391˚. Interestingly, this finding emulates results from a recently published study by Shah, et al. (2020) who reported a reduction in turn angle in PwMS over the course of seven days of continuous monitoring (Shah, McNames et al. 2020). Although our study design was different from Shan, et al. (2020) we postulate that PwMS perform reduced turn angles in a variety of circumstances due to an abundance of caution. Such that if PwMS were to lose balance, they may not be able to produce an appropriate compensatory step response (Peterson, Huisinga et al. 2016). However, the true functional significance of reduced turn angles in PwMS remains unknown.

For the 180˚ turns performed while walking at a self-selected pace, no significant differences for any of the turn variables were observed between groups. These results are contrary to previously reported 180˚ turns differences between PwMS and neurotypical controls. For instance, Spain, el al. (2012) reported significantly longer 180˚ turn durations in PwMS compared to neurotypical controls during an instrumented Timed Up and Go (iTUG) test (Spain, St George et al. 2012). Although, a recent report demonstrated that 180˚ turn duration during the iTUG is influenced by disease severity, such that no significant differences in turn duration were observed for PwMS having an EDSS between 0-1.5, although for those with greater severity (i.e., between 2-3.5 and 4-6.5) significantly longer turn durations were observed in PwMS (Pau, Porta et al. 2017). However, for peak turn velocity studies have demonstrated similarities between neurotypical controls and PwMS, which mimics the results from the current study (Spain,

St George et al. 2012). Lastly, it should be noted that the current study is capturing 180˚ turn variables during a self-selected pace two-minute walk test, while other studies are reporting the same turning variables captured during the iTUG, which inherently could provide different results due to the task objectives. For example, the objective of iTUG is to complete the test as quickly as possible, with a shorter total duration suggesting better mobility. While the self-selected pace two-minute walk test we performed was not based on performance (i.e., time or distance covered), rather we sought to capture a number of 'natural' 180˚ turns.

Together, these results may indicate that PwMS demonstrate an altered turning characterisitcs and subsequently reduced turning performance when compared to neurotypical control participants for 360˚ in-place turns at different speeds. However, turns performed while walking did not demonstrate any significant differences between groups. Possibly suggesting that task complexity and turn style may provide important turn related kinematic differences between PwMS and neurotypical controls.

CONCLUSION

In summary, a variety of spatiotemporal measures of turning demonstrated significance between neurotypical control participants and PwMS. PwMS demonstrated an altered turning strategy which involved an increased turn duration and reduced peak turn velocity and turn angle for 360˚ turns. Conversely, turning strategies appeared to be similar for 180˚ turns while walking between neurotypical controls and PwMS. Additionally, as MS is a neurodegenerative disease the neural underpinnings associated with the observed kinematic differences remain to be explored.

CHAPTER 3 – NEUROPHYSIOLOGICAL DIFFERENCES BETWEEN NEUROTYPICAL CONTROLS AND PEOPLE WITH MULTIPLE SCLEROSIS

INTRODUCTION

In 1985 Dr. Anthony T. Barker invented transcranial magnetic stimulation (TMS) which allows for the non-invasive assessment of neurophysiology and biochemical properties of humans *in vivo* (Barker, Jalinous et al. 1985). TMS is performed by eliciting a nearly two centuries old physics law known as Faraday's Law of electromagnetic induction. Briefly, electromagnetic induction works by inducing a voltage into a closed-circuit conductor by passing the voltage through a magnetic field or by moving the magnetic field past the closed-circuit conductor, ultimately eliciting an electrical current. Since the 1980s, vast technological improvements have been developed allowing for various TMS paradigms to be implemented in research and clinical settings. These developments have established a myriad of paradigms for the assessment of neurophysiology, neural transmission, and neural plasticity in both neurotypical and atypical populations including multiple sclerosis (MS).

In 2019, it was estimated that nearly 1 million individuals in North America live with MS (Wallin, Culpepper et al. 2019). MS is a progressive neuroinflammatory, immune-mediated disease of the central nervous system (CNS) which affects a variety of neural structures, functions, and biochemical processes. People who suffer from MS often develop non-localized lesions and vast axonal demyelination throughout the CNS, subsequently impacting the function of the peripheral nervous system. Due to the heterogeneity of neurodegeneration throughout the CNS people often display a diverse set of symptoms and complications. Although diverse, some similarities exist, such as

muscle weakness, fatigue, impaired cognition, lack of coordination, and mobility deficits (Kent-Braun, Ng et al. 1997, Hamilton, Rochester et al. 2009, Sosnoff, Socie et al. 2014, Cameron and Nilsagard 2018, Mordillo-Mateos, Soto-Leon et al. 2019, Richmond, Swanson et al. 2020). Due to the neural impairments associated with MS, researchers have employed various neuroimaging techniques to elucidate some of the neural underpinnings accompanying the aforementioned devastating symptoms seen in people with MS (PwMS).

In fact, in 1986 Dr. Barker (inventor of TMS) published the first account of TMS to be used in PwMS to assess neural conduction times in neurotypical individuals and those with MS (Barker, Freeston et al. 1986). Since these early investigations, researchers have developed a greater understanding of the neurophysiological measurement capacities TMS offers. While conventional use of TMS was primarily implemented to measure conduction times within the motor tracts of the CNS, modern studies often perform TMS to assess relative levels of excitatory and inhibitory synaptic activity. Specifically, researchers typically use TMS to measure glutamatergic (excitatory) and GABAergic (gamma-aminobutyric acid, inhibitory) neurotransmission (Figure 3.1).

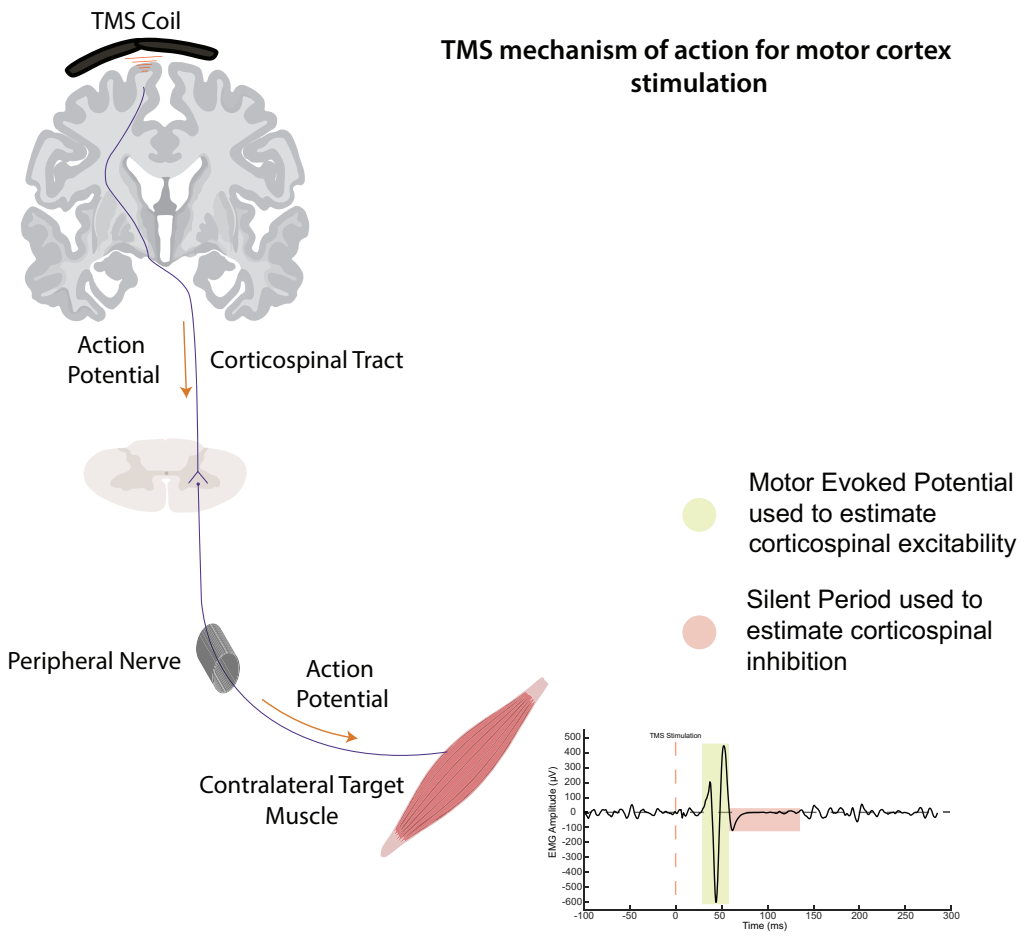

Figure 3.1. Transcranial magnetic stimulation (TMS) applied over the motor cortex activates interneurons oriented parallel to the brain surface. This coil placement leads to a trans-synaptic activation of pyramidal cells evoking descending volleys in the corticospinal tract. Motoneuron activation in response to corticospinal volleys leads to a brief contraction in the target muscle termed a motor evoked potential (MEP), which is recorded through electromyography (EMG) surface electrodes applied over the muscle belly. The MEP can be used to estimate excitability of the corticospinal tract, while the silent period which follows the MEP can be used to estimate corticospinal inhibition.

Corticospinal excitability is often referred to as the dynamic balance between excitatory and inhibitory (E-I) inputs within the corticospinal circuits and typically measured through the quantification of motor evoked potentials (MEPs). While corticospinal excitability is largely, although not entirely (e.g., dopaminergic and cholinergic, etc.), driven by glutamatergic (excitation) and GABAergic (inhibitory)

interactions, motor evoked potentials (MEPs) are a common measure representing the state of post-synaptic cortical excitability and pre-synaptic intracortical processes (Bestmann and Krakauer 2015). Thus, providing a measure of relative excitability often referred to as glutamatergic activity.

MEPs are derived by delivering a single suprathreshold stimulus (typically 120% above resting motor threshold) over a specific region of the motor cortex known as the 'hotspot' resulting in the depolarization of neurons and a subsequent action potential descending the corticospinal tract. The result is then recorded as a brief burst of EMG such that the target muscle of interest often visibly twitches. From a single TMS stimulation, a number of MEP characteristics can be assessed including, latency, amplitude, duration, and area which all are largely thought to represent glutamatergic activity (Ziemann 2004) (Figure 3.2).

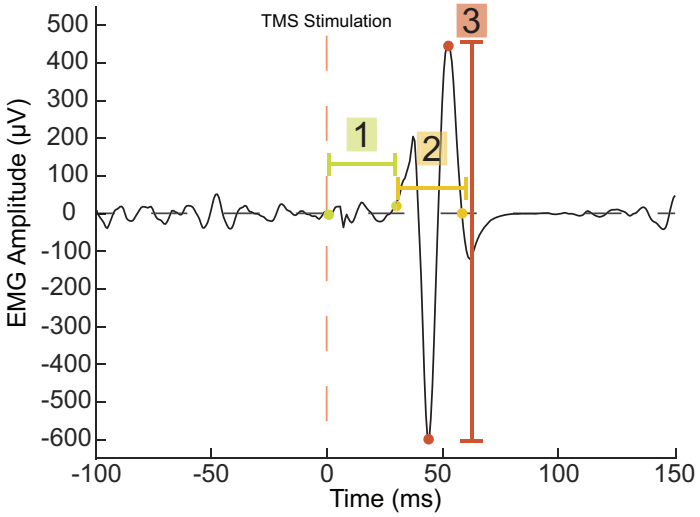

Figure 3.2. Motor evoked potential (MEP) characteristics. 1. MEP latency (ms), 2. MEP duration (ms), 3. peak-to- peak MEP amplitude (μV or mV). MEP area (ms/amp) is be measured as the sum of deflections above and below the baseline EMG, which can also be measured via rectified EMG trace.

GABA is the primary inhibitory neurotransmitter in the CNS and plays a vital role in inhibiting neurotransmission. Measuring GABAergic function through the use of TMS

can be assessed using several TMS paradigms, although the assessment of the cortical silent period (cSP) duration remains the most common for single pulse TMS. The cSP is a measure of corticospinal inhibition specifically thought to measure the metabotropic $GABA_B$ receptor (Fuhr, Agostino et al. 1991, Cantello, Gianelli et al. 1992, Siebner, Dressnandt et al. 1998, Werhahn, Kunesch et al. 1999) which is derived by stimulating the motor cortex while a participant performs a low-level voluntary muscle contraction (≤20% maximal voluntary contraction (MVC)) contralateral to the stimulated hemisphere. Given that the cSP is a measure of both spinal and cortical inhibitory mechanisms the first 0 – 50ms of the silent period are thought to reflect spinally mediated inhibition via recurrent inhibition by Renshaw cell activation, motoneuron after-hyperpolarization, or disynaptic inhibition via Ia inhibitory interneurons (Fuhr, Agostino et al. 1991, Cantello, Gianelli et al. 1992, Roick, Vongiesen et al. 1993, Hupfeld, Swanson et al. 2020). While the following 50 – 200ms is thought to be the result of intracortical inhibitory suppression of corticospinal outputs (Fuhr, Agostino et al. 1991, Inghilleri, Berardelli et al. 1993, Chen, Lozano et al. 1999). As such, the stimulation results in a brief disruption to the ongoing EMG signal, known as a silent period (Figure 3.3). As mentioned, the cSP is typically quantified as the duration of the EMG disruption, such that a shorter duration suggests reduced GABAergic activity while longer silent periods suggest greater levels of GABAergic synaptic activity.

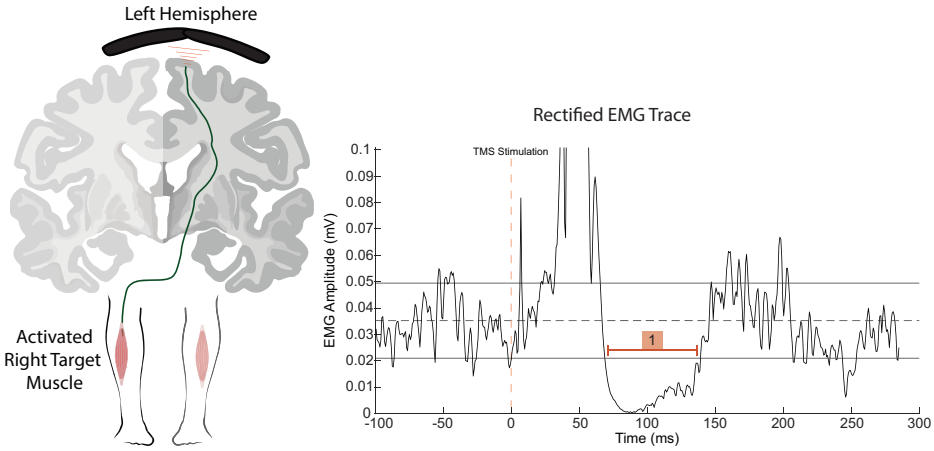

Figure 3.3. Cortical mechanism for cSPs. Here we depict left hemisphere cortical stimulation to the leg region of the primary motor cortex concurrently to an isometrically activated right tibialis anterior (TA) target muscle. Rectified EMG from the active TA is shown to the right, where the cortical response then includes $GABA_B$-receptor mediated intracortical inhibition resulting in an EMG disruption lasting up to a couple hundred milliseconds in the target muscle (1. represents cSP duration).

In a typical nervous system, these two neurotransmitters work together, maintaining a homeostatic balance, where GABAergic neurotransmission inhibits excitatory activity allowing for harmonious neural responses. However, in MS it is believed the dysregulation of these two neurotransmitters have pathophysiological implications which may contribute to disease severity, progression, and motor impairments (Bassi, Mori et al. 2017). A number of human and animal based histopathological studies have documented the dysregulation of the glutamatergic system in MS, discovering excess amounts of extracellular glutamate (Pitt, Werner et al. 2000, Pitt, Nagelmeier et al. 2003, Macrez, Stys et al. 2016). Importantly, this upregulation of extracellular glutamate is implicated in a variety of neural impairments observed in MS, and believed to contribute to excitotoxicity (i.e., harmful accumulation of extracellular glutamate) which at substantial levels (often seen in MS) leads to significant myeline damage, MS related relapses, and extending disease severity (Macrez, Stys et al. 2016). While the majority of extracellular glutamatergic studies in MS have been performed either postmortem or through animal

models the use of non-invasive TMS *in-vivo* has gained attention as an alternative method for assessing neurophysiological imbalances in PwMS. However, it must be noted that TMS is unable to directly quantify levels of extracellular neurotransmitters, although the technique does provide a measure of neurophysiological synaptic activity. As such, researchers have been assessing various adaptations between excitatory and inhibitory neurotransmitters in PwMS, and furthermore associating them to disease related behaviors (Nantes, Zhong et al. 2016).

The majority of TMS studies conducted in PwMS have focused on the neurophysiology of both cortical and corticospinal motor systems, particularly for the upper limbs. This is primarily a result of being able to easily stimulate the upper limb somatotopy (Figure 3.4) without much difficulty (Kukowski 1993, Boroojerdi, Hungs et al.

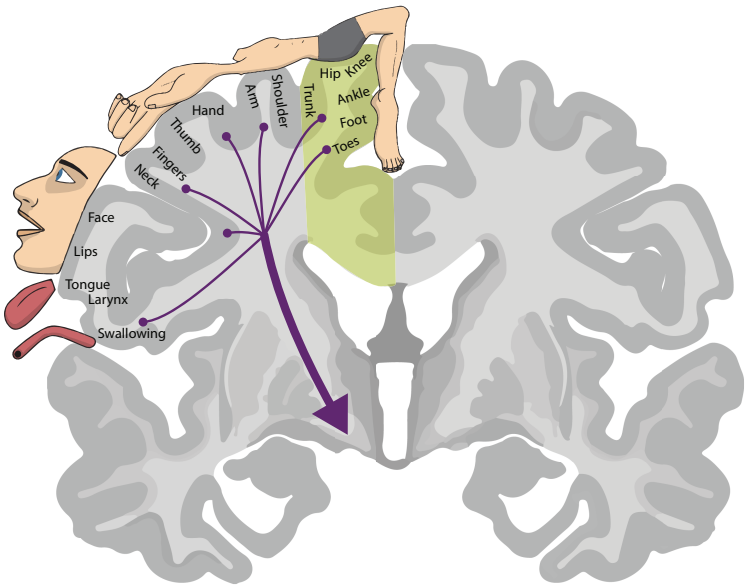

Figure 3.4. Somatotopic representation of the motor cortex (i.e., precentral and paracentral gyrus). Detailing the cortical regions responsible for descending command via the corticospinal tract (CST) for muscle activation. For instance, the region in green represents the paracentral gyrus and is the cortical region responsible for descending command to the lower extremities. Furthermore, this is the region targeted via single pulse TMS in the current study to assess neurophysiological mechanisms to the tibialis anterior (TA).

1998, Hoppner, Kunesch et al. 1999, Cruz-Martinez, Gonzalez-Orodea et al. 2000, Petajan and White 2000, Caramia, Palmieri et al. 2004, Conte, Lenzi et al. 2009, Kale, Agaoglu et al. 2009, Mori, Kusayanagi et al. 2013, Nantes, Zhong et al. 2016, Nantes, Zhong et al. 2016, Neva, Lakhani et al. 2016, Chaves, Wallack et al. 2019). This research has led to a number of important discoveries regarding neuropathophysiology in MS. For instance, upper extremity TMS studies have documented associations between biochemical processes, cognitive function, fatigue, disease severity, and upper limb motor control (Hallett 2000, Jahanshahi and Rothwell 2000, Liepert, Mingers et al. 2005). For instance, the assessment of excitatory neurotransmission to the upper limbs has identified decreases in excitability (i.e., reduced MEP characteristics) in PwMS (Cruz-Martinez, Gonzalez-Orodea et al. 2000, Petajan and White 2000, Gagliardo, Galli et al. 2007, Kale, Agaoglu et al. 2009, Neva, Lakhani et al. 2016). Functionally, these decreases have been associated with a variety of behavioral outcomes including greater disease severity (Cruz-Martinez, Gonzalez-Orodea et al. 2000, Kale, Agaoglu et al. 2009, Neva, Lakhani et al. 2016). In addition, inhibitory neurotransmission has been associated with functional behavior in PwMS. Specifically, one study assessing intrahemispheric GABAergic function of the upper limbs in PwMS found associations between levels of inhibition and manual dexterity impairments, where those with greater inhibitory activity demonstrated greater levels of impairment (Nantes, Zhong et al. 2016).

While the majority of studies have assessed the neurophysiological associations to the upper limbs, few have explored the lower limbs for possible similarities or differences. Interestingly, the first study to investigate the lower limbs using TMS in PwMS was conducted in 1991 and discovered longer neural transmission times and the presence of

“abnormal” MEPs (Jones, Streletz et al. 1991). Since this initial recording, the majority of follow-up studies assessing the lower limbs have focused on the neurophysiological assessment of excitability (Schmierer, Niehaus et al. 2000, Tataroglu, Genc et al. 2003, Jorgensen, Nielsen et al. 2005, Groppa, Oliviero et al. 2012, Di Sapio, Bertolotto et al. 2014). Similar to the upper limb results, the initial lower limb studies of glutamatergic function have discovered reduced glutamatergic activity (Kukowski 1993, Cruz-Martinez, Gonzalez-Orodea et al. 2000, Schmierer, Niehaus et al. 2000, Tataroglu, Genc et al. 2003, Jorgensen, Nielsen et al. 2005, Sahota, Prabhakar et al. 2005, Gagliardo, Galli et al. 2007). While studies have demonstrated reduced glutamatergic activity for the lower limbs, to our knowledge only one study (conducted nearly 20 years ago) has assessed inhibitory function related to the lower limbs (Tataroglu, Genc et al. 2003, Kiylioglu, Parlaz et al. 2015). This study demonstrated similarities to the upper limb inhibitory literature, showing longer cSP durations in three phenotype specific clinical MS cohorts compared to neurotypical controls (Tataroglu, Genc et al. 2003).

Since the initial TMS investigations in PwMS, the assessment of neurophysiology related to the upper limbs remains substantially more comprehensive in the literature. The more recent findings suggest the effects of neurophysiological alterations (e.g., excitatory and inhibitory neurotransmitters) are impacted in PwMS, and associated with upper limb motor control and more broadly disease severity (Kukowski 1993, Cruz-Martinez, Gonzalez-Orodea et al. 2000, Liepert, Mingers et al. 2005, Sahota, Prabhakar et al. 2005, Gagliardo, Galli et al. 2007, Goetz, Tilley et al. 2008, Chaves, Wallack et al. 2019). While neurophysiological assessments to the upper limbs continue to be assessed there remains a dearth of literature examining similar associations between neurophysiology

and motor control and disease severity to the lower limbs in PwMS. However, the foundation for exploring neurophysiological associations with lower limb motor control resides in the healthy aging literature which has documented relationships between inhibitory activity and lower limb motor control (Fling and Seidler 2012, Swanson and Fling 2018). For instance, greater levels of inhibition via longer cSP durations have demonstrated associations between linear gait coordination in neurotypical older adults (Swanson and Fling 2018). Moreover, levels of inhibition have been associated with nonlinear styles of ambulation such as measures of turning (Swanson and Fling 2019). Specifically, Swanson, et al. (2020) demonstrated significant associations between the cSP duration and turning performance, demonstrating that older adults with greater inhibition perform turns more characteristic of younger adults (Swanson and Fling 2019). These results demonstrated that inhibitory activity is not only associated with upper limb motor control but also appears to play an important role in lower limb dynamic movements. While initial studies have demonstrated altered neurophysiological function in PwMS these studies are few in number and remain inconclusive. Additionally, it remains to be determined whether altered neurophysiological function of the corticospinal system in PwMS is associated with dynamic lower limb motor performance.

Therefore, the purpose of this study was to assess excitatory and inhibitory neurophysiology in PwMS and determine whether there are any associations to dynamic lower limb movements, thus providing a systemic context as to the neurophysiological adaptations associated lower limb motor control in MS. As such we have identified three primary hypotheses: 1) we hypothesize that PwMS will demonstrate reduced glutamatergic activity to the lower limbs compared to neurotypical controls. Specifically,

we hypothesize that PwMS will demonstrate reduced MEP amplitude compared to neurotypical controls. 2) we hypothesize that PwMS will demonstrate altered inhibitory (GABAergic) activity as measured via the cortical silent period to the lower limbs compared to neurotypical controls. Specifically, we hypothesize longer silent period durations similar to previously published literature, in addition we will be assessing two other measures of inhibitory function termed average percent depth (%dcSP Ave) and max percent depth (%dcSP Max) of the silent period, which we hypothesize will be reduced in the MS cohort. 3) we hypothesize that PwMS will demonstrate a positive association between corticospinal excitation and turning performance, whereas PwMS will demonstrate a negative association between corticospinal inhibition and turning performance.

METHODS

Participants were seated in an adjustable upright chair with their feet placed on a stationary platform (Figure 3.5). Motor evoked potentials were elicited bilaterally from the tibialis anterior (TA) muscle MagPro ×100 stimulator (MagVenture, Farum, Denmark) using a 2 × 95 mm angled butterfly coil (120-degree, Cool D-B80). For consistency across participants the scalp was mapped using a permanent marker. The center of the head (Cz) was determined by measuring from the nasion to inion and from the tragus of each ear (Homan, Herman et al. 1987). Once the Cz was marked, additional marks were made laterally to each side from the Cz at 2 cm and then anteriorly 5.5 cm to account for the size of the coil in order to place the center of the coil over the 2 cm mark (Groppa, Oliviero et al. 2012). After initial markings were made, a sagittal line was placed for consistent visual coil placement. The coil was placed tangentially against the scalp at roughly 45°

from the mid-sagittal line which is roughly perpendicular to the central sulcus allowing for optimal current direction for stimulus (Groppa, Oliviero et al. 2012). With participants seated and relaxed, the 'hot spot' for cortical stimulation of the TA was determined as the location where the TMS stimulus evoked a maximal EMG response from the TA contralateral to the hemisphere being stimulated. To limit the potential for hemispheric propagation (i.e., stimulation overflow on to the opposing hemisphere) during testing trials, MEPs during hot spot detection were assessed in both leg muscles independently. The resting motor threshold (RMT) was determined in both hemispheres and defined as the lowest stimulator intensity to evoke a muscle response of at least 50 μV in five out of ten trials. Muscle activity was recorded via bipolar EMG electrodes (Ag/AgCl, 11 mm diameter (part number: EL503), BIOPAC Systems, Inc) sampled at 2000 Hz, and transmitted to a laboratory computer.

Prior to TMS collection participants were asked to produce a series of maximal voluntary contractions (MVCs) to determine their maximal force output for each TA. Participants' legs (individually) were secured to the foot platform using a strap secured around the dorsum of the foot (Figure 3.5). Additionally, a Velcro strap was secured around the heel to account for any posterior foot slip during the MVC and TMS trials. The strap around the dorsum of the foot was attached to a high-capacity carabiner and a force transducer (part number: TSD121C, BIOPAC Systems, Inc) which was securely attached to the base of the foot platform. The strap was tightened around the foot to limit any ability for dorsiflexion or the foot being able to lift off the platform. Each participant conducted between two-five MVCs which were transmitted to the laboratory computer for instant measurement analysis. MVCs were concluded when force production no longer

increased across attempts and the two highest force producing values were within 10% of each other. The same process was replicated for the opposite leg.

TMS COLLECTION

A variety of protocols have been developed to elucidate the neurophysiology and utility of TMS for the study of the lower limbs. The most common NIBS technique and the technique used in this study was single pulse TMS (spTMS) which delivers a single suprathreshold electromagnetic stimulation to the primary motor cortex to measure the response via EMG at a particular muscle(s) of interest, the TA for this study.

Nearly all forms of spTMS require stimulation of the primary motor cortex to assess the neurophysiological properties of the central and peripheral nervous systems. Once MVCs were collected participants underwent a randomized spTMS protocol. The spTMS protocol included the collection of the excitatory and inhibitory measures. Specifically, we collected MEP amplitude which is an excitatory measure, and three inhibitory measures termed the cortical silent period duration (cSP duration), %dcSP Ave, and %dcSP Max.

The cSP was tested in both hemispheres and the corresponding TA. To elicit the silent period response, participants were asked to maintain an isometric contraction at 15% of their MVC. For the cSP trials, participants were asked to maintain an isometric contraction of the contralateral TA for the duration of the trial (three minutes). To ensure 15% MVC participants were given visual biofeedback on a screen directly in front of them, which depicted a circular dial and a translucent blue wedge (Figure 3.5). Within the dial a

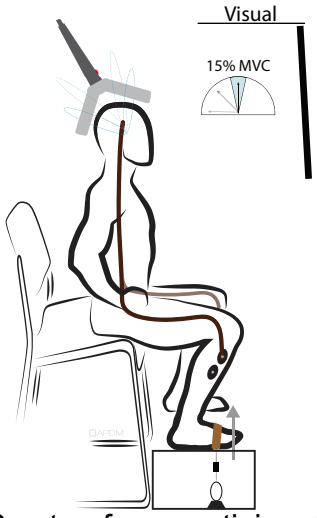

Figure 3.5. Representative TMS setup for a participant. Participants foot was secured to a platform connected to a force transducer all while gazing at a visual target to maintain constant 15% MVC force output while receiving TMS stimulations.

gauge would rotate around the axis depending on the amount of force being produced. The blue wedge would indicate to the participant where the gauge should be maintained (i.e., 15% MVC) for the duration of the trial. Participants were asked to maintain the gauge within the blue wedge to the best of their ability, if a simulation produced a muscle twitch causing the gauge to exit the wedge, they were asked to adjust their force output accordingly to place the gauge back into the wedge prior to the next stimulation. Each of the trials were 3 minutes in duration, during which time the researcher gave a stimulation at 120% of the RMT every 7–10 seconds with a total number of stimulations averaging

around 20 simulations per hemisphere (40 total). Concurrent to the cSP trials, MEPs were collected as they are also a product of the stimulation.

TURNING COLLECTION

Please refer back to Chapter 2 for turning collection details.

ANALYSIS

TMS DATA ANALYSIS

Prior to analyzing the TMS measures, the EMG signal from each trial was filtered using a Nyquist combination band filter (60Hz) offline using AcqKnowledge software (BIOPAC Systems, Inc., Santa Barbara, CA). Filtered data was then imported and rectified using an automated custom MATLAB (MathWorks, Nantick, MA) script to identify and quantify TMS characteristics.

For the quantification of MEPs and cSP variables, 100ms prior to the simulation and 350ms post stimulation were extracted from the EMG trace for every stimulation. Those EMG segments were then imported into an alternative vector. Each of the EMG traces were then visually inspected for quality; the trials which did not demonstrate a clear or expected trace were removed from further analysis (Figure 3.6). The remaining traces were withheld and subsequently averaged together to produce one averaged EMG trace which was used to quantify the MEP amplitude and silent period variables. For each subject the variables measured included silent period duration, max percent depth of SP (dcSP% Max), and average percent depth of SP (dcSP% Ave).

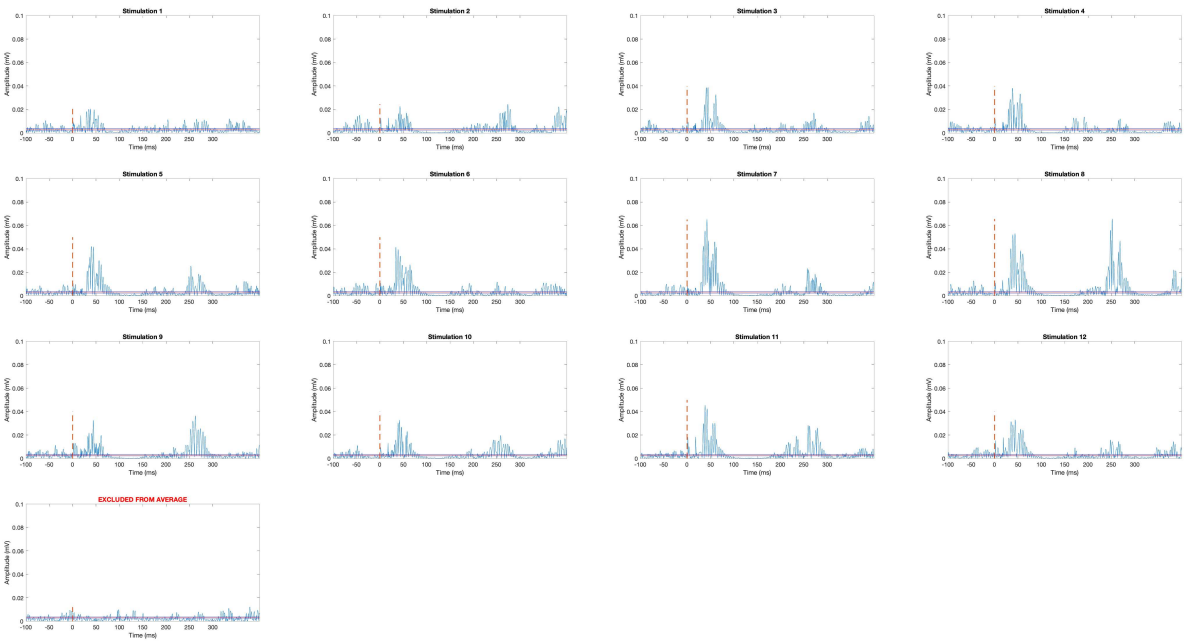

Figure 3.6. Representative example of our TMS analysis and the exclusion (stimulation 13 for this example) of particular stimulations which did not capture a typical MEP or silent period response.

MEAN CONSECUTIVE DIFFERENCE QUANTIFICATION

The cSP characteristics were quantified first by determining the mean consecutive difference (MCD) of 100ms prior to stimulation. The MCD is the mean successive difference between individual EMG data points. For interpretation, the smaller the difference is between successive data points the smaller the MCD, while larger differences (greater variability) between EMG data points, the larger the MCD would be from the mean EMG data. The absolute value for those differences was then calculated, and a mean of those absolute values was quantified thus providing the MCD for that trial.

CALCULATION OF MCD:

```
%  1) Calculate difference column; this "difference" column is the difference between successive data points (D1-D2, D2-D3, D3-D4, etc.)
Prestim_Difference = (SP_prestim_EMG(1:end-1) - SP_prestim_EMG(2:end));

%  2) Take the absolute value of the difference column.
Prestim_absDifference = abs(Prestim_Difference);
```

```
% 3) Take the mean of the absolute values of the difference column. This is the peristimulus MCD.
Prestim_MCD = mean(Prestim_absDifference);
```

Following the calculation of the MCD, standard deviation limits were set by multiplying the MCD by 2.66 (or three standard deviations from the mean), which remains a common multiplicative level used to set the threshold limits for determining silent period characteristics (Hupfeld, Swanson et al. 2020).

MOTOR EVOKED POTENTIAL QUANTIFICATION

Similar to the quality check of the silent period data, the individual MEPs were visually inspected based on their characteristics. Of those withheld they were averaged together, producing one average MEP trail to quantify the MEP amplitude. The amplitude of the MEP was assessed as the maximum EMG amplitude achieved relative to the MCD (Figure 3.7 – C).

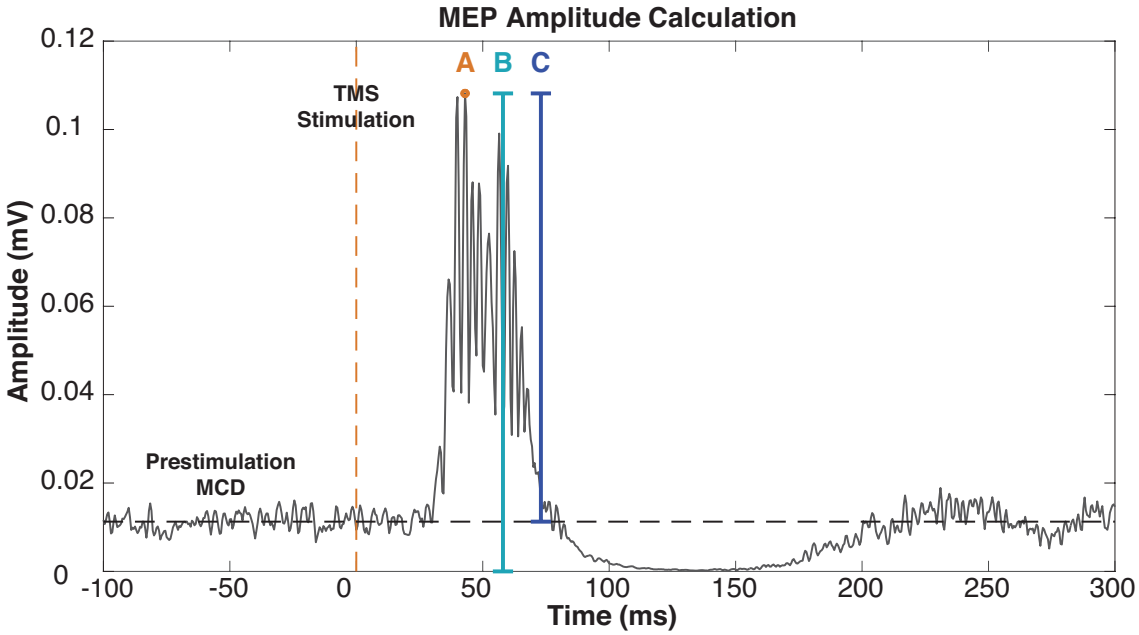

Figure 3.7. Representative MEP Calculation: A) Max amplitude measures on the y-axis. B) Maximum amplitude from 0 mV. C) Relative maximum amplitude from the MCD.

CALCULATION OF MEP AMPLITUDE:

```
SP_ymax = (max(SP_Average_EMG(100:500)));
```

CALCULATING THE SILENT PERIOD METRICS

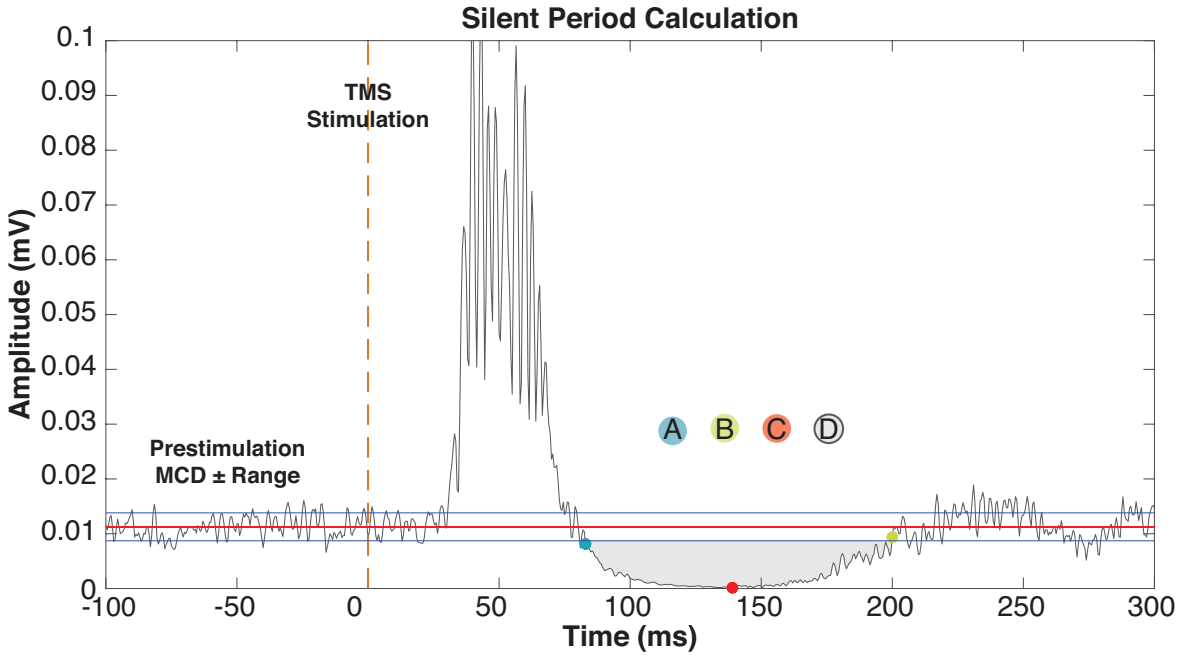

Figure 3.8. Representative silent period metric calculations: A) Silent period onset. B) Silent period offset. C) %dSP Max. D) %dSP Ave.

CALCULATION OF SILENT PERIOD (ONSET):

After the MCD limits were set, the *onset* of the silent period was determined. The *onset* was defined as the first data point to fall *below* the lower variation limit for five consecutive data points (Figure 3.8 – A).

```
for i = (250:length(SP_Average_EMG))

% Starting from stim (i.e., data point #200) scroll through the data
  if SP_Average_EMG(i)<SP_NegLine && SP_Average_EMG(i+1)<SP_NegLine
&&                SP_Average_EMG(i+2)<SP_NegLine                &&
SP_Average_EMG(i+3)<SP_NegLine && SP_Average_EMG(i+4)<SP_NegLine
&& SP_Average_EMG(i+5)<SP_NegLine
% Marks the point where the trace goes below the negative limit line (based on
MCD) for 5 consecutive data points.

        SP_start(i)= i;
```

```
% The point where the trace goes below the negative limit line for 5 consecutive
points is called SP_start and tells us the row # of the SP start.
  break
  end
end
```

CALCULATION OF SILENT PERIOD (OFFSET):

The *offset* was defined as the first data point to fall *above* the lower variation limit. To limit false positive offsets and natural variation of the EMG signal, 50% of the following 5ms of data points (upon first offset detected data point) must be above the variation limit (Figure 3.8 – B).

```
for i = SP_Start_Location:(length(SP_Average_EMG))
% Set the origin as the start location of SP onset, then have it travel through the
length of the data.
  if SP_Average_EMG(i)>SP_NegLine
    for k = 1:10
      pts(k)=SP_Average_EMG(i+k)>SP_NegLine==true;
% Get true=1 / false=0 value in matrix to indicate whether each of 10 points after
the identified offset value are > lower limit line.
    end
       if sum(pts) > 5
% If sum of the pts matrix > 5, you have >5/10 points after the identified offset
that were > lower limit line.
      SP_end(i)= i;
% This is the off point for the silent period.
      break
% This ends the loop once you've found the first spot that qualifies as an offset.
    end
  end
end

SP_End_Location = find(SP_end);
% We now know where the start location is. It is the FIRST value that has a # in
the matrix we just created. The first time the criterion for offset was true.
```

CALCULATION OF SILENT PERIOD (DURATION):

After the *onset* and *offset* were identified in the data, the calculation of silent period duration was determined by subtracting the *offset* from the *onset*.

```
% Calculate SP Duration
SP_Duration = (SP_end_time - SP_start_time);
% SP duration is the end time subtracted by the start time
```

PERCENT DEPTH OF CORTICAL SILENT PERIOD (DCSP%MAX & DCSP%AVE):

Depth values of the silent period represent measures of the neurophysiological inhibitory capacity and the resultant inhibitory effect on muscle activity (Wassermann, Fuhr et al. 1991, Jung and Ziemann 2006, Fling and Seidler 2012). These measures are quantified as percentages of the pre-stimulation EMG mean and quantified as the lowest (i.e., %dcSP Max) and the average (%dcSP Ave) EMG amplitude during the silent period. To quantify the dcSP% Max (minimum EMG amplitude during the silent period) the lowest EMG amplitude is determined and divided by the pre-stimulation average then multiplied by 100 (Figure 3.8 – C).

```
% Calculate %dcSP Max
SP_dSPmax = (100-((SP_ymin/SP_prestimave)*100));
```

Whereas the dcSP% Ave is a measure of the average EMG amplitude during the silent period. This value is quantified by calculated the average EMG amplitude during the silent period and dividing that value by the pre-stimulation average multiplied by 100 (Figure 3.8 – D).

```
% Calculate %dcSP Average
SP_dSPavg_matrix=(100(((SP_Average_EMG(SP_Start_Location:SP_End_Lo
cation)/SP_prestimave))*100));
```

TURNING DATA ANALYSIS

Refer back to Chapter 2, for turning analysis.

STATISTICAL ANALYSIS

All statistical analysis was performed in JMP Pro 15 with an alpha level set at 0.05 unless otherwise noted. A total of three participants (one neurotypical control, two MS)

had one hemisphere (left hemisphere, left and right hemisphere, respectively) that did not produce any quantifiable TMS measures. Additionally, two participants demonstrated noisy MEP measures which were unable to be characterized, although their silent period data was unaffected. Participants with one quantifiable hemisphere or had full inhibitory data were kept for all further analysis.

Between-group differences for age were examined via a one-way ANOVA while between-group sex differences were examined via a chi-square test. To determine between-group differences for all other demographic measures independent t-tests were performed. For TMS measures the effect of age was assessed as a potential covariate but analysis showed no statistical significance and was therefore, removed from further analysis. As such, a linear mixed-effects model was developed based on the combination of fixed (groups and hemispheres) and random (subject) effects within the study design. Fixed effects and interactions were further examined for significance. Post-hoc analysis of interactions was assessed using a one-way ANOVA. Turning analyses was described earlier (Chapter 2), although briefly independent t-tests were used to determine group differences.

Linear regression was used to assess correlations between hemisphere specific TMS measures and turning variables. Correlation strength between TMS and turning metrics were calculated using Pearson correlation coefficients and corrected for multiple comparisons using a Bonferroni correction with an alpha level set to 0.025 unless otherwise noted. Correlations strengths were classified as very strong (0.9–1.0), strong (0.7–0.9), moderate (0.5–0.69), weak (0.3–0.49) and negligible (< 0.30) (Mukaka 2012). All data are presented as mean ± SD unless noted otherwise.

RESULTS

PARTICIPANT CHARACTERISTICS

Complete participant characteristics, demographics, and questionnaire results of study participants are presented in Table 3.1. Age, weight, height and BMI were not significantly different between groups.

Table 3.1. Characteristics and demographic data of participants included in the study.

	Control		MS		
	Mean	SD	Mean	SD	p-value
Gender	15F | 8M		19F | 7M		
Age (yrs.)	46.76	15.93	48.19	11.95	0.72
Height (in.)	66.87	2.99	65.25	2.88	0.06
Weight (lbs.)	158.48	29.09	150.77	20.61	0.29
BMI	24.79	3.35	24.99	3.84	0.84
Disease Duration (yrs.)	-	-	11.69	10.72	
EDSS (mean, range)	-	-	3.5	0-4	
Falls in the Last Month (#)	0	0	0.07	0.27	0.18
Falls Last 6 Months (#)	0	0	0.5	0.99	**0.02**
Dominate Leg (% Right)	96		96		
Most Affected Side	-	-	15 Right | 8 Left | 3 Unknown		
Left Hemisphere RMT (%MSO)	68.95	8.09	66.77	7.42	0.32
Right Hemisphere RMT (%MSO)	69.1	7.68	67.5	7.91	0.48
Left Tibialis Anterior (N)	307.52	104.77	229.64	89.3	**0.007**
Right Tibialis Anterior (N)	293.33	95.98	218.59	66.71	**0.0017**
Exercise (% Yes)	96		96		
Exercise per Week (hrs.)	4.9	2.58	5.3	3.99	0.67
Type of Exercise					
Aerobic	9		14		
Anaerobic	1				
Aerobic + Anaerobic	12		10		
Other			1		
Education					
Highschool	0		1		
Associates	0		4		
BA/BS	10		8		
Masters	10		9		

Doctorate	3		4		
Questionnaires					
Becks Depression Inventory	2.43	3.16	7.77	7.2	**0.002**
Short Form-36					
General Health	83.26	11.24	59.04	22.89	**<0.0001**
Physical Function	96.28	5.06	75.96	23.02	**0.0001**
Energy/Fatigue	70.22	14.18	51.54	18.64	**0.0003**
Roles Limitations	94.56	18.39	60.58	38.84	**0.0004**
Social Functioning	92.93	14.51	74.52	21.65	**0.0012**
Pain	90.43	11.22	73.46	21.97	**0.0017**
Emotional Problems	91.3	20.64	61.54	42.89	**0.004**
Emotional Wellbeing	83.3	11.61	75.23	12.85	**0.026**
Change in Health	56.52	13.52	60.58	21.42	0.439
Modified Fatigue Impact Scale - Total Score (0-84)	-	-	31.85	14.67	
MS Walking Scale-12 (%)	-	-	26.12	24.9	
MS Walking Scale-12 Total Score	-	-	24.54	11.95	

Notes: BMI – Body Mass Index; %MSO – percent maximal stimulation output; RMT – resting motor threshold; N – Newtons

TMS MEASURES

Strength measures for the TA were statistically different for both legs between groups (Table 3.1). Specifically, neurotypical controls demonstrated greater maximal TA strength for the left ($t_{(47)}$ = -2.810, p = 0.007) and right ($t_{(47)}$ = -3.323, p = 0.002) legs compared to PwMS. No significant effects were found for RMT for group ($t_{(47)}$ = 0.92, p = 0.36) or hemisphere ($t_{(47)}$ = -.50, p = 0.62) (Table 3.1).

Motor cortex excitability was measured via MEP amplitude relative to the pre-stimulation mean muscle activity. For MEP amplitude there was an effect of group ($F_{(1, 44.1)}$ = 4.94, p= 0.031), such that PwMS demonstrated reduced MEP amplitude compared to neurotypical controls. No significant effect of hemisphere nor interaction was revealed.

Regarding inhibitory TMS measures, no significant effect of group, hemisphere, or interaction was found for cortical silent period (cSP) duration (p = ≥ 0.23). As for average depth of cSP (%dcSP Ave) there was a significant effect of group ($F_{(1, 47.7)}$ = 6.55, p= 0.014); where PwMS demonstrated significantly reduced %dcSP Ave compared to their neurotypical counterparts. Additionally, for %dcSP Ave there was no effect of hemisphere nor an interaction between group x hemisphere. Maximum depth of cSP (%dcSP Max) demonstrated a significant effect of group ($F_{(1, 45.9)}$ = 6.63, p= 0.013), such that PwMS demonstrated reduced %dcSP Max. No further effect of hemisphere or interaction for %dcSP Max were significant. A full description of TMS measures is located in Table 3.2.

Table 3.2. Average excitatory and inhibitory TMS measures separated by group and hemisphere.

		Control		MS		
TMS Measure	Hemisphere	Mean	SD	Mean	SD	p-value[a]
MEP Amp (mV)	Left	0.054	0.03	0.040	0.02	0.032
	Right	0.052	0.03	0.035	0.02	
Silent Period Duration (ms)	Left	115.66	46.24	108.26	50.04	0.915
	Right	117.83	42.54	123.94	67.88	
dcSP Average (%)	Left	76.89	7.27	70.29	9.63	0.014
	Right	75.13	7.81	71.09	7.84	
dcSP Max (%)	Left	95.98	2.87	92.89	5.32	0.013
	Right	95.35	3.10	93.02	4.55	

Notes: [a] – p-values represent the effect of group.

TURNING RESULTS

Refer to Chapter 2, for a comprehensive description of turning results.

CORRELATIONS BETWEEN 360˚ FAST TURN METRICS AND NEUROPHYSIOLOGICAL MEASURES

The measure of turn angle demonstrated no group differences and was therefore not included in any of the following correlation analyses. Furthermore, all correlation analyses presented are specific to the left hemisphere, as excitatory and inhibitory

outcomes elicited from the right hemisphere revealed no significant associations with the turning performance results.

In PwMS, MEP amplitude did not demonstrate any associations between turn duration or peak velocity. However, all three inhibitory measures: cSP duration, %dcSP Ave, and %dcSP Max demonstrated significant moderate negative correlations with turn duration for PwMS (Table 3.3, Figure 3.9). Additionally, turn velocity was significantly associated with %dcSP Max in PwMS. For neurotypical controls, none of the TMS measures demonstrated any significant associations with measures of turning performance.

Table 3.3. Associations between 360˚ fast pace turn variables and left hemisphere TMS measures

	Turn Duration (s)				Turn Velocity (˚/s)			
	Control		MS		Control		MS	
	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.
MEP Amp (mV)	-0.45	0.034	-0.15	0.510	0.38	0.08	0.07	0.772
SP Duration (ms)	0.03	0.895	**-0.52**	**0.008**	-0.004	0.99	0.40	0.048
%dcSP Ave	0.14	0.537	**-0.50**	**0.012**	0.02	0.93	0.37	0.071
%dcSP Max	-0.04	0.860	**-0.60**	**0.001**	0.08	0.73	**0.45**	**0.025**

Notes: Values in **bold** remained significant after Bonferroni Correction (p=0.025)

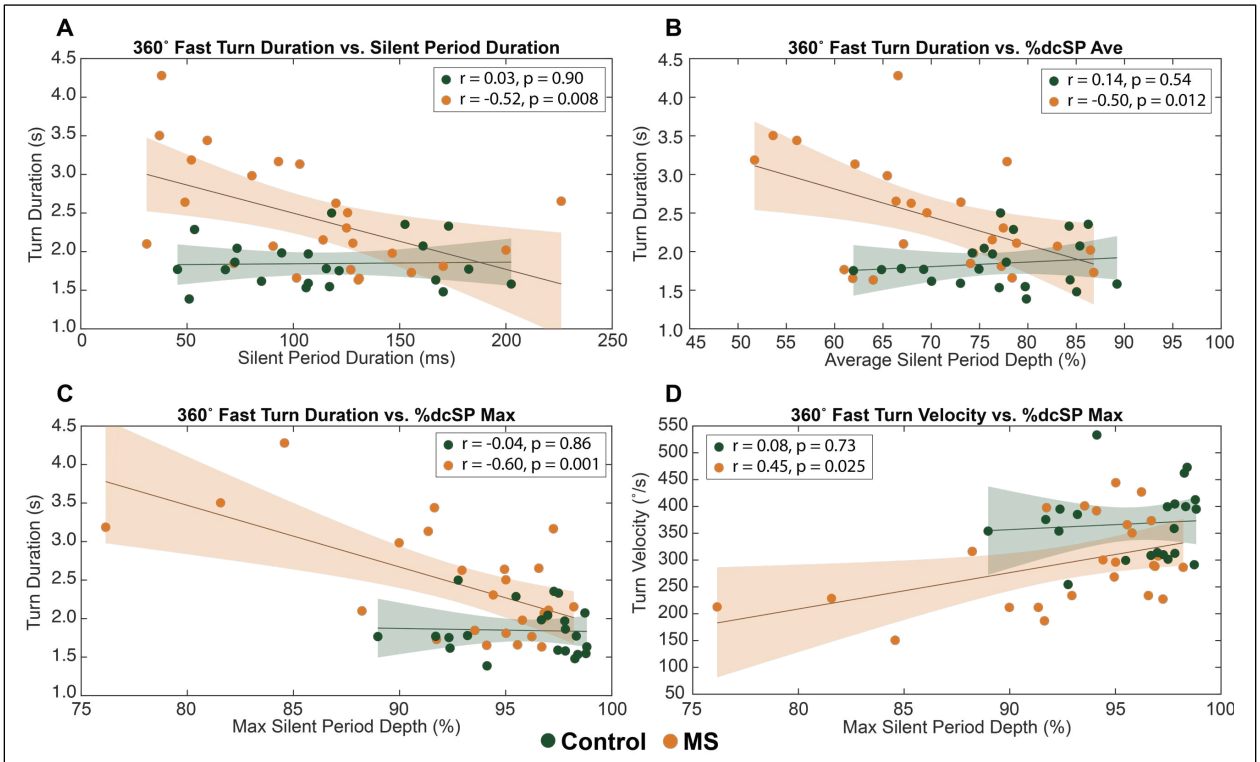

Figure 3.9. Associations between 360˚ fast turn and inhibitory measures. A) depicts an association between turn duration and silent period duration. B) depicts an association between turn duration and %dcSP Ave. C) depicts an association between turn duration and %dcSP Max. D) depicts an association between peak turn velocity and %dcSP Max. All significant associations were only observed in the MS group.

CORRELATIONS BETWEEN 1-MINUTE 360˚ TURN METRICS AND NEUROPHYSIOLOGICAL MEASURES

Turn duration and peak turn velocity were included in the following correlation analysis between 1-minute of consecutive but alternating 360˚ turns and left hemisphere TMS captured neurophysiological measures. In addition, 'Number of Turns' completed during the 1-minute consecutive but alternating 360˚ turns was included as a measure of overall turning performance. Although turn angle was not included in the correlation analysis has no TMS measures demonstrated associations upon initial investigation.

All left hemisphere TMS and 1-minute 360˚ turning associations are detailed in Table 3.4. MEP amplitude demonstrated no associations for any turn variables in either

group. Moreover, cSP duration did not demonstrate any significant associations between the turning measures for either group. Although not significant, weak associations were revealed between cSP duration, and turn duration and peak turn velocity for PwMS. However, for measures of inhibitory capacity (i.e., %dcSP Ave and Max) significant associations for turn duration and number of turns completed were observed. Specifically, PwMS demonstrated significant moderate negative associations between turn duration and both %dcSP Ave and Max measures (Figure 3.10 – A,B). Interestingly, neurotypical controls demonstrated trending associations in the opposing direction, although none met the corrected 0.025 significance threshold. Specific to number of turns completed and %dcSP Ave both neurotypical controls and PwMS demonstrated significant and opposing associations, such that neurotypical controls demonstrated a negative association while PwMS demonstrated a positive association (Figure 3.9 – C). For %dcSP Max, PwMS demonstrated a significant positive association with number of turns, while neurotypical controls demonstrated a non-significant negative association (Figure 3.9 – D). Interestingly, and contrary to the 360˚ in-place fast turns there were no significant associations between peak turn velocity and any of the TMS measures for either group.

These results indicate that PwMS who demonstrated greater inhibitory capacity performed faster turns similar to their neurotypical counterparts. Additionally, PwMS who demonstrated greater levels of inhibitory capacity accrued more total turns compared to those with less inhibitory capacity. Conversely, neurotypical controls who demonstrated less inhibitory capacity performed more total turns compared to those with greater inhibitory capacity.

Table 3.4. Associations between 1-minute of consecutive but alternating 360˚ self-selected pace turn variables and left hemisphere TMS measures

	Turn Duration (s)				Turn Velocity (˚/s)				Number of Turns (#)			
	Control		MS		Control		MS		Control		MS	
	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.
MEP Amp (mV)	-0.14	0.549	0.23	0.307	-0.03	0.905	-0.18	0.416	0.07	0.743	-0.10	0.649
SP Duration (ms)	0.29	0.190	-0.40	0.046	-0.18	0.415	0.41	0.041	-0.27	0.218	0.34	0.092
dcSP Ave (%)	0.42	0.052	**-0.56**	**0.004**	-0.21	0.360	0.43	0.034	**-0.48**	**0.025**	**0.51**	**0.009**
dcSP Max (%)	0.21	0.341	**-0.51**	**0.009**	-0.11	0.634	0.43	0.032	-0.25	0.268	**0.53**	**0.006**

Notes: Values in **bold** remained significant after Bonferroni Correction (p=0.025)

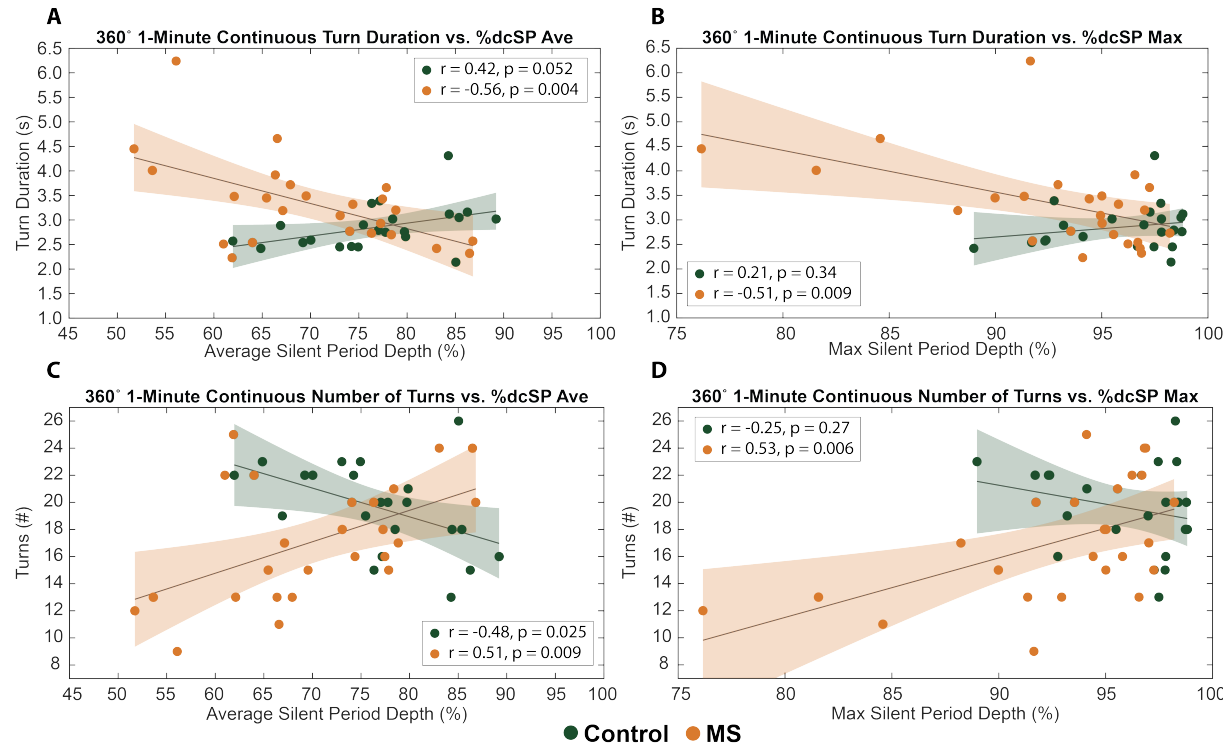

Figure 3.10. Associations between 1-minute 360˚ turn and inhibitory measures. A) depicts a significant negative association between turn duration and %dcSP Ave in PwMS. B) depicts a significant negative association between turn duration and %dcSP Max in PwMS. C) depicts significant associations between number of turns and %dcSP Ave for both groups in opposing directions. D) depicts a significant positive association between number of turns and %dcSP Max in PwMS.

CORRELATIONS BETWEEN 180˚ TURNS WHILE WALKING AND NEUROPHYSIOLOGICAL MEASURES

For this analysis, turn duration, peak turn velocity, and number of steps in the turn were included to assess the associations between 180˚ turns while walking and left

hemisphere TMS measures (Table 3.5). MEP amplitude and %dcSP Max did not demonstrate any significant association between the turning variables for either group. Although, silent period duration and %dcSP Ave demonstrated significant associations to peak turn velocity (Figure 3.11 – A,B, respectively). Specifically, these associations were only significant for the MS cohort, where greater inhibition was associated with greater peak turn velocity. However, number of steps in the turn and turn duration demonstrated no significant associations between any of the TMS measures. Although, %dcSP Ave and turn duration demonstrated a trend towards significance for the MS group, such that greater inhibitory capacity was associated with a shorter turn duration. Moreover, the neurotypical control cohort demonstrated no significant associations between any turn variables and TMS measures.

Table 3.5. Associations between 180˚ turn variables and left hemisphere TMS measures

	Turn Duration (s)				Turn Velocity (˚/s)				Steps in Turn (#)			
	Control		MS		Control		MS		Control		MS	
	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.
MEP Amp (mV)	-0.12	0.61	0.3	0.181	-0.12	0.606	-0.4	0.063	-0.26	0.246	0.170	0.450
SP Duration (ms)	0.05	0.821	-0.29	0.167	0.23	0.311	**0.57**	**0.003**	0.17	0.454	-0.123	0.557
dcSP Ave (%)	0.09	0.705	-0.41	0.042	0.04	0.869	**0.5**	**0.011**	0.14	0.546	-0.278	0.178
dcSP Max (%)	0.29	0.189	-0.23	0.274	-0.02	0.914	0.42	0.037	0.38	0.083	-0.095	0.653

Notes: Values in **bold** remained significant after Bonferroni Correction (p=0.025)

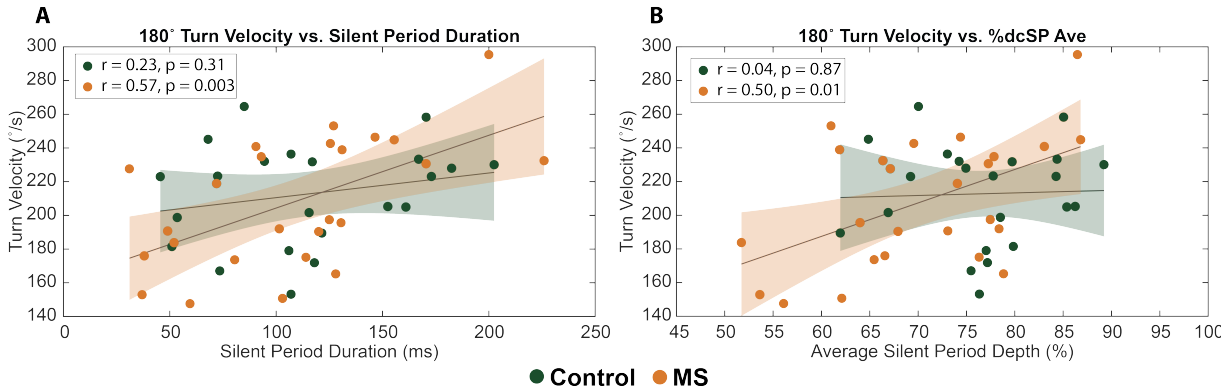

Figure 3.11. Associations between 180˚ turn and inhibitory measures. A) depicts a significant positive association between turn velocity and silent period duration in PwMS. B) depicts a significant positive association between turn velocity and %dcSP Ave.

DISCUSSION

The purpose of the current study was to assess excitatory and inhibitory corticospinal neurophysiology specific to the lower limbs in neurotypical controls and PwMS. Additionally, we sought to determine whether excitatory and inhibitory measures were associated with turning performance in both groups. Corticospinal excitation and inhibition were measured via single pulse TMS. Specifically, excitation was measured via the MEP amplitude, while inhibition was measured via the silent period duration, %dcSP Ave, and %dcSP Max. For full turning analysis and collection please refer to Chapter 2. Briefly, we analyzed three distinct types of turning; 360˚ in-place fast turns, 1-minute of 360˚ self-selected pace turns, and 180˚ turns while walking.

For MEP amplitude our results demonstrated a significant effect of group where PwMS demonstrated reduced MEP amplitude compared to neurotypical controls which is in full agreement with our first hypothesis. For silent period duration, our results revealed no significant effect of group, hemisphere or interaction, although inhibitory capacity revealed significant effects of group for both %dcSP Ave and %dcSP Max which were both reduced in the MS cohort, and in partial agreement with our second hypothesis. Right hemisphere TMS measures did not demonstrate any significant associations between turning measures for either group; therefore, associations for each turning measures were focused on left hemisphere inhibitory measures. Moreover, MEP amplitude did not demonstrate any significant associations between turning variables for any of the turning measures. For the 360˚ in-place fast turns the correlation analysis demonstrated significant associations between turn duration and peak turn velocity and left hemisphere inhibitory measures for the MS cohort. For 1-minute of consecutive but

alternating 360˚ self-selected pace turns our results demonstrated significant associations between turn duration and number of turns completed for both inhibitory capacity measures of the left hemisphere in both groups. Lastly, for the 180˚ turns while walking our results demonstrated significant associations between peak turn velocity and cSP duration and %dcSP Ave of the left hemisphere in the MS group.

EXCITATORY AND INHIBITORY MEASURES BETWEEN NEUROTYPICAL CONTROLS AND PWMS

Theoretically, since our data did not demonstrate a hemispheric difference, we could have averaged the results between the two hemispheres; however, we decided to maintain independent hemispheric values. Principally, this decision was guided by evidence of brain lateralization, or the notion of that each hemisphere integrates specific responses and actions (Fling, Dutta et al. 2014). As such, it is well documented that each hemisphere has innate responsibilities, including the sensorimotor region and the regulation of purposeful movements (Mutha, Haaland et al. 2012, Fling, Dutta et al. 2014). For instance, a recent report by Fling, et al. (2014) demonstrated associations between proprioceptive balance control and microstructural integrity of Brodmann Area (BA) 3a (i.e., the nadir of the central sulcus, associated with the somatosensory cortex) to be restricted entirely to the right hemisphere in PwMS (Fling, Dutta et al. 2014). Moreover, voluntary inhibition of manual movements (via go-no-go tasks) has been postulated to be right-lateralized to the frontal-basal ganglia-thalamic pathway (Aron 2007). Although, contradicting that assumption other studies have placed doubt on the existence of a right-lateralized network promoting inhibitory control, suggesting that the two hemispheres work in cooperation (Mirabella, Fragola et al. 2017). Furthermore, there is convincing

evidence for the role that the left hemisphere plays regarding particular aspects of upper extremity motor control. For instance, it has been postulated that the left hemisphere may be responsible for the planning motor actions, and subsequently has become specialize in regulating well-established patterns of behavior that are often characterized as routine, familiar, and internally directed (Mutha, Haaland et al. 2012, Mirabella, Fragola et al. 2017). While the contributions for each hemisphere remain inconclusive especially for lower limb directed movements the evidence certainly suggests both hemispheres are both functionally independent while simultaneously interconnected. Therefore, given the existing literature suggesting specialized hemispheric lateralization, we felt obligated to present findings from both hemispheres.

EXCITABILITY

As previously stated, MEPs are generally thought to reflect corticospinal excitability driven by the dynamic balance between excitatory and inhibitory (E-I) inputs within corticospinal circuits. A necessary measure prior to any TMS study is the determination of motor thresholds, thereby normalizing the observed TMS responses across study participants. The majority of studies report higher motor thresholds for PwMS compared to neurotypical controls (Cruz-Martinez, Gonzalez-Orodea et al. 2000, Jorgensen, Nielsen et al. 2005, Gagliardo, Galli et al. 2007), although similarities between neurotypical controls and PwMS have also been observed (Di Sapio, Bertolotto et al. 2014). Our results mimic the latter, we report no significant difference for RMT between the two groups. While our results are unlike the majority, research has consistently shown that motor thresholds do not correlate with clinical outcomes (i.e., disease severity, motor impairments, etc.), suggesting that motor thresholds may have relatively low clinical utility

(Snow, Wadden et al. 2019). This lack of observed association between RMT and clinical outcomes may be due to the proposed idea that motor thresholds are thought to reflect the excitability of the cortico-cortico and thalamo-cortical axons rather than cortico-motor axons (Ziemann 2004).

In contrast, MEP characteristics, including amplitude have consistently demonstrated overall reductions in PwMS and demonstrate some of the most robust measures for distinguishing between neurotypical controls and PwMS (Snow, Wadden et al. 2019). These reductions are synonymous with reduced corticospinal excitability which is typically observed in MS. Our results are in agreeance with prior reports demonstrating reduced MEP amplitude in both the upper and lower limbs in PwMS (Kukowski 1993, Cruz-Martinez, Gonzalez-Orodea et al. 2000, Schmierer, Niehaus et al. 2000, Tataroglu, Genc et al. 2003, Jorgensen, Nielsen et al. 2005, Sahota, Prabhakar et al. 2005, Gagliardo, Galli et al. 2007). Specifically, we report significant MEP amplitude reductions in the lower limbs for both hemispheres in the MS cohort. While the underlying mechanisms of diminished excitability in PwMS has yet to be fully elucidated it has been postulated that this reduction may reflect a loss of motor neurons in the corticospinal system, decreased connectivity between motor commands and the corticospinal system, or altered balance between excitation and inhibition (Snow, Wadden et al. 2019).

INHIBITION

In the motor cortex of neurotypical individuals, GABAergic neurotransmission has been related to motor learning, neural plasticity, and motor control (Levy, Ziemann et al. 2002, Stagg, Bachtiar et al. 2011). Moreover, levels of GABAergic neurotransmission appear to play a critical role in the regulation of cortical reorganization after lesion

formation in stroke survivors (Levy, Ziemann et al. 2002). However, the role of GABAergic neurotransmission within the motor cortex of PwMS remains inconclusive. For instance, MS related inhibitory adaptations not exclusively associated with TMS measures demonstrate inconsistent findings, such that studies have reported greater inhibition (Tataroglu, Genc et al. 2003, Nantes, Zhong et al. 2016, Neva, Lakhani et al. 2016) while other studies have documented no differences or reduced inhibition in PwMS (Vucic, Burke et al. 2012, Cawley, Solanky et al. 2015, Santarnecchi, Rossi et al. 2015, Zoupi, Booker et al. 2021). Interestingly, a recently published histological study reported that PwMS demonstrate a selective loss of inhibitory interneurons within the sensorimotor cortex which was postulated to be secondary to cortical demyelination (Zoupi, Booker et al. 2021). Together these reports suggest that PwMS demonstrate inhibitory dysregulation as a product of pathology, likely influenced by cortical demyelination.

Regardless there remains a substantial lack of consistent reporting of inhibitory differences between neurotypical individuals and PwMS particularly in relation to TMS measures to the lower limbs. For instance, only one other study performed by Tataroglu, et al. (2003) has explored inhibitory activity via single pulse TMS to the lower limbs and demonstrated significantly longer cSP durations for PwMS compared to neurotypical controls and furthermore, greater durations for those with progressive versus relapsing phenotypes of the disease (Tataroglu, Genc et al. 2003). Our results contradict their silent period duration significance between neurotypical controls and PwMS; however, the reported silent period durations demonstrate similarities to our results. For instance, the Tataroglu, et al. (2003) study reported an average silent period duration of 118.1 ± 74 ms in their RRMS group, while the results from our MS cohort showed durations of 108.3 ±

50 ms and 123.9 ± 68 ms for the left and right hemispheres respectively. Interestingly and dissimilar to their results were the silent period durations for the neurotypical controls. For instance, they documented a hemispheric average silent period duration of 78.6 ± 26 ms for the lower limbs. While our results for silent period duration were 115.7 ± 46 ms for the left hemisphere and 117.8 ± 42 ms for the right hemisphere (right TA and left TA, respectively). Silent period durations ≤100 ms for the lower limbs have been reported in the literature for neurotypical adults of a similar age range to the Tataroglu, et al. (2003) study (average age of 33.4 [range 22-49yrs]) (Beynel, Chauvin et al. 2014), silent period durations ≥100ms for that age range appear to be more common (Oliviero, Profice et al. 2006, Fujiyama, Garry et al. 2009, Fujiyama, Hinder et al. 2012, Swanson and Fling 2018, Swanson and Fling 2019). Silent period durations are known to be influenced by a variety of factors including age, such that older adults (i.e., adults ≥65) often demonstrate reduced durations, although their average age and range were comparable to young adults (Sale and Semmler 2005, Oliviero, Profice et al. 2006, Swanson and Fling 2019). Additionally, stimulation intensity can influence silent period duration, where higher intensities often produce longer durations (Hupfeld, Swanson et al. 2020). However, the Tataroglu, et al. (2003) study performed a suprathreshold intensity of 150% motor threshold, while our study utilized a suprathreshold intensity of 120% motor threshold (Tataroglu, Genc et al. 2003). As such, it is difficult to determine what influenced their shortened silent period durations, or if it was merely a product of their sample. Regardless, we are confident the silent period durations for our neurotypical control cohort parallel the existing literature for lower limb silent period durations, although direct

comparisons are difficult due to the average age of our cohort and the paucity of middle-age TMS research.

A particularly novel approach for quantifying corticospinal inhibition was the implementation using the calculation of %dcSP Ave and %dcSP Max. These two measures are not entirely novel and often reported in the interhemispheric inhibitory literature when assessing the ipsilateral silent period (iSP) which is a measure of transcallosal inhibition through the corpus callosum. Specifically, this technique is performed by inducing a single TMS pulse to the ipsilateral hemisphere while a participant is performing an isometric contraction of an ipsilateral muscle. The TMS pulse results in excitatory transcallosal motor fibers synapsing on contralateral inhibitory interneurons within the homologous motor cortex (Wassermann, Fuhr et al. 1991), resulting in a net inhibitory effect and a brief depression to the EMG signal of the ipsilaterally contracted muscle. Similar to cSPs, iSPs are often characterized by their duration; however, percent average depth of the iSP (i.e., %diSP Ave) and percent maximal depth of the iSP (i.e., %diSP Max) of the silent period has been used to quantify levels of inhibitory activity (Jung and Ziemann 2006, Fling and Seidler 2012). Additionally, %diSP Ave and Max have demonstrated greater sensitivity for delineating between young and older adults than the conventionally reported silent period duration, although to our knowledge these measures have not included in intrahemispheric (i.e., cSP) inhibitory analyses (Fling and Seidler 2012, Kuo, Dubuc et al. 2017, Hupfeld, Swanson et al. 2020). It is thought that since cSPs tend to reach higher levels of inhibition (i.e., EMG amplitude during the silent period nears 100% muscle deactivation) compared to iSPs, researchers may feel these measures would be less likely to demonstrate group differences (Hupfeld, Swanson et al. 2020).

While the distinct physiological mechanisms for %dcSP Ave and Max ultimately remain unclear, we propose that these measures represent inhibitory capacity, such that they measure the influence inhibition has on a target muscle of interest. Where greater amplitudes of %dcSP Ave and Max translate to a greater inhibitory capacity or influence of inhibition to particular muscles. For the interpretation of our results, we demonstrate significant differences between neurotypical controls and PwMS for both %dcSP measures, such that PwMS demonstrate significantly reduced inhibitory capacity compared to neurotypical controls. These results appear to be consistent with prior reports of reduced inhibitory activity as measured using TMS and magnetic resonance spectroscopy (MRS) in PwMS compared to neurotypical adults (Vucic, Burke et al. 2012, Cawley, Solanky et al. 2015, Santarnecchi, Rossi et al. 2015, Zoupi, Booker et al. 2021). Additionally, in the iSP literature, these two measures have been reported to demonstrate less variability and more reliability, suggesting they may provide greater sensitivity and reproducibility (Fleming and Newham 2017, Sankarasubramanian, Machado et al. 2017, Cunningham, Knutson et al. 2019, Lin, Cunningham et al. 2020).

The current inhibitory results augment the existing literature by providing similar silent period durations to previously published data for lower limb related TMS studies in PwMS. Additionally, our results extend the current understanding of inhibitory activity in PwMS by incorporating two new silent period quantification measures capable of describing intrahemispheric inhibitory capacity. As such we demonstrate temporally similar silent period durations to the lower limbs, although reduced inhibitory capacity such that the influence of inhibition is less impactful to the TA in PwMS.

ASSOCIATIONS BETWEEN TURNING PERFORMANCE AND TMS RELATED NEUROPHYSIOLOGY

A number of neurotransmitters (i.e., dopamine, serotonin, and acetylcholine) are relevant to motor performance; although aside from acetylcholine, TMS is only able to measure glutamatergic (excitatory) and GABAergic (inhibitory) neurotransmission in the motor cortex (Stagg, Bachtiar et al. 2011). Moreover, glutamate and GABA are critical for the development and regulation of descending motor commands. While intrahemispheric glutamatergic activity describes the balance between excitation and inhibition, TMS related glutamatergic measures are often less sensitive to motor control assessments (Stagg, Bachtiar et al. 2011). However, studies assessing GABAergic neurotransmission have consistently demonstrated associations, such that GABAergic neurotransmission is believed to be vitally important for proper motor control in humans (Stagg, Bachtiar et al. 2011, Stagg, Bachtiar et al. 2014).

Although PwMS in our study demonstrated reduced excitability (i.e., smaller MEP amplitudes), neither group revealed any significant associations between MEP amplitude and the various turning measures or variables. These results appear to be consistent with the upper limb research, for example, PwMS demonstrated reduced MEP amplitudes although no associations between MEP amplitude and complex dexterous movements were observed (Thickbroom, Byrnes et al. 2002). Interestingly, it has been shown that excitability in PwMS is not static and that exercise can augment MEP amplitudes, which may reflect an exercise induced change in the balance of intracortical excitation and inhibition (Thickbroom, Sacco et al. 2008). However, it remains unknown if the exercise induced increase alters the associations between complex motor tasks in PwMS.

While excitability has yet to demonstrate strong associations with motor performance, research has demonstrated weak to moderate associations between disease severity and MEP amplitude, such that smaller amplitudes correlate with greater disease severity (Cruz-Martinez, Gonzalez-Orodea et al. 2000, Snow, Wadden et al. 2019). While not a primary outcome of the current study, our results demonstrate weak and non-significant ($rs \leq -0.36$, $p \geq 0.09$) associations between hemispheric MEP amplitude and the cumulative EDSS score and disease duration. The lack of association is not surprising given that our cohort of MS participants had an EDSS range of 0-4 and a skewed distribution with 19 (73%) participants presenting with an EDSS between 3.5-4.0. Moreover, the EDSS is a broad scale encompassing a variety of functional outcomes, which likely reflects the often reported small to moderate correlations between MEP amplitude. Lastly, given the heterogeneity of disease progression as a product of age, and the advancements in immunotherapeutic disease modifying drugs, disease progression can be exceptionally variable. The current results complement and add to the existing literature demonstrating reduced excitability in PwMS that furthermore, no significant associations with lower limb turning performance, disease severity, or disease duration.

While glutamatergic activity was not associated with lower limb turning measures the associations between corticospinal inhibitory activity and turning performance in PwMS did demonstrate significant associations. Specifically, for PwMS all of the significant associations demonstrated that greater inhibitory activity (i.e., longer cSP durations, or greater %dcSP Ave, and %dcSP Max) related to turns more characteristic of their healthy counterparts.

To our knowledge, this is the first study attempting to associate these specific measures in PwMS. Not surprisingly the comparable literature demonstrates inconsistent results. For instance, a study performed by Nantes and colleagues (2016), demonstrated a positive association between cSP duration of the dominant hand first dorsal interosseous (FDI) muscle and completion time of the 9-Hole Pegboard Test (9HPT) (i.e., greater inhibition associated with worse performance) (Nantes, Zhong et al. 2016). Furthermore, Nantes, et al. (2016) subdivided their full MS cohort based on the completion time of the 9HPT into either a "preserved function" (time to complete 9HPT was *within* ±2 SD of the control group) and "impaired function" (time to complete 9HPT was *greater* than 2 SD of the control group) group, respectively named RRMS-P and RRMS-I. Interestingly, those individuals placed into the RRMS-P group did not demonstrate a significant cSP duration difference compared to the healthy controls, while those in the RRMS-I group demonstrated significantly longer cSP durations compared to the healthy controls and RRMS-P groups. However, for their regression analysis the MS groups (i.e., RRMS-P and RRMS-I) were combined and demonstrated a positive association between the 9HPT and cSP duration. Importantly, it is unclear based on their results if one of the MS subgroups demonstrated particular leverage for this association. Paradoxically, the reason this may be important is that younger neurotypically adults demonstrate a similar positive association between motor performance (upper and lower limb) and inhibitory activity (Seidler, Bernard et al. 2010, Fling, Kwak et al. 2012, Swanson and Fling 2018, Swanson and Fling 2019). Therefore, these results are difficult to interpret, as they may be influenced by age, or more likely driven by the RRMS-P group not requiring significant inhibition for proper completion of the task due to their relative

lack of impairment. Moreover, the authors only presented associations for their MS cohort and neglected to present comparative associations for their healthy control cohort, leaving the reader speculating that either no associations were observed or that they were in the opposite direction. Conversely, the results from the present study parallel previously reported results in PwMS. Specifically, an MRS study conducted by Cawley and colleagues (2015) demonstrated that greater levels of GABA within the left sensorimotor cortices, specifically the hand region to be associated with stronger grip strength and better manual dexterity (via 9HPT). Interestingly, Cawley, et al. (2015) did not demonstrate a significant association to the Timed 25-foot Walk Test, which theoretically may be a product of the MRS assessment window being focused on the hand knob of the motor cortex (Cawley, Solanky et al. 2015).

While neurotypical aging and MS are distinct, they do demonstrate similar mobility disabilities, for instance both groups demonstrate very similar turning characteristics (Swanson., et al. (2021) – Under Review). Specifically, turn duration for both 360˚ and 180˚ turns are similar as is peak turn velocity for 360˚ turns. Moreover, a recent investigation from our group revealed significant associations between these same turning measures and corticospinal inhibition (i.e., cSP duration) of the lower limbs in neurotypical younger and older adults (Swanson and Fling 2019). Specifically, we reported that greater corticospinal inhibition (i.e., longer cSP durations) in neurotypical older adults (≥ 65 years) was associated with faster 180˚ turn velocities and shorter 360˚ turn durations (Swanson and Fling 2019). Inferring that more corticospinal inhibition in older adults is related to turning performance more characteristics of their younger counterparts.

The associations from the present study demonstrate similarities to our previously published healthy aging data, such that greater inhibition in the impaired group (e.g., PwMS and older adults, respectively) relates to better turning performance. Regarding the present study, this pattern was observed for all significant associations for the three different turning measures, although to varying degrees for particular turning variables. For instance, the current results revealed that 360˚ turn variables as opposed to the 180˚ turn variables demonstrated more significant associations. We believe this could be a product of greater gait automaticity and reliance of spinally mediated inhibitory mechanisms for 180˚ turns, whereas the 360˚ turns require higher order and multi-level neural control for proper completion.

Although cSP duration did not demonstrate a groupwise difference, cSP duration did reveal a positive association with 360˚ in-pace fast turn duration and 180˚ peak turn velocity. These results may suggest that the temporal influence of inhibition to lower limb muscles is important for temporally mediated turning movements in PwMS. Interestingly, the two measures of inhibitory capacity revealed significant differences between groups and furthermore, demonstrated associations with more turning variables within the three turning measures. These results suggest that the amplitude of inhibition rather than the temporal influence of inhibition may be a sensitive GABAergic measure and an important mediating factor for lower limb dynamic movements. Such that, the amplitude of inhibition to particular muscles may be important for the successful completion of dynamic tasks requiring higher level neural command.

While there were many associations of MS groups our results only revealed one significant association for the neurotypical control group. Specifically, we reported a

significant negative association for number of turns completed to left hemisphere %dcSP Ave. Directionally this association opposed that of the MS cohort, such that neurotypical adults with greater inhibitory capacity completed fewer total turns. The results of the present study, however, are similar to previously published observations for the inhibitory control of turning in neurotypically healthy young adults (Swanson and Fling 2019). Specifically, those results showed that younger adults with less inhibition displayed better turning performance. While the current study results presented a similar pattern, it must be noted that the inhibitory adaptations associated with neurotypical middle-age adults remain unknown. Although, we postulate that neurotypically healthy middle-age adults may rely on different neural resources such as subcortical and/or spinal level modulation for successful bilateral lower limb control, although those neural mechanisms have yet to be fully elucidated.

As described, the brain demonstrates significant amounts of lateralization (i.e., hemispheric specialization), largely thought to promote efficient neural computation and integration (Mutha, Haaland et al. 2012). Lateralization regarding the current study will be discussed in greater detail in the following chapter (see Chapter 4 – Discussion). Although briefly, all of the associations between the TMS and turning measures/variables which met significance after correction (i.e., $p \leq 0.025$) were lateralized to the left hemisphere. These findings are particularly interesting and demonstrate novel findings regarding inhibitory lateralization and turning performance in PwMS.

While the significance of left hemisphere lateralization for the associations between intracortical inhibition and turning performance in PwMS remains unknown, left hemispheric specialization is thought to play a significant role in enhancing movement

planning and execution (Mutha, Haaland et al. 2012). Further, studies have provided evidence suggesting a particular role for the left hemisphere in motor learning tasks which require movement planning and execution for future actions. These ideas are consistent with pathological populations that have demonstrated greater left, rather than right hemisphere damage resulting in ideomotor apraxia; a disorder where the observed spatiotemporal motor deficits are thought to arise from impaired planning due to damage of the left frontal and parietal regions (Haaland, Harrington et al. 2000, Mutha, Haaland et al. 2012). Additionally, the parietal cortex of left hemisphere has been implicated for its role in the preparation of selected overt movements and the decision of which limb to use for a particular task (Castiello and Paine 2002, Oliveira, Diedrichsen et al. 2010).

Collectively it appears that the left hemisphere is particularly well suited for movement planning and execution, which has been shown to be disrupted in PwMS (Russo, Crupi et al. 2015). As such these results may indicate a compensatory inhibitory lateralization, meant to assist in properly executing the turning task. Interestingly the neurotypical control group did not demonstrate similar associations suggesting the utilization of different neural mechanisms to perform the same task, which we speculate are likely subcortical and/or spinal in origin. However, it must be noted that the neurotypical control group in the present study did not demonstrate associations to the right hemisphere which has previously been reported (Swanson and Fling 2019). We suggest these differences are likely the product of the age range and mean age of the recruited neurotypical control cohort. For instance, the current study recruited healthy participants between the ages of 23-76 years, while the prior investigation assessed age related differences between young adults (≤ 30 years) and older adults (≥ 65 years). For those particular age-related

results, we demonstrated significant but opposite interactions, such that younger adults with less inhibition performed turns better and older adults with more inhibition performed turns better (Swanson and Fling 2019). Therefore, given the range of participant ages in the current study any possible associations are likely diminished. These results might suggest that neurophysiological mechanisms of dynamic movements for healthy middle-aged adults are variable, likely a product of the natural aging process and future studies incorporating multiple age ranges may be able to shed light on this phenomenon.

CONCLUSION

Together these results demonstrate neurophysiological differences affecting the lower limbs between PwMS and neurotypical controls. Specifically, this cohort of PwMS showed reduced glutamatergic and GABAergic activity. Moreover, while we do not demonstrate silent period duration differences, we demonstrated for the first time reduced intrahemispheric inhibitory capacity in PwMS, using a quantifiable TMS measure typically performed in the interhemispheric iSP literature. Additionally, we report a number of lateralized left hemisphere associations between turning and inhibitory measures in PwMS, while only one relationship was observed in the neurotypical control cohort. These results may indicate that reduced inhibitory activity in PwMS is associated with reduced lower limb motor performance. Since this is the first study to assess these associations, further investigations are warranted to elucidate the neural mechanisms of lower limb motor performance in PwMS.

CHAPTER 4 – NEUROANATOMICAL STRUCTURAL DIFFERENCES BETWEEN NEUROTYPICAL CONTROLS AND PEOPLE WITH MULTIPLE SCLEROSIS

INTRODUCTION

Multiple Sclerosis (MS) is a chronic immune-mediated demyelinating disease of the central nervous system (CNS). While the etiology of MS is unknown, it is believed to involve a combination of genetic and environmental factors (Noseworthy, Lucchinetti et al. 2000, Sospedra and Martin 2005, Ascherio and Munger 2007, Ascherio and Munger 2007, Consortium 2013). Pathologically, MS is characterized by inflammation, demyelination, neuronal and axonal damage, and gliosis (non-neuronal cell scaring) within the CNS (Lassmann, Brück et al. 2007, Dutta and Trapp 2011). Importantly, the pathogenic mechanisms causing these damaging abnormalities remain largely unknown. The result of these pathological observations typically entails focal demyelinating lesions associated with both the white and grey matter of the cerebrum and spinal cord.

Conventionally, white matter lesions were considered a hallmark of the disease, and thus MS was broadly considered a white matter disease. Overtime this commonly accepted concept has been reconceived, largely due to technological advancements of evaluating lesions through modern neuroimaging techniques (Geurts, Calabrese et al. 2012, Klaver, De Vries et al. 2013). While MS maintains this label, recent evaluations of grey matter within the cerebrum have provided compelling evidence that MS is much more than a white matter condition. It has even been reported that total grey matter demyelination likely exceeds white matter demyelination, especially in the progressive phenotypes (Geurts, Calabrese et al. 2012). Thus, not necessarily surprising, early histological evidence found vast grey matter damage and cell loss, which aberrantly

contributes to the neurological dysfunction seen in people with MS (PwMS). In fact, James W. Dawson a physician and pathologist in the early twentieth century was the first to describe MS pathology of the cortex as a lack of glial proliferation and scant cellular abundance in areas demonstrating demyelination (Dawson 1916). These discoveries led Dawson to ponder: “Is then, the process that attacks the cortex different in its nature and origin from that which affects the rest of the central nervous system?” (Dawson 1916). This question, a century later remains under investigation and largely lacked exploratory momentum until a seminal paper in 2001 by Peterson, J.W. and colleagues who discovered vast cortical and subcortical grey matter damage in PwMS (Peterson, Bö et al. 2001). Since these findings, efforts have begun to detail the prevalence of grey matter damage and uncover the associated effects of grey matter damage in MS (Kidd, Barkhof et al. 1999, Peterson, Bö et al. 2001). Moreover, grey matter damage has generated interest in elucidating the progression and cause of neuronal dysfunction. Leading to question whether grey matter and white matter damage are linked, or whether grey matter damage to some degree is independent of white matter damage.

There have been a number of studies suggesting a directionality associated with cortical damage in a variety of diseases including MS. To bolster this idea, studies using various magnetic resonance imaging (MRI) techniques and clinical populations have reported significant correlations between white matter damage and grey matter atrophy (a broad term used for grey matter damage). For example, studies conducted in people with transected spinal cord injuries demonstrated reduced grey matter volume and thickness in various cortical and subcortical regions including the thalamus, precentral gyrus, and postcentral gyrus, suggesting cortical atrophy to be a product of retrograde

degeneration (i.e., thalamic, pre-, and postcentral gyrus atrophy was secondary to the spinal cord injury) (Freund, Weiskopf et al. 2011, Wenzhao, Shi et al. 2019). In MS, this assumption of retrograde degeneration has also been postulated, suggesting that the distinct regions of cortical atrophy within the motor cortex along with areas of the frontal and temporal lobes may be a product of white matter lesions (Sailer, Fischl et al. 2003). Further support for this hypothesis is that the corticospinal tract has direct white matter fiber connections to the precentral gyrus (i.e., motor cortex), and the frontal periventricular white matter houses fibers that connect to the frontal and temporal lobes, both of which are common sites for white matter lesion in MS (Narayanan, Fu et al. 1997). Moreover, researchers have suggested that lesions within the thalamocortical white matter tract may cause upstream and downstream retrograde effects, such that lesions within this particular tract may explain the thalamic atrophy commonly observed in younger individuals early in the disease progression (Azevedo, Cen et al. 2019). Additional support for the connection between grey matter atrophy and white matter lesions have been supported through various cross-sectional studies which have identified correlations between global grey matter volume and total white matter lesion volume (De Stefano, Matthews et al. 2003, Sanfilipo, Benedict et al. 2005, Roosendaal, Bendfeldt et al. 2011). Aside from cross sectional investigations, one longitudinal study in people with relapsing remitting MS (RRMS) discovered associations between grey matter atrophy and white matter lesion load in the frontal and parietal cortices, suggesting the association to be partially dependent upon white matter lesion load (Bendfeldt, Kuster et al. 2009).

While there is research to support the notion of retrograde degradation; alternatively, another theory suggests that grey matter and white matter defects are

independent of one another. Those who prescribe to this theory believe that the abundant grey matter alterations are not well explained by focal demyelinating white matter lesions, as such a number of studies using histopathological and neuroimaging evidence supports this concept. For instance, research assessing cortical structures have observed a gradient of neuronal loss of the primary motor cortex by demonstrating extensive subpial demyelination in the outermost layers of the neocortex (Magliozzi, Howell et al. 2010). Importantly, no associations were observed between the gradient neuronal loss and white matter lesion load nor location, and in many cases, the white matter appeared to be fully intact (Calabrese, Magliozzi et al. 2015). These results suggest very little, if any influence of white matter lesion on gray matter subpial demyelination, however additional research indicates the grey matter damage could be due to inflammatory processes (Howell, Reeves et al. 2011). Moreover, it has been postulated that grey matter degradation could result in anterograde (i.e., cortical atrophy is primary to secondary corticospinal tract damage) axonal loss of the corticospinal tract, suggesting the opposite direction of demyelinating impacts (Calabrese, Magliozzi et al. 2015). Similarly, histopathological post-mortem studies conducted in PwMS discovered substantially greater grey matter demyelination compared to white matter demyelination throughout various parts of the CNS including the spinal cord, cerebellum, and cerebrum (Kutzelnigg, Faber-Rod et al. 2007, Gilmore, DeLuca et al. 2009, Gilmore, Donaldson et al. 2009). Aside from post-mortem histopathological investigations, neuroimaging studies have observed grey matter lesions in the early stages of the disease where white matter lesion load appeared to be small or not observed (Calabrese, Atzori et al. 2007, Calabrese and Gallo 2009). However, studies reporting both grey matter and white matter lesions only report

moderate associations between the two measures (Chard, Griffin et al. 2002). Additionally, a longitudinal study reported greater associations between white matter lesions and white matter volume compared to associations between grey matter atrophy (Tiberio, Chard et al. 2005). This was further exemplified in a more recently published report demonstrating no associations between grey matter atrophy and various measures of white matter damage in people with RRMS (Sbardella, Petsas et al. 2013). While numerous studies have demonstrated varying degrees of associations between grey matter atrophy and white matter lesion volume and location, these associations appear to only describe less than half of the total variance (Bendfeldt, Blumhagen et al. 2010).

While evidence exists for retrograde neuronal loss as a result of white matter damage, opposing evidence for anterograde axonal loss as a result of grey matter atrophy similarly exists (Lansley, Mataix-Cols et al. 2013, Bergsland, Horakova et al. 2018). However, these mechanisms do not clearly explain the extent of neuronal damage observed in MS, and likely point to some level of independence from each other. In support of partial independence, there are thought to be three likely pathogenic mechanisms involved in grey matter pathology, ultimately leading to grey matter atrophy. These independent mechanisms include meningeal and/or cortical inflammation, pathology originating in white matter affecting the distribution of sodium channels over the length of axons, and mitochondrial abnormalities leading to imbalanced cellular energy demand and supply causing axonal dieback (Geurts, Calabrese et al. 2012, Zivadinov, Ramasamy et al. 2017). Thus, indicating an amalgamation of neurodegenerative processes are likely involved and contribute to the extent of neuronal damage seen in MS (Bendfeldt, Blumhagen et al. 2010, Calabrese, Magliozzi et al. 2015).

Independent of causality, grey matter atrophy is abundant and has been observed in nearly all brain regions including the cortex (frontal, parietal, temporal lobes), basal ganglia, cerebellum, and brainstem (Peterson and Fling 2018). The rate of atrophy varies by diagnosis although more progressive forms of the disease typically demonstrate greater rates of atrophy compared to relapsing remitting forms (Roosendaal, Bendfeldt et al. 2011). While grey matter atrophy progressively occurs in PwMS, it has also been shown to affect individuals very early on in the disease and even prior to clinical diagnosis (Calabrese, Atzori et al. 2007). Although atrophy affects all subtypes of MS, the location of atrophy seems to partially vary based on the diagnosis. For instance, a recently published, multi-phenotype large cohort (n=1,424) study assessed the progression of cortical atrophy using an event-based model to determine which regions are temporally affected throughout the disease course and by phenotype (Eshaghi, Marinescu et al. 2018). The results of this study demonstrated a number of neuroanatomical structures having common atrophic similarities which appeared to be key regions across MS phenotypes and disease stages. These key regions of early atrophy included the posterior cingulate cortex, precuneus, thalamus, and brainstem (Eshaghi, Marinescu et al. 2018). While there are sequential atrophic similarities between phenotypes, differences were reported. For instance, in the relapse-onset forms of MS (i.e., relapsing-remitting and secondary progressive) the posterior cingulate cortex and precuneus atrophied first followed by the middle cingulate cortex, brainstem, and thalamus (Eshaghi, Marinescu et al. 2018, Eshaghi, Prados et al. 2018). Lastly, the pallidum and medial precentral gyrus (aka., paracentral gyrus) demonstrated atrophy in the relapsing forms of MS (Eshaghi, Marinescu et al. 2018). Patients diagnosed with primary progressive MS (PPMS) showed

primary atrophy in the thalamus, cuneus, precuneus, and pallidum (Eshaghi, Marinescu et al. 2018). Atrophy to those regions was followed by the brainstem, precentral gyrus, and posterior cingulate cortex; and finally, the frontal operculum and middle temporal gyrus demonstrated atrophy (Eshaghi, Marinescu et al. 2018).

Given that regional atrophy is both sequential and partially phenotype specific there are a number of (aforementioned) regions demonstrating atrophy that have been associated with motor performance. One common measure of motor performance and functional ability in PwMS is the Expanded Disability Status Scale (EDSS) which is used to characterize disease severity on a scale of 0 (no disability due to the disease) to 10 (death due to MS) in increments of .5 (Kurtzke 1983). Importantly, ambulatory ability is heavily weighed within this scale such that scores between 4.0 (when quantifiable mobility limitations are first acknowledged) – 6.0 (when a walking aid is required) measure levels of ambulatory disability. Since the scale heavily weights ambulatory ability, it is not necessarily surprising that global and regional atrophy has been associated with the EDSS (Sailer, Fischl et al. 2003, Calabrese, Atzori et al. 2007, Charil, Dagher et al. 2007, Narayana, Govindarajan et al. 2013). For instance, atrophy in regions associated with the control of locomotion such as the basal ganglia (i.e., putamen, thalamus, caudate), cortex (i.e., pre-central gyrus, post-central gyrus, precuneus, anterior cingulate, temporal pole), brainstem, and cerebellum have all been associated with the EDSS (Sailer, Fischl et al. 2003, Lansley, Mataix-Cols et al. 2013, Narayana, Govindarajan et al. 2013, Peterson and Fling 2018). Additionally, volume of brainstem has been associated with gait related indices including the Timed 25-Foot Walk test and the Ambulation Index, which are both commonly reported clinical gait outcomes (Edwards, Gong et al. 1999, Jasperse, Vrenken

et al. 2007, Shiee, Bazin et al. 2012, Peterson and Fling 2018). Together these results suggest that grey matter atrophy plays an important role in disease severity which is linked to lower limb motor control.

While there are different theories describing the basis of grey matter atrophy, certainly overwhelming evidence describes vast grey matter atrophy in all phenotypes of MS. Furthermore, grey matter atrophy is an important predictor for the progression of the disease which has been strongly associated with reduced motor performance. While regions such as the brainstem and cerebellum have been associated with reduced linear walking performance, it is conceivable that atrophy may also be associated with additional components of mobility, such as turning related movements. However, to date studies have yet to establish whether cortical atrophy either global or regional are associated with specific measures of turning. Therefore, this study was built on the evidence of vast cortical grey matter atrophy in MS and to further assess which regions of the cortex demonstrate cortical thinning as a result of the disease. Additionally, we aimed to assess whether neurophysiological function to the lower limbs and turning performance measures were related to cortical thickness of the sensorimotor region. As such, we developed three primary hypotheses, first we hypothesized that PwMS would demonstrate global clusters of reduced cortical grey matter thickness and specific thinning within the sensorimotor cortex for both hemispheres compared to neurotypical control participants. Second, we hypothesized that PwMS would demonstrate a positive association between motor cortex (i.e., precentral and paracentral gyrus) cortical thickness and excitation, whereas motor cortex thickness would be negatively associated with inhibition. Lastly, we hypothesized that PwMS would demonstrate a positive

association between cortical thickness of the sensorimotor cortex and turning performance.

METHODS

Participants came into the lab for two testing sessions which occurred on separate days no more than 10 days apart. For session one, participants came to the Intermountain Neuroimaging Consortium in Boulder, Colorado and completed the informed consent procedure, the magnetic resonance imaging (MRI) protocol, and the turning while walking protocol. Session two occurred at the Human Performance Clinical Research Laboratory at Colorado State University, participants completed the self-administered EDSS, questionnaires, transcranial magnetic stimulation (TMS) protocol, and the turning in-place protocol.

MAGNETIC RESONANCE IMAGING

Imaging acquisition was completed with a 3.0 T Siemens MAGNETOM Prismafit (Siemens Medical Solutions USA, Inc., Malvern, PA) MRI scanner. Parallel imaging was conducted using a 32-channel head coil used to assist with spatial localization and subsequently reduce image acquisition time. All participants underwent the same imaging protocol and remained in the scanner between images. The imaging protocol included the following scans: diffusion weighted imaging, T1 – weighted anatomical, T2 – weighted fluid-attenuated inversion recovery (FLAIR), and a resting state functional scan. For the purposes of this study only the T1 and T2 weighted scan parameters will be discussed.

The high resolution T1 – weighted anatomical scan was collected using a single – slab and the following scan parameters: TR = 2400 ms; TE = 2.07 ms; TI = 1000 ms; flip angle = 8˚, echo train length = 0.49 ms; field-of-view = 256 mm (180 mm (RL), 256 mm

(AP), 256 mm (FH)); slice thickness = 0.8 mm; slices = 224 (sagittal); and voxel dimensions = 0.8 mm x 0.8 mm x 0.8 mm. (TR = repetition time; TE = echo time; TI = inversion time)

The T2 – weighted FLAIR was collected using a single – slab and the following scan parameters: TR = 6000 ms; TE = 428 ms; TI = 2000 ms; echo train length = 933 ms; field-of-view = 256 mm (176 mm (RL), 256 mm (AP), 256 mm (FH)); slice thickness = 1.0 mm; slices = 176 (sagittal); and voxel dimensions = 1.0 mm x 1.0 mm x 1.0 mm. (TR = repetition time; TE = echo time; TI = inversion time)

TURNING

Please refer to Chapter 2 for full description of turning methods.

TRANSCRANIAL MAGNETIC STIMULATION

Transcranial Magnetic Stimulation (TMS) methods are fully described in Chapter 3, although briefly participants underwent single pulse TMS to measure excitatory (glutamatergic) and inhibitory (gamma-aminobutyric acid, GABAergic) neurophysiological activity specific to the tibialis anterior (TA) muscles. To assess excitatory neurophysiology, we quantified the motor evoked potential (MEP) amplitude and for inhibitory neurophysiology, we quantified the cortical silent period duration, average percent depth of the silent period (%dcSP Ave), and max percent depth of the silent period (%dcSP Max).

PROCESSING

IMAGE PROCESSING

Models of whole brain characteristics including the white and grey matter surface (parcellation) as well as subcortical grey matter (segmentation) were reconstructed using

FreeSurfer (Version 6.0.0) and the *-recon-all* processing pipeline with the *-FLAIR* flag (Dale and Sereno 1993, Dale, Fischl et al. 1999, Fischl, Sereno et al. 1999). Both the T1 and T2 weighted images underwent the processing pipeline and were combined using the *-FLAIR* flag to inform FreeSurfer of the image acquisition type and to account for proper configuration and contrast of the FLAIR image. This process reconstructed individual surface models of grey and white matter, subsequently mapping measures of cortical thickness, surface area, and volume (Fischl and Dale 2000). In FreeSurfer cortical thickness (our primary outcome) was quantified as the distance between the white matter border and the pial surface (Figure 4.1). Vertex-wise cortical surface area was calculated

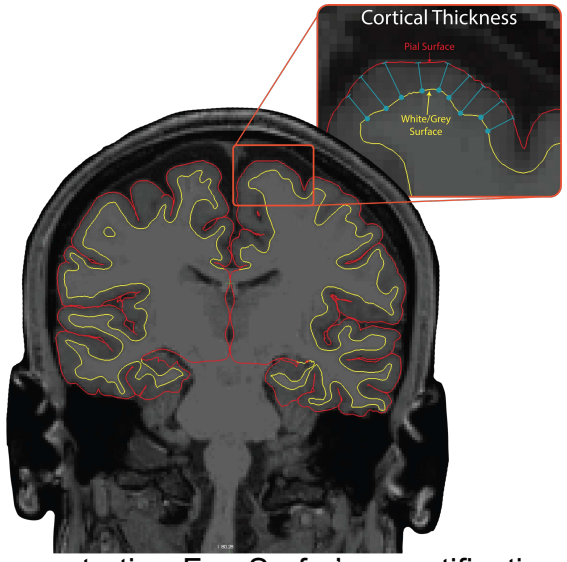

Figure 4.1. Graphic demonstrating FreeSurfer's quantification of cortical grey matter thickness.

as the mean area of the associated triangular region, which when mapped onto the standard template the measure indicates the vertex-wise areal expansion (Joyner, J. et al. 2009). The measure of cortical volume is a product of cortical thickness and area (Feczko, Augustinack et al. 2009, Postelnicu, Zollei et al. 2009). Cortical parcellation and subcortical segmentation are both automatically accomplished through the *-recon-all*

processing pipeline (Fischl, Salat et al. 2002, Fischl, van der Kouwe et al. 2004, Destrieux, Fischl et al. 2010). Cortical thickness measures using FreeSurfer have proven to demonstrate strong agreement with histological and manual measurement studies (Rosas, Liu et al. 2002, Kuperberg, Broome et al. 2003, Salat, Buckner et al. 2004, Cardinale, Chinnici et al. 2014).

Preprocessing procedures were performed separately for each subject. First, each T1-weighted image was run using the *-recon-all* command then each T2 was run using the *-recon-all* + *-FLAIR* command, which takes roughly 24 hours of processing per participant. After initial processing each scan was manually inspected for inconsistencies and defects as a result of the processing pipeline. Depending on the volumetric size of observed defects manual editing was performed to adjust image contrasts (i.e., highlight lesions or unaccounted grey matter) or remove unwanted voxels (i.e., skull). To limit bias this step was performed blinded as to participant group. After manual correction, an abbreviated pipeline was run (i.e., *-autorecon2-cp, -autorecon2-wm,* or *-autorecon2-pial*) depending on the corrections that were made, averaging four hours of additional processing. The intermediate processing step automatically recalculates all parcellation and segmentation metrics (i.e., thickness and volume). After troubleshooting, each scan was reassessed for defect correction. Although rare, in cases where defects remained, additional manual editing was implemented, and subsequent processing was performed. Once the image was successfully edited the individual surface maps were registered to the FreeSurfer ‘fsaverage’ template to allow for subject comparisons. The ‘fsaverage’ template was smoothed using a Gaussian kernel with a full-width at half-maximum of 10 mm prior to further analysis (Steenwijk, Geurts et al. 2016, Greve and Fischl 2018).

TURN DATA PROCESSING

Refer to Chapter 2 for full details.

TMS DATA PROCESSING

Refer to Chapter 3 for full details.

STATISTICAL ANALYSIS

Groupwise cortical thickness analysis was performed in FreeSurfer through their assessment pipeline and with additional assistance from FreeSurfer engineers. All other statistical analysis was conducted in JMP Pro 15 with an alpha level set at 0.05 unless otherwise documented. For ROI analysis normality was assessed using a Shapiro-Wilks test and QQ plots, which demonstrated a normal distribution for all three regions (i.e., precentral gyrus, paracentral gyrus, and postcentral gyrus). Between-group differences for age were assessed via a one-way analysis of variance (ANOVA) while sex was assessed via a chi-square test. For all other demographic variables independent t-tests were performed. All data are presented as mean ± SD unless noted otherwise.

GROUPWISE CORTICAL THICKNESS STATISTICAL ANALYSIS

After normalizing and smoothing the cortical thickness maps, source-based morphometry analysis was performed. A general linear model was fit to the data set, this was performed by creating a FreeSurfer Group Descriptor (FSGD) file which was designed to assess for thickness differences between groups. The current analysis assessed the effects of age and sex on cortical thickness, which did not demonstrate any significant differences at a conservative corrected threshold (0.01). Given that age and sex did not demonstrate significant effects on cortical thickness measures between the two groups, the final analysis model assessed cortical thickness differences as a product

of disease. As such a contrast file was created, to specify the contrast weights for each group in the model.

A cluster-wise correction was performed on the data to limit any false positive cortical thickness differences. This was performed using a permutation analysis (1000 permutations), which has recently been shown to reduce false positive rates when compared to the more conventional Monté-Carlo correction for multiple comparisons (Greve and Fischl 2018). For this particular analysis, a vertex-wise cluster threshold was set to a p-value of 0.01 with positive *a-priori* statistical direction, such that we suspect neurotypical controls will display greater cortical grey matter thickness.

REGION OF INTEREST STATISTICAL ANALYSIS

Although the effects of age and sex were not significant in the whole brain analysis they were included as covariates in the ROI analysis. Therefore, a linear mixed-effects model was developed based on the combination of fixed (groups and hemispheres) and random (subject) effects with age and sex included as covariates within the study design. Fixed effects and interactions were further examined for significance. Post-hoc analysis of interactions was assessed using a one-way ANOVA.

LINEAR REGRESSION STATISTICAL ANALYSIS

Linear regressions were used to assess correlations between cortical thickness of the ROIs and neurophysiological measures as well as objective turning measures (for both groups). Correlation strength between MRI, TMS, and turning metrics were calculated using Pearson correlation coefficients and corrected for multiple comparisons using a Bonferroni correction. Correlations strengths were classified as very strong (0.9–

1.0), strong (0.7–0.9), moderate (0.5–0.69), weak (0.3–0.49) and negligible (< 0.30) (Mukaka 2012).

RESULTS

PARTICIPANT DEMOGRAPHICS

Table 4.1 outlines the demographic and clinical characteristics of study participants. No significant differences were observed for age ($F_{(1,48)} = 0.13$, $p = 0.72$) or sex ($\chi^2_{(2)} = 0.36$, $p=0.55$). Both the neurotypical controls and PwMS demonstrated similar levels of weekly physical exercise, although the MS group demonstrated more falls 6 months prior to participating in the study, with three experiencing one fall and four experiencing repeated falls (i.e., between 2-4 falls).

Table 4.1. Characteristics and demographic data of participants included in the study.

	Control		MS		
	Mean	SD	Mean	SD	p-value
Gender	15F | 8M		19F | 7M		
Age (yrs.)	46.76	15.93	48.19	11.95	0.72
Height (in.)	66.87	2.99	65.25	2.88	0.06
Weight (lbs.)	158.48	29.09	150.77	20.61	0.29
BMI	24.79	3.35	24.99	3.84	0.84
Disease Duration (yrs.)	-	-	11.69	10.72	
EDSS (mean, range)	-	-	3.5	0-4	
Falls in the Last Month (#)	0	0	0.07	0.27	0.18
Falls Last 6 Months (#)	0	0	0.5	0.99	**0.02**
Dominate Leg (% Right)	96		96		
Most Affected Side	-	-	15 Right | 8 Left | 3 Unknown		
Left Hemisphere RMT (%MSO)	68.95	8.09	66.77	7.42	0.32
Right Hemisphere RMT (%MSO)	69.1	7.68	67.5	7.91	0.48
Left Tibialis Anterior (N)	307.52	104.77	229.64	89.3	**0.007**
Right Tibialis Anterior (N)	293.33	95.98	218.59	66.71	**0.002**
Exercise (% Yes)	96		96		

Exercise per Week (hrs.)	4.9	2.58	5.3	3.99	0.67
Type of Exercise					
Aerobic	9		14		
Anaerobic	1				
Aerobic + Anaerobic	12		10		
Other			1		
Education					
Highschool	0		1		
Associates	0		4		
BA/BS	10		8		
Masters	10		9		
Doctorate	3		4		
Questionnaires					
Becks Depression Inventory	2.43	3.16	7.77	7.2	**0.002**
Short Form-36					
General Health	83.26	11.24	59.04	22.89	**<0.001**
Physical Function	96.28	5.06	75.96	23.02	**<0.001**
Energy/Fatigue	70.22	14.18	51.54	18.64	**<0.001**
Roles Limitations	94.56	18.39	60.58	38.84	**<0.001**
Social Functioning	92.93	14.51	74.52	21.65	**0.001**
Pain	90.43	11.22	73.46	21.97	**0.002**
Emotional Problems	91.3	20.64	61.54	42.89	**0.004**
Emotional Wellbeing	83.3	11.61	75.23	12.85	**0.026**
Change in Health	56.52	13.52	60.58	21.42	0.439
Modified Fatigue Impact Scale - Total Score (0-84)	-	-	31.85	14.67	
MS Walking Scale-12 (%)	-	-	26.12	24.9	
MS Walking Scale-12 Total Score	-	-	24.54	11.95	

Notes: BMI – Body Mass Index; %MSO – percent maximal stimulation output; RMT – resting motor threshold; N – Newtons

WHOLE BRAIN RESULTS

The whole brain analysis demonstrated no significant effects of age or sex; therefore, the results reflect cortical grey matter thickness as a product of disease. Thickness measures of each participant's brain were mapped on to the inflated surface and reconstructed to provide statistical thickness differences across the entire cortical

surface (i.e., sulci and gyri). This revealed distinct clusters spanning various cortical regions in both hemispheres corresponding to distinct areas of cortical thinning in PwMS (Figure 4.2). Table 4.2 reflects the cluster-wise significance for each cluster that met the cluster-wise corrected threshold (p=0.05).

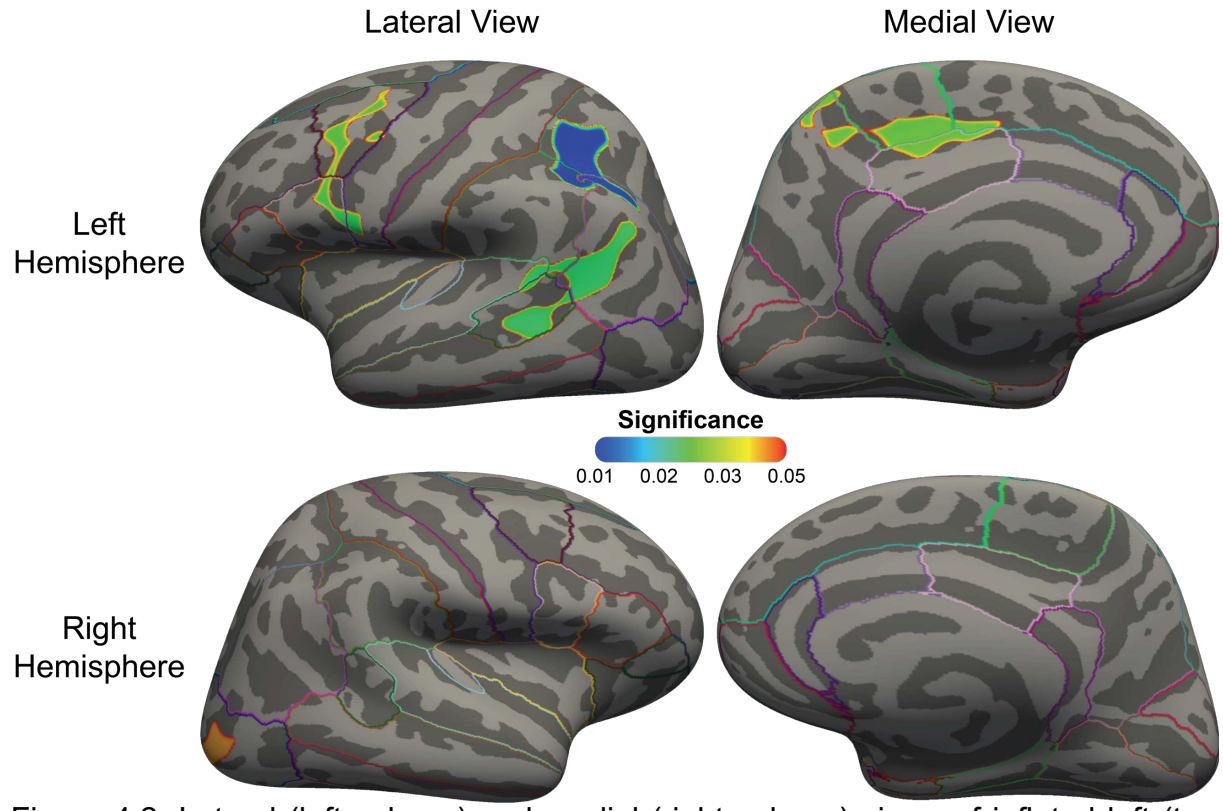

Figure 4.2. Lateral (left column) and medial (right column) views of inflated left (top row) and right (bottom row) hemispheres. Images depict the group difference maps highlighting significantly thinner cortical regions in 26 PwMS compared to 23 neurotypical controls. Colors on the inflated brains correspond to the significance map in the center, representing p-values.

Table 4.2. Cluster region, size, location, and significance for clusters demonstrating significant cortical thinning.

Hemisphere	Cluster Region	Size (mm^2)	MNI Coordinates (X,Y,Z)			CWPLow	CWPHigh	CWP
Left	Superiorparietal	1393.61	-34.7	-45.9	34.2	0.004	0.016	0.010
	Inferiorparietal	1254.66	-37.3	-57.4	18.9	0.010	0.026	0.018
	Precentral	1110.29	-53.8	3.9	10.2	0.016	0.032	0.024
	Paracentral	1033.53	-13	-16.8	45	0.018	0.036	0.026
Right	Lateraloccipital	796.52	28.1	-96.8	-2.4	0.028	0.050	0.038

Notes: Size- surface area (mm^2) of significant cluster; MNI(XYZ) - (MNI305) coordinates of significant cluster max vertex; CWPLow and CWPHigh – 90% confidence interval for cluster-wise p-value; CWP – cluster-wise p-value.

In PwMS, the superiorparietal cluster demonstrated a cluster-wise corrected significance (p = 0.01) representing particular cortical thinning in the supramarginal gyrus (Brodmann Area (BA) 40), superior parietal lobule (BA7), and the superior portion of the inferior parietal lobule (BA40). The inferiorparietal cluster (p = 0.018) presented with cortical thinning of the inferior parietal lobule (BA40), inferior aspect of the supramarginal gyrus (BA40), dorsal superior temporal gyrus (BA22), and the dorsal middle temporal gyrus (BA21). The precentral cluster (p = 0.024) demonstrated cortical thinning in the precentral gyrus (BA4), pars opercularis (BA44), and the caudal middle frontal gyrus (BA9). The paracentral cluster (p = 0.036) demonstrated cortical thinning of the precuneus (BA7), posterior cingulate gyrus (BA23), superior frontal gyrus (BA8), and the paracentral gyrus (BA4). For the right hemisphere one cluster aptly named the lateral occipital cluster (p = 0.038) demonstrated significant cortical atrophy of the lateral occipital lobe (BA17) in PwMS. Taken together, the whole brain analysis demonstrated multiple regions of significant cortical thinning in PwMS, and the majority of this thinning was lateralized to the left hemisphere. For cortical thickness values of specific cortical regions within each cluster refer to Table 4.3.

Table 4.3. Cortical thickness (mm) differences for the whole brain analysis and associated regions and clusters.

			Control		MS	
Hemisphere	Cortical Region	Associated Cluster(s)	Mean	SD	Mean	SD
Left	Superior Parietal Lobule	Superiorparietal	2.60	0.15	2.48	0.20
Left	Supramarginal Gyrus	Superiorparietal, Inferiorparietal	2.92	0.15	2.79	0.22
Left	Inferior Parietal Lobule	Superiorparietal, Inferiorparietal	2.80	0.13	2.65	0.25

Left	Bank of Superior Temporal Sulcus	Inferiorparietal	2.81	0.16	2.61	0.24
Left	Superior Temporal Gyrus	Inferiorparietal	3.10	0.15	2.95	0.33
Left	Caudal Middle Frontal Gyrus	Precentral	2.98	0.14	2.82	0.20
Left	Precentral Gyrus	Precentral	3.01	0.16	2.88	0.17
Left	Pars Opercularis	Precentral	2.99	0.15	2.83	0.26
Left	Paracentral Gyrus	Paracentral	2.86	0.15	2.70	0.18
Left	Posterior Cingulate Gyrus	Paracentral	2.75	0.20	2.56	0.29
Left	Superior Frontal Gyrus	Paracentral	3.07	0.15	2.93	0.22
Left	Precuneus	Paracentral	2.75	0.12	2.63	0.19
Right	Lateral Occipital Lobe	Lateraloccipital	2.50	0.15	2.40	0.17

Notes: all mean and SD measures represent cortical thickness (mm – millimeter)

REGION OF INTEREST RESULTS

We further performed an *a-priori* region of interest (ROI) analysis for specific cortical regions associated with the sensorimotor cortices. Specifically, we assessed cortical thickness differences of the precentral gyrus, paracentral gyrus, and postcentral gyrus. For the precentral gyrus, there was a significant effect of group ($F_{(1,45)}$ = 9.41, p = 0.004) and hemisphere ($F_{(1,47)}$ = 9.10, p = 0.004), although no group x hemisphere interaction was observed. Moreover, age and sex were not identified as significant covariates. For the paracentral gyrus the results demonstrated a significant effect of group ($F_{(1,45)}$ = 10.86, p = 0.002), although no significant effect of hemisphere or interaction between group x hemisphere was observed. Likewise, age and sex demonstrated no significance. Lastly, for the postcentral gyrus there was no significant effect of group, although there was a trend towards significance ($F_{(1,45)}$ = 3.76, p = 0.059). Moreover, no significance was observed for an effect of hemisphere or an interaction for group x hemisphere. While sex did not demonstrate a significant covariate effect, age was significantly related to postcentral gyrus thickness ($F_{(1,45)}$ = 4.13, p = 0.048).

Although mean cortical grey matter thickness and white matter hypo-intensity volume were not primary measures we performed group-wise analyses for the two measures. For mean grey matter cortical thickness there was an effect of group ($F_{(1,45)}$ = 5.89, p = 0.019), although no effect of hemisphere, interaction, or covariates. For white matter hypo-intensity volume there was a significant effect of group ($F_{(1,45)}$ = 10.28, p = 0.003) and a covariate effect of age ($F_{(1,45)}$ = 7.49, p = 0.009), although no significant effect for sex. For reference, Table 4.4 details the ROI cortical thicknesses for each hemisphere and provides the mean global cortical grey matter thickness and white matter hypo-intensity volume.

These results indicate that PwMS demonstrate greater cortical thinning, particularly in regions of the brain associated with movement. Interestingly, the postcentral gyrus did not demonstrate a significant effect of group, indicating that MS pathology may differentially effect motor regions (pre- and paracentral gyri) rather than sensory regions (i.e., postcentral gyrus) in RRMS.

Table 4.4. Hemispheric ROI cortical thickness measures in PwMS relative to neurotypical controls.

		Control		MS	
Structure	Hemisphere	Mean	SD	Mean	SD
Precentral Gyrus (mm)	Left	3.01	0.16	2.88	0.17
	Right	2.95	0.17	2.82	0.18
Paracentral Gyrus (mm)	Left	2.86	0.15	2.70	0.18
	Right	2.87	0.18	2.74	0.21
Postcentral Gyrus (mm)	Left	2.49	0.14	2.37	0.22
	Right	2.44	0.13	2.37	0.23
Mean Cortical Thickness (mm)	Left	2.82	0.11	2.70	0.19
	Right	2.81	0.12	2.71	0.22
Total T2- hyperintensity (mm^3)	Combined	1035.34	841.43	3513.59	3734.55

Notes: mm – millimeters; mm^3 – volume.

TURNING RESULTS

Refer to Chapter 2 for detailed turning results.

TRANSCRANIAL MAGNETIC STIMULATION RESULTS

Refer to Chapter 3 for detailed TMS results.

CORRELATIONS BETWEEN NEUROANATOMICAL STRUCTURE AND NEUROPHYSIOLOGICAL FUNCTIONAL

As the cortical thickness differences in the whole brain analysis were primarily lateralized to the left hemisphere the correlations between neuroanatomical structure and neurophysiological function were focused to the left hemisphere. Additionally, because the TMS analysis targeted the leg region of the motor cortex, only the precentral and paracentral gyri measures were included in the following correlation analysis. Significance was corrected for multiple comparisons via Bonferroni correction and set to 0.025.

No associations were observed between MEP amplitude and cortical thickness for either ROI. However, the correlation analysis demonstrated significant associations between inhibitory TMS measures and precentral and paracentral gyri thickness. Specifically, a moderate positive association was overserved between silent period duration and thickness of the precentral gyrus ($r = 0.53$, $p = 0.008$), such that those with greater thickness demonstrated longer silent periods in PwMS (Figure 4.3 – A). For the paracentral gyrus both neurotypical controls and PwMS demonstrated significant associations with silent period duration (Figure 4.3 – B). Specifically, neurotypical controls demonstrated a moderate negative association ($r = -0.56$, $p = 0.007$), while PwMS revealed a weak positive association ($r = 0.48$, $p = 0.015$). This interaction revealed that greater thickness in the neurotypical controls was associated with reduced silent period durations while in the MS group greater thickness was associated with longer silent period

durations. Moreover, the control group demonstrated a weak negative association between %dcSP Ave and paracentral gyrus thickness ($r = -0.48$, $p = 0.024$), such that greater thickness was related to reduced inhibitory capacity (Figure 4.3 – C). Although not significant after correction, the neurotypical controls demonstrated a similar trend for %dcSP Max, such that greater thickness of the paracentral gyrus related to reduced %dcSP Max (i.e., inhibitory capacity). Together these results indicated that inhibitory activity was associated with cortical thickness, although the direction of those associations were opposite between neurotypical controls and PwMS.

Table 4.5. Associations between left hemisphere precentral and paracentral gyrus thickness and left hemisphere TMS measures.

	Precentral Gyrus (mm)				Paracentral Gyrus (mm)			
	Control		MS		Control		MS	
	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.
MEP Amplitude (mV)	-0.03	0.886	0.02	0.914	-0.09	0.705	0.04	0.851
SP Duration (ms)	-0.23	0.302	**0.52**	**0.008**	**-0.56**	**0.007**	**0.48**	**0.015**
dcSP Ave (%)	-0.20	0.381	0.20	0.349	**-0.48**	**0.024**	0.04	0.847
dcSP Max (%)	-0.16	0.466	0.20	0.328	-0.47	0.027	0.19	0.363

Notes: Values in **bold** remained significant after Bonferroni Correction ($p = 0.025$)

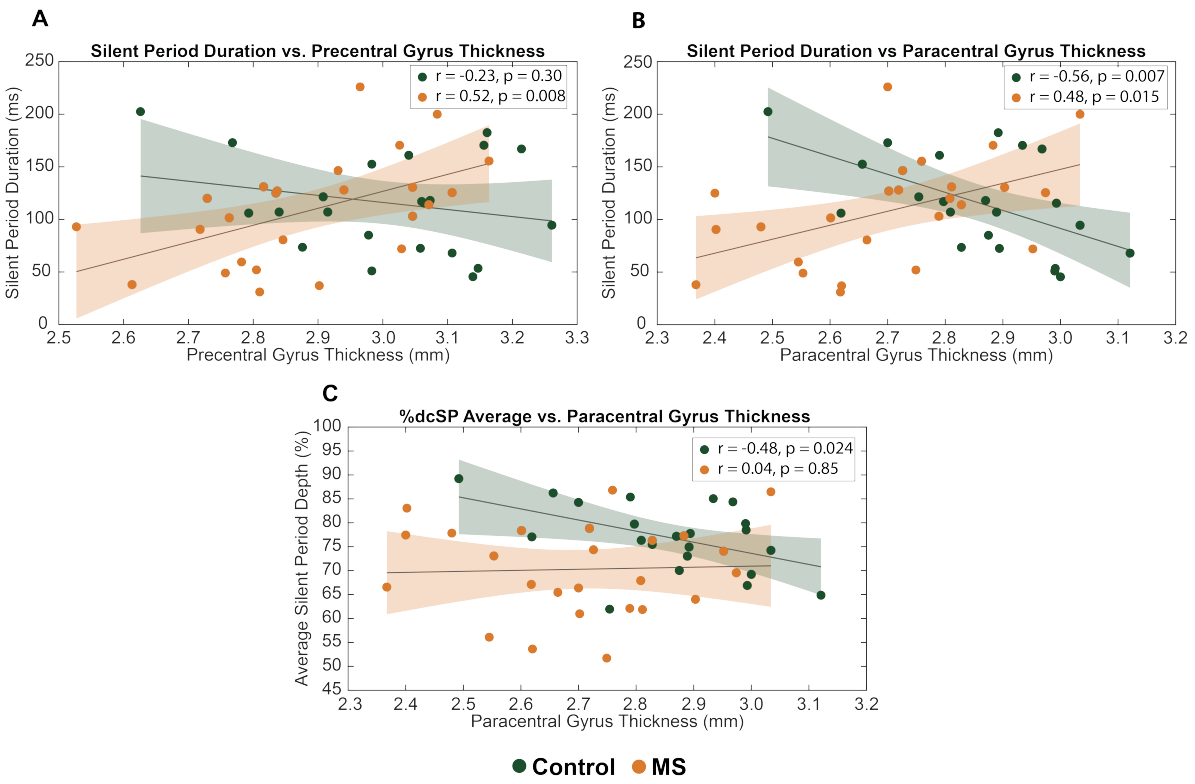

Figure 4.3. Associations between left hemisphere neuroanatomical structure and neurophysiological function. A) depicts a significant association between left hemisphere silent period duration and left hemisphere precentral gyrus thickness in PwMS. B) depicts significant associations between left hemisphere silent period duration and paracentral gyrus thickness for both groups. C) depicts a significant association between left hemisphere %dcSP Ave and paracentral gyrus thickness for neurotypical controls.

CORRELATIONS BETWEEN TURNING MEASURES AND NEUROANATOMICAL STRUCTURE

As previously described, the whole brain analysis demonstrated multiple areas of cortical thinning within the left hemisphere in PwMS. Therefore, to maintain consistency only left hemisphere ROI thickness measures were associated with turning measures and variables. While the postcentral gyrus was not included for the association between neuroanatomical structure and function, this region was included in the turning associations given that all three ROIs make up the sensorimotor cortex and influence descending motor commands.

CORRELATIONS BETWEEN 360˚ FAST TURN METRICS AND SENSORIMOTOR THICKNESS OF THE LEFT HEMISPHERE

Turn angle did not demonstrate a group difference for 360˚ in-place fast turns between groups, and therefore was not included in of the following correlation analysis. As such, the associations between ROI thickness and turning variables were limited to turn duration and peak turn velocity with a corrected p-value of 0.025. Cortical thickness for each ROI demonstrated no significant associations for either turn variable in the neurotypical control cohort. However, the MS group demonstrated significant associations between grey matter thickness and turn duration and peak turn velocity (Table 4.6). Specifically, turn duration demonstrated weak ($r \leq -0.44$, $p \leq 0.023$) negative associations for each of the three ROIs (Figure 4.4 – A,B,C). Peak turn velocity similarly demonstrated weak ($r \leq 0.44$, $p \leq 0.023$) but positive associations between the precentral and paracentral gyri (Figure 4.4 – D,E). While not significant ($r = 0.43$, $p = 0.029$) the postcentral gyrus demonstrated a trend towards significance in the MS cohort. Together, these results demonstrated that cortical atrophy is associated with reduced 360˚ fast turning performance in PwMS.

Table 4.6. Associations between left hemisphere ROI thickness and 360˚ fast turning measures.

	Precentral Thickness (mm)				Paracentral Thickness (mm)				Postcentral Thickness (mm)			
	Control		MS		Control		MS		Control		MS	
	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.
Turn Duration (s)	0.04	0.846	**-0.44**	**0.023**	-0.08	0.711	**-0.47**	**0.017**	0.16	0.480	**-0.45**	**0.022**
Turn Velocity (˚/s)	-0.12	0.581	**0.46**	**0.019**	0.01	0.968	**0.44**	**0.023**	-0.21	0.333	0.43	0.029

Notes: Values in **bold** remained significant after Bonferroni Correction (p = 0.025)

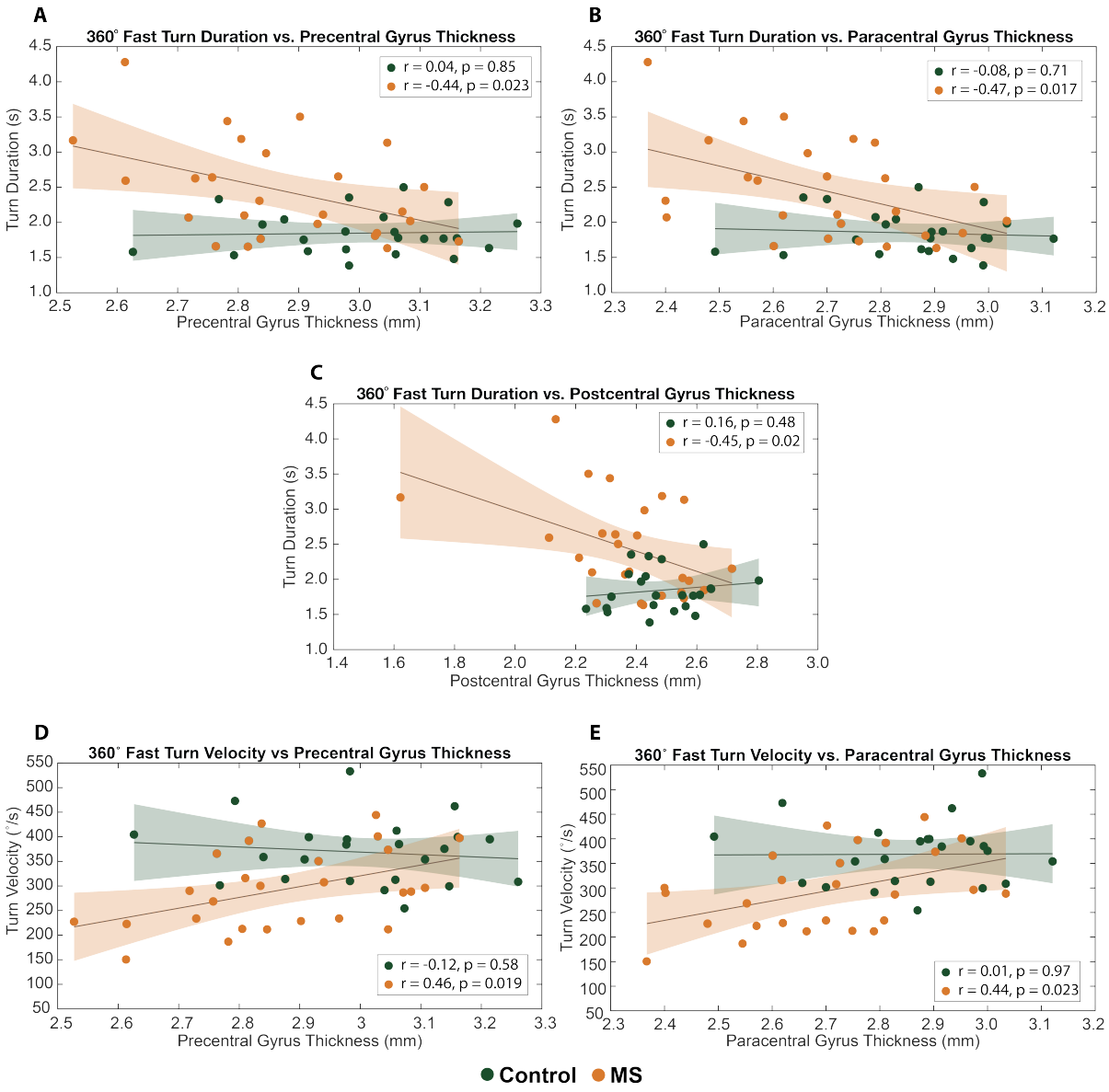

Figure 4.4. Associations between left hemisphere ROI thickness and 360˚ fast pace turning measures. A) depicts a significant association between turn duration and left hemisphere precentral gyrus thickness in PwMS. B) depicts a significant association between turn duration and left hemisphere paracentral gyrus thickness in PwMS. C) depicts a significant association between turn duration and left hemisphere postcentral gyrus thickness in PwMS. D) depicts a significant association between peak turn velocity and left hemisphere precentral gyrus thickness in PwMS. E) depicts a significant association between peak turn velocity and left hemisphere paracentral gyrus thickness in PwMS.

CORRELATIONS BETWEEN 1-MINUTE 360˚ TURN METRICS AND SENSORIMOTOR THICKNESS OF THE LEFT HEMISPHERE

For the following correlations significance was corrected for multiple comparisons and set to 0.0125. Similar to the prior analysis, the neurotypical control group demonstrated no significant associations between ROI cortical thickness and turning variables, whereas PwMS demonstrated significant associations for turn angle and peak turn velocity. Specifically, turn angle revealed a moderate positive association with postcentral cortical thickness ($r = 0.68$, $p < 0.001$) (Figure 4.5 – D). As observed in Figure 4.5 – C,D, one particular individual likely leveraged the association, although upon further investigation that particular individual was not identified as an outlier for cortical thickness or the turning variables and was therefore, included in the analysis. Peak turn velocity demonstrated moderate positive associations for each of the three ROIs ($r \geq 0.54$, $p \leq 0.004$) (Figure 4.5 – A,B,C). These results suggest that PwMS who presented with greater cortical thinning of the postcentral gyrus demonstrated reduced turn angles, while greater thinning within each of the ROIs was associated with reduced peak turn velocity. The associations between ROI thickness and turn variables obtained during the self-selected pace 1-minute consecutive and alternating 360˚ turns are presented in Table 4.7.

Table 4.7. Associations between left hemisphere ROI thickness and 1-minute 360˚ turning measures.

	Precentral Thickness (mm)				Paracentral Thickness (mm)				Postcentral Thickness (mm)			
	Control		MS		Control		MS		Control		MS	
	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.
Turn Duration (s)	-0.43	0.041	-0.41	0.038	-0.41	0.055	-0.40	0.041	-0.17	0.449	-0.38	0.055
Turn Angle (˚)	0.12	0.588	0.40	0.041	0.42	0.045	0.39	0.048	0.02	0.912	**0.68**	**0.0001**
Turn Velocity (˚/s)	0.26	0.229	**0.54**	**0.004**	0.29	0.186	**0.56**	**0.003**	0.02	0.939	**0.57**	**0.003**
Number of Turns (#)	0.47	0.025	0.31	0.121	0.49	0.018	0.33	0.099	0.22	0.303	0.36	0.073

Notes: Values in **bold** remained significant after Bonferroni Correction ($p = 0.0125$)

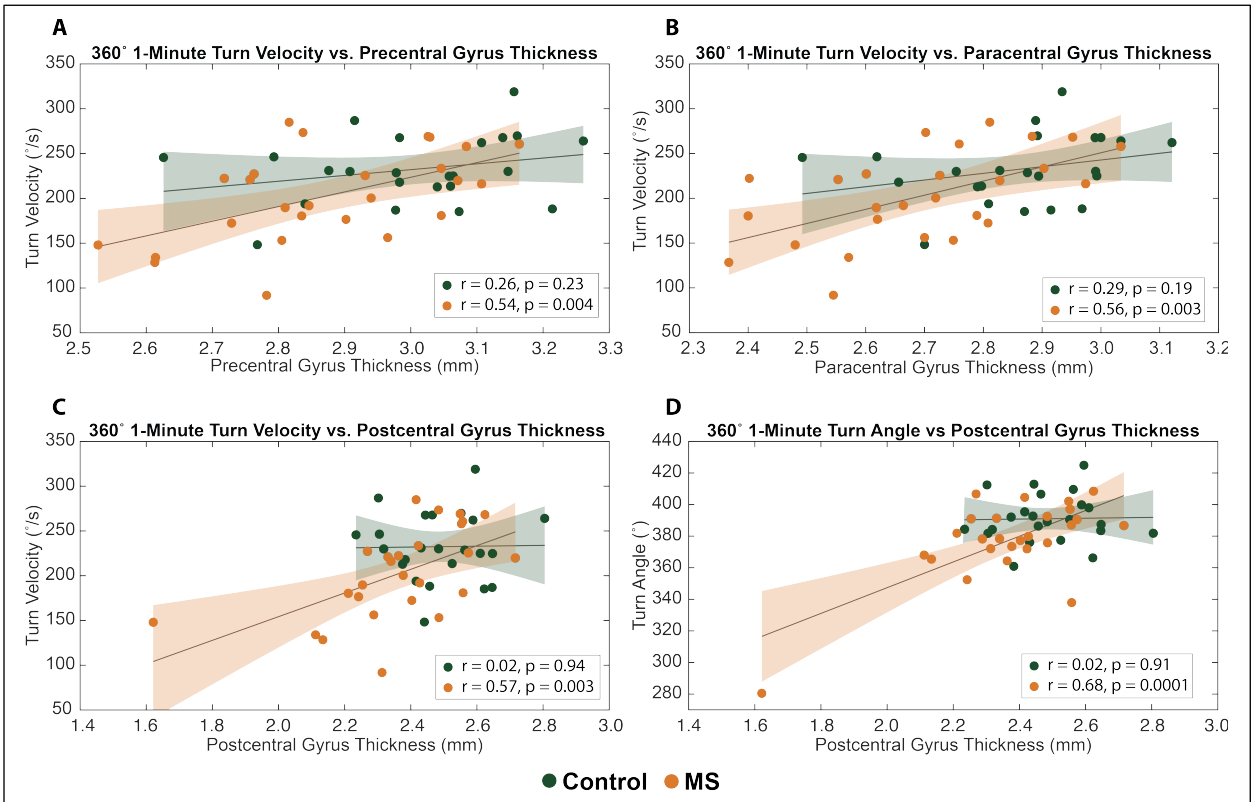

Figure 4.5. Associations between left hemisphere ROI thickness and 1-minute 360˚ measures. A) depicts a significant association between peak turn velocity and left hemisphere precentral gyrus thickness for PwMS. B) depicts a significant association between peak turn velocity and left hemisphere paracentral gyrus thickness for PwMS. C) depicts a significant association between peak turn velocity and left hemisphere postcentral gyrus thickness in PwMS. D) depicts a significant association between turn angle and left hemisphere postcentral gyrus thickness in PwMS.

CORRELATIONS BETWEEN 180˚ TURN METRICS AND SENSORIMOTOR THICKNESS OF THE LEFT HEMISPHERE

The correlation results between 180˚ turn variables and cortical thickness of distinct sensorimotor ROIs demonstrated no significant associations for either group. Table 4.8 details all quantified associations with a corrected p-value set to 0.0125.

Table 4.8. Associations between left hemisphere ROI thickness and 180˚ turning measures.

	Precentral Thickness (mm)				Paracentral Thickness (mm)				Postcentral Thickness (mm)			
	Control		MS		Control		MS		Control		MS	
	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.

Turn Duration (s)	-0.39	0.064	-0.07	0.739	-0.24	0.274	-0.17	0.403	-0.19	0.378	-0.08	0.701
Turn Angle (˚)	-0.15	0.508	0.28	0.164	-0.25	0.241	0.24	0.238	-0.25	0.251	0.11	0.596
Turn Velocity (˚/s)	0.33	0.126	0.25	0.220	0.22	0.324	0.34	0.092	0.25	0.249	0.12	0.575
Steps in Turn (#)	-0.10	0.660	0.00	0.995	-0.16	0.463	-0.06	0.761	0.08	0.726	0.01	0.952

Notes: No associations demonstrated significance after Bonferroni Correction (p = 0.0125)

CORRELATIONS BETWEEN NEUROANATOMICAL FUNCTION AND TURNING MEASURES

Refer to Chapter 3 for detailed associations between neurophysiological function and turning performance.

DISCUSSION

The primary aim of the present study was to assess the effects of MS on global and regional cortical thickness. The secondary aims of the current study were to determine whether cortical thickness revealed any distinct atrophic patterns in MS and furthermore, to analyze associations between neurophysiological function and turning performance. Both global and regional grey matter thickness measures were derived using structural MRI analysis. Results demonstrated diffuse cortical thinning in PwMS localized to left fronto-temporo-parietal regions and the right occipital pole. The ROI analysis demonstrated significant thinning of the pre- and paracentral gyri in MS, although no significance was observed between groups postcentral gyrus thickness. Both 360˚ and 180˚ turning measures were obtained using wireless inertial sensors which demonstrated distinct spatiotemporal differences between groups. Excitatory and inhibitory neurophysiological function was measured at the tibialis anterior (TA) via single pulse TMS which revealed significantly reduced excitability and inhibitory activity in the MS cohort. The correlations between left hemisphere cortical thickness of the pre- and paracentral gyri were significantly associated with left hemisphere inhibitory TMS

measures. In addition, left hemisphere cortical thickness of the specified ROIs (i.e., precentral gyrus, paracentral gyrus, and postcentral gyrus) demonstrated associations with turning performance specific to the 360˚ turn variables of the MS cohort. Together these results demonstrated a propensity for left hemisphere atrophy of the primary motor cortex which was strongly associated with inhibitory neurophysiological function. Additionally, while the postcentral gyrus did not demonstrate thickness differences between groups all three ROIs representing the sensorimotor cortex were significantly associated with 360˚ turning performance in PwMS.

WHOLE BRAIN

The results from the current study demonstrated similar regional thinning and augment prior indications of atrophic lateralization in PwMS. Our results are consistent with other reports demonstrating significant thinning in the superior parietal, supramarginal, inferior parietal, superior temporal, middle frontal, precentral, paracentral, pars opercularis, posterior cingulate, superior frontal, precuneus, and occipital cortical regions in PwMS (Sailer, Fischl et al. 2003, Calabrese, Atzori et al. 2007, Charil, Dagher et al. 2007, Bendfeldt, Kuster et al. 2009, Ramasamy, Benedict et al. 2009, Lansley, Mataix-Cols et al. 2013, Narayana, Govindarajan et al. 2013, Steenwijk, Geurts et al. 2016, Rocca, Battaglini et al. 2017, Eshaghi, Marinescu et al. 2018). Specifically, our results showed greater cortical atrophy in the left hemisphere when compared to the right, which appears to be consistent with results from a published meta-analysis assessing localization of grey matter atrophy in PwMS (Lansley, Mataix-Cols et al. 2013). Additionally, published results have documented greater atrophy in the left hemisphere compared to the right, further confirming a propensity of left hemisphere atrophy in MS

(Ramasamy, Benedict et al. 2009, Prinster, Quarantelli et al. 2010, Narayana, Govindarajan et al. 2013, Preziosa, Pagani et al. 2017).

This pattern of localized and asymmetric atrophy is not unique to MS, in fact neurotypical aging and other neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease have demonstrated similar localized structural adaptations which are thought to be associated with the underlying progression of age and disease pathology (Derflinger, Sorg et al. 2011, Lemaitre, Goldman et al. 2012, Claassen, McDonell et al. 2016). While the regional predominance and lateralization of grey matter atrophy in MS remain unknown, this pattern of atrophy may be the result of neural susceptibility to disease manifestations such as chronic inflammation, or improper neuro-protective/neuro-plastic responses (Lansley, Mataix-Cols et al. 2013). For instance, it has been theorized that thalamic atrophy (which presents early in the disease course) may be an indirect consequence of cortical grey matter loss (Lansley, Mataix-Cols et al. 2013, Eshaghi, Prados et al. 2018). This is assumed to be due to the thalamus having extensive reciprocal connections with the cortex and other subcortical structures, making it exceptionally sensitive to the effects of pathology in those areas (i.e., cortex and subcortical structures). Therefore, the thalamus may act as a primary indicator of diffuse neuropathologic damage as a result of MS (Cifelli, Arridge et al. 2002, Minagar, Barnett et al. 2013). Conversely, research has suggested that thalamic grey matter atrophy may be the product of localized axonal lesions within the thalamocortical tract, resulting in upstream and downstream retrograde degenerative effects (Henry, Shieh et al. 2009, Azevedo, Cen et al. 2019).

SENSORIMOTOR REGION OF INTEREST

In addition to the whole brain analysis, we performed a ROI analysis with the following *a-prior* regions – precentral, paracentral, and postcentral gyri. These particular regions were decided based on prior evidence demonstrating particular susceptibility to cortical atrophy in MS, and the known influence and integration of motor commands from those particular cortical structures (Sailer, Fischl et al. 2003, Steenwijk, Geurts et al. 2016). Additionally, TMS stimulation was targeted towards to the leg region of the motor cortex which neuroanatomically aligns with the paracentral gyrus.

Although postcentral gyrus thickness between groups did not reach significance there was a trend towards cortical thinning for that particular region in the MS cohort. We, therefore, believe these results to be consistent with prior investigations demonstrating cortical atrophy of the sensorimotor cortex in PwMS (Reddy, Narayanan et al. 2002, Sailer, Fischl et al. 2003, Lansley, Mataix-Cols et al. 2013, Narayana, Govindarajan et al. 2013, Bergsland, Laganà et al. 2015, Nantes, Zhong et al. 2016, Steenwijk, Geurts et al. 2016). Moreover, a recent meta-analysis reported these particular regions consistently emerged as areas that demonstrated cortical atrophy in PwMS and were furthermore significantly associated with functional disability (Sailer, Fischl et al. 2003, Prinster, Quarantelli et al. 2010, Lansley, Mataix-Cols et al. 2013).

ASSOCIATIONS BETWEEN NEUROANATOMICAL STRUCTURE AND NEUROPHYSIOLOGICAL FUNCTION

The effects of MS on the nervous system are diverse, although it has been established that as a product of MS pathology both cortical grey matter and excitatory/inhibitory neurophysiology are influenced. Given that neurophysiological activity (excitatory and inhibitory) occurs within the grey matter (cortical and subcortical)

the consequences of MS pathology should theoretically demonstrate associations between cortical grey matter atrophy and neurophysiological activity. To our knowledge, only one study has explored this relationship in PwMS. Nantes, et al. (2016) performed structural analysis assessing cortical thickness differences of the precentral gyrus between neurotypical controls and PwMS, additionally, the authors performed single pulse TMS to quantify cSP duration from the dominate (i.e., right) hand first dorsal interosseus (FDI) muscle (Nantes, Zhong et al. 2016). Their results showed left lateralized cortical thinning and longer silent period durations in the impaired MS cohort compared to the neurotypical control group. However, their results demonstrated non-significant associations between left hemisphere precentral gyrus thickness and cSP duration. It is worth noting that the Nantes and colleague's study contradicts aspects of the current study results. First, our results did not reveal longer silent period durations in the MS cohort, in fact, the durations were nearly identical between groups (for further information see Chapter 3 discussion). Second, the direction of their associations between cortical thickness of the motor cortex and silent period duration for the MS cohort was distinctly inverse to ours. For instance, Nantes and colleagues reported a non-significant association in the negative direction, while we reported a moderate, significant positive association between motor cortex thickness and silent period duration. However, Nantes, et al. (2016) did report a negative association between motor cortex thickness and silent period duration for the neurotypical control cohort, which reflects the results from our study. Interestingly, since silent period durations were similar between groups in our study, we postulate that the effect of cortical structure degradation may be an important mechanism mediating corticospinal inhibitory activity. These results suggest that left

lateralized cortical thickness of the motor cortex (i.e., pre- and paracentral gyri) may impact inhibitory activity in MS, such that reduced thickness results in reduced inhibitory activity.

Additionally, our results demonstrate an inverse relationship between groups for the associations between cortical thickness of the paracentral gyrus and both silent period duration and %dcSP Ave of the TA. Given the relative lack of both upper and lower limb studies in this area, this inverse relationship between cortical thickness and inhibitory activity remains difficult to interpret, especially for the neurotypical adults. In the healthy aging literature, older adults often demonstrate reduced cortical thickness, and moreover, those who demonstrate greater inhibitory activity perform motor control tasks better than those with reduced inhibition (Seidler, Bernard et al. 2010, Fling and Seidler 2012, Swanson and Fling 2018, Swanson and Fling 2019). Alternatively, neurotypical younger adults typically do not present with cortical atrophy, and furthermore, those with reduced inhibitory activity demonstrate better motor performance (Seidler, Bernard et al. 2010). While the current neurotypical control cohort spans a wide range of ages these results could suggest proper inhibitory adjustments as a product of cortical thickness and, likely age. Alternatively, literature suggests that the first ~50ms of the cortical silent period are spinally mediated whereas the remaining duration is cortically mediated inhibition (Fuhr, Agostino et al. 1991, Cantello, Gianelli et al. 1992). Therefore, greater thickness and shorter silent periods may represent optimal neuronal efficiency, such that the allocation of commands are mediated at the spinal and/or subcortical levels allowing higher order neural mechanisms (i.e., the cortex) to manage other complex operations. Together, these results suggest a fundamental difference concerning neuroanatomical structure

and neurophysiological function between neurotypical controls and PwMS. Such that greater cortical thickness in neurotypical individuals results in reduced but optimal (or necessary) inhibitory activity. While in MS, inhibitory capacity is reduced as a result of pathology; therefore, greater cortical thickness provides enhanced opportunity for inhibitory neurotransmission which is likely necessary for proper compensation.

ASSOCIATIONS BETWEEN SENSORIMOTOR CORTICAL THICKNESS AND TURNING PERFORMANCE

Grey matter atrophy has been associated with clinical indicators including physical disability, cognitive decline, and disease duration (Sailer, Fischl et al. 2003, Chen, Narayanan et al. 2004, Amato, Portaccio et al. 2007, Calabrese, Atzori et al. 2007, Benedict, Holtzer et al. 2011, Roosendaal, Bendfeldt et al. 2011). Importantly, grey matter atrophy demonstrates independence from white matter degradation and furthermore, stronger associations with clinical indicators (De Stefano, Matthews et al. 2003, Calabrese, Atzori et al. 2007, Benedict, Holtzer et al. 2011, Roosendaal, Bendfeldt et al. 2011, Calabrese, Magliozzi et al. 2015). While grey matter atrophy demonstrates clinically relevant correlations in MS, recent reports suggest atrophic patterns are predominately regional rather than diffuse and global (Lansley, Mataix-Cols et al. 2013, Steenwijk, Geurts et al. 2016). Furthermore, regional patterns of atrophy appear to provide distinct relationships with functional disability (Lansley, Mataix-Cols et al. 2013, Steenwijk, Geurts et al. 2016). Specifically, it has been observed that left lateralized atrophic clusters of the sensorimotor cortex demonstrate significant negative associations with the EDSS (Prinster, Quarantelli et al. 2010, Steenwijk, Geurts et al. 2016). Given that the EDSS has a bias towards locomotor disability, this negative association suggests atrophy of these

regions influences functional disability. Consistent with prior results demonstrating an association between the EDSS and cortical thickness of the sensorimotor cortex, our results further demonstrate left lateralized cortical atrophy associated with specific characteristics of turning performance in PwMS. Such that reduced cortical thickness of the sensorimotor cortex (i.e., pre-, para- and postcentral gyrus) correlates with 360˚ turn duration, velocity, and angle. Therefore, providing further evidence that left hemisphere atrophy impacts locomotor movements, specifically turning characteristics.

One other study has assessed the effects of cortical atrophy on turning variables in PwMS. Although this study performed by Lorefice and colleagues measured global cortical atrophy and its association with components of the instrumented Timed Up and Go (iTUG) (Lorefice, Coghe et al. 2017). Specifically, Lorefice, et al. (2017) showed a significant negative association between global cortical grey matter volume and the iTUG completion time (Lorefice, Coghe et al. 2017). Moreover, the authors further demonstrated significant positive associations between mean and peak turn velocity and global cortical grey matter volume (Lorefice, Coghe et al. 2017). Thus, revealing a relationship between cortical grey matter structure and lower limb dynamic motor performance, where greater cortical grey matter volume relates to faster turns and better iTUG performance. It must be noted that turns performed during the iTUG are characteristically 180˚, which in our study none of the 180˚ turn variables demonstrated significant associations with cortical thickness of the specified ROIs. While these results are inconsistent, we believe this may be a product of associating specific turning variables to global, rather than regional measures of atrophy. These results may also demonstrate inconsistencies given the different task objectives. For example, the iTUG performance

is solely based on completion time, such that faster times equate to better mobility performance. While in the current study participants were observed completing 'naturally occurring' 180˚ turns during a self-selected pace two-minute walk test. Moreover, the authors suggested that given the relative complexity of turning, which requires whole body coordination and the ability to anticipate/plan forthcoming movements that these correlations may likely be associated with 'the cognitive control' of movement (Lorefice, Coghe et al. 2017). While cognitive control is necessary for the production of an intrinsically motivated motor plan, that term is likely meant to suggest numerous subregions within the prefrontal cortex spanning a relatively large and complex neural network (Miller 2000, Clark 2015). However, we contest that given their associations were specific to temporal characteristics of movement (in this case, turn velocity), regions such as the sensorimotor cortex may offer more compelling evidence for their observed associations. Furthermore, given that the EDSS (which has a bias towards locomotion) has been associated with the left hemisphere sensorimotor cortex in PwMS, specific characteristics of locomotor movements may likely be influenced by atrophy of the sensorimotor region.

Given that turning is a complex movement not only requiring strength, balance and coordination, but also the ability to anticipate, correct for postural perturbations, and incorporate programming and planning skills, it is important to assess cortical regions that are known influence components of this complex transitionary movement (Lorefice, Coghe et al. 2017). As such, literature has identified the left frontoparietal region as a particular area known to influence motor planning, adaptation, learning of sequences, and skills (Mutha, Haaland et al. 2012). Moreover, research has shown that damage to the

left frontoparietal region can result in ideomotor apraxia, which causes spatiotemporal motor deficits thought to arise from impaired motor planning due damage of internal motor representations (Haaland, Harrington et al. 2000, Ochipa and Rothi 2000, Mutha, Haaland et al. 2012). Which have also been described as movement formulas that specify the spatiotemporal components of skilled purposeful limb movements (Ochipa and Rothi 2000). As such, we suspect our associations between cortical thickness and spatiotemporal characteristics of 360˚ turning are consistent with damage to the frontoparietal region. Moreover, given that motor cortical neurons, specifically pyramidal tract neurons influence spinal interneuronal networks and lower motor neurons, disruption or degradation likely results in reduced synergistic muscle activity and subsequently affects lower limb movement patterns (Drew and Marigold 2015). While we did not demonstrate thickness differences between neurotypical controls and PwMS for the postcentral gyrus our results did reveal associations between that cortical region and 360˚ turning characteristics. We believe this is likely a product of reduced integration and/or functional connectivity of the ipsilateral sensorimotor network, such that the connections between the somatosensory cortex and motor cortex are somehow disrupted (Peterson and Fling 2018, Tavazzi, Bergsland et al. 2018). Lastly, our results did not reveal any associations between cortical thickness of the specified ROIs and turning performance measures in the neurotypical control cohort. We believe this is likely due to a diverse motor network properly integrating for the control of dynamic lower limb movements. For instance, contributions from cortical and subcortical structures including the posterior parietal cortex, cerebellum, basal ganglia, and premotor cortex, among others converge on the motor cortex for proper movement execution and adaptive gait modifications (Drew

and Marigold 2015). Whereas in MS pathology, atrophy of the motor cortex provides a distinct region influencing locomotor impairments. Additionally, given that motor cortex atrophy only explains roughly 50% of the turning results, it goes without saying that the aforementioned structures likely influence turning in MS.

CONCLUSION

Together, we believe the current results support the previously reported observation of left hemisphere lateralized atrophy in PwMS. Additionally, we expand upon previous observations demonstrating significant associations between the left pre-, para-, and postcentral gyrus thickness and aspects of locomotion by specifically assessing associations between cortical thickness and turning characteristics. Lastly, we demonstrate consistency for the role of the left frontoparietal region influencing planned motor skills and spatiotemporal characteristics of movement. Thus, adding specificity to regional control of lower limb movements and the effects of atrophy in PwMS.

CHAPTER 5 – OVERALL CONCLUSIONS

SUMMARY

In this series of studies, we examined the effect relapsing remitting multiple sclerosis (RRMS) has on turning performance, neurophysiological function, neuroanatomical structure and the associations between these distinct components. The combined series of studies included 26 individuals with clinically definite RRMS and 23 age and sex matched neurotypical control participants. In the first study, we used validated wireless inertial sensors to measure turning variables from three discrete measures of turning. Specifically, we measured self-selected fast pace 360˚ in place, 1-minute of consecutive but alternating 360˚ in place self-selected natural pace, and 180˚ self-selected natural pace turns. Our results revealed that PwMS demonstrated reduced turning performance compared to neurotypical healthy controls. For the 360˚ in place fast pace turns PwMS demonstrated significantly longer turn durations and reduced peak turn velocities, although no differences were observed for turn angle. For the 1-minute of consecutive but alternating 360˚ turns, PwMS demonstrated significantly longer turn duration, reduced peak turn velocity, reduced turn angles, and fewer total turns compared to their neurotypical counterparts. Lastly, for the 180˚ turns, no significant differences were observed for any of the measured turning variables (i.e., turn duration, peak turn velocity, turn angle, and number of steps within the turn). The results from Chapter 2 indicate that PwMS demonstrated reduced turn performance although the reductions appeared to be limited to more complex 360˚ turns.

In the second study, we measured neurophysiological activity and the association between turning performance. Specifically, we assessed corticospinal excitation via glutamatergic activity and inhibition via GABAergic activity using spTMS measured at the TA muscle of each leg individually. Our excitatory TMS results revealed that PwMS demonstrated reduced MEP amplitude, suggesting a reduction of glutamatergic activity in PwMS compared to neurotypical controls. For the inhibitory TMS assessment, we assessed three characteristics of the resultant silent period. These results showed similarities between PwMS and neurotypical controls for the temporally determined cortical silent period duration, while measures of inhibitory capacity (i.e., the degree to which the muscle was deactivated as a result of inhibition following stimulation) demonstrated significant reductions in PwMS. Together the neurophysiological measures revealed that individuals with RRMS demonstrated reduced lower limb related glutamatergic activity and GABAergic capacity. The correlation analysis demonstrated left hemisphere lateralized significant associations between inhibitory neurophysiological measures and turning performance. Excitatory measures demonstrated no associations between the turning measures for either group. Conversely, inhibitory measures demonstrated significant associations between all turning measures, primarily in the MS cohort. Specifically, for the 360˚ fast pace turns, turn duration was significantly associated with silent period duration, percent average depth of the silent period (%dcSP Ave), and percent maximal depth of the silent period (%dcSP Max) in the MS group, while turn velocity was only associated with %dcSP Max in the MS group. For the 1-minute consecutive but alternating 360˚ turns, turn duration and was significantly associated with %dcSP Ave and %dcSP Max in the MS group, while for number of turns a significant

association was observed between %dcSP Ave and both groups and %dcSP Max in the MS group. Lastly, for the 180˚ turns, peak turn velocity demonstrated a significant association between silent period duration and %dcSP Ave in MS group. Together, the results from Chapter 3 demonstrated significant differences for both excitatory and inhibitory TMS measures and furthermore, associations between corticospinal inhibition and turning performance. Thus, the current study showed that PwMS who had greater inhibition performed turn more similarly to their neurotypical counterparts, whereas neurotypical controls with greater inhibition completed fewer total turns, revealing an opposing effect of inhibition on turning performance.

In the third study, we assessed global and region of interest (ROI) cortical grey matter atrophy in PwMS compared to neurotypical controls. Additionally, we assessed the associations between ROI cortical thickness and neurophysiological and turning measures. Specifically, we assessed group differences in global cortical thickness and cortical thickness of the sensorimotor cortex via three distinct ROIs (pre-, para-, and postcentral gyri) between groups via structural MRI analysis. The global cortical thickness differences demonstrated a district pattern of atrophy lateralized to the left cortical hemisphere, revealing significant clusters of atrophy in the fronto-temporo-parietal region. The sensorimotor ROI analysis revealed significant cortical atrophy in PwMS for the precentral and paracentral gyrus, although the postcentral gyrus did not demonstrate thickness differences between groups. The associations between neuroanatomical structure and neurophysiological function demonstrated significant associations between cortical silent period duration and the thickness of the precentral and paracentral gyrus, and furthermore an association between %dcSP Ave and paracentral gyrus thickness.

The results from the prior association indicated that PwMS with greater thickness demonstrated greater levels of inhibition, while in the neurotypical controls the opposite was observed, such that greater thickness was associated with reduced levels of inhibition. The correlation analysis between sensorimotor cortical thickness and turning produced a number of significant associations that were specific to the MS cohort and 360˚ turn measures. For 360˚ fast pace turns, turn duration was significantly associated with pre-, para-, and postcentral gyrus thickness, while peak turn velocity was associated with the pre- and paracentral gyrus thickness. For the 1-minute consecutive but alternating 360˚ turns, turn angle demonstrated a significant association with postcentral gyrus thickness, while peak turn velocity was significantly associated with thickness of all three ROIs. The correlations between 360˚ turn performance and sensorimotor cortical thickness indicated that PwMS who had greater sensorimotor cortical thickness performed turns more similarly to their neurotypical control counterparts. Conversely, sensorimotor cortical thickness was not associated with turning performance in the neurotypical control cohort. Taken together the results from Chapter 4 demonstrated localized left hemisphere fronto-temporo-parietal cortical atrophy along with specific cortical thinning of the motor cortex. Additionally, these results demonstrated an association between neuroanatomical structure and neurophysiological function and 360˚ turning performance in PwMS.

To date, no studies have assessed both cortical thickness and neurophysiological function in PwMS specific to the lower limbs. Moreover, no studies have incorporated objective measures of dynamic lower limb movements to identify neural mechanisms associated with performance. As such this study provides a novel approach to assess

the neural underpinnings of movement in PwMS, and furthermore provides compelling evidence to suggest a relationship between neuroanatomical structure and neurophysiological function of the sensorimotor cortex and turning performance in PwMS.

FUTURE DIRECTIONS

TURNING IN MULTIPLE SCLEROSIS

The study performed in Chapter 2 provides novel insights regarding spatiotemporal turning differences between PwMS and neurotypical controls. Given that turning is a dynamic task requiring both kinematic and systematic integration, a number of questions remain in explaining the results uncovered in Chapter 2. While our study identified that PwMS take longer to perform turns and do so at a reduced angle and velocity, these measures are the result of an amalgamation of various factors that together, demonstrate reduced performance. As such, we believe further evaluation of turning through the identification of distinct components participating in the movement could be augmented to uncover various nuances of turning in PwMS.

During a turn, there is a defined temporal sequence of the axial skeletal segments initiating movement and reorienting the body's position in space. First, the movement begins at the head in the yaw direction along the longitudinal axis, which is followed by the trunk, pelvis, and finally the movement of the feet. As such, this movement is completed in a cranio-to-caudal sequence (Hollands, Ziavra et al. 2004). In neurotypical older adults, the components of this cranio-to-caudal sequence have been shown to be impaired, for instance older adults completing 130˚ turns have demonstrated less head on trunk, or cervical spine rotation compared to their younger counterparts, although appears to be compensated for by increasing the lumbo-pelvic rotation (Baird and Van

Emmerik 2009). Moreover, for 90˚ turns it has been shown that older adults often initiate a turn with all segments of the axial skeleton (i.e., head, trunk, pelvis) simultaneously, which has been termed as an en-bloc style turn. Furthermore, those older adults who demonstrate greater rates of falling, present with an en-bloc style of turning for 360˚ turns (Wright, Peters et al. 2012). The en-bloc style of turn is not only observed in neurotypical older adults but has also been observed in neurologically impaired populations including Parkinson's disease and stroke (Lamontagne, Paquette et al. 2007, Hong, Perlmutter et al. 2009). Importantly, the en-bloc style is thought to be adopted as a compensatory adaptation to simplify the control of segmental movements and reduce imbalance, however this compensation increases an individual's risk of falling (Wright, Peters et al. 2012). While this style of turn has been observed in a variety of populations and associated with mobility disability, no studies to-date have assessed whether or not PwMS demonstrate an en-bloc style turn. Therefore, investigating segmental turning in MS may provide additional context regarding the observed reduced turning performance in this population.

Aside from the segmental components of turning, it is well established that turning requires proper balance control which incorporates three primary and distinct systems – vision, somatosensory, and vestibular. Moreover, all three systems have been shown to be affected to varying degrees as a result of MS pathology (Hebert, Corboy et al. 2011, Jasse, Vukusic et al. 2013, Huisinga, St George et al. 2014). However, it remains unknown whether one particular balance related system is predominantly influencing the observed reduction in turning performance. From a clinical perspective, identifying whether segmental alterations are associated with turning deficits in MS, may provide

targeted rehabilitation opportunities. Moreover, it remains to be investigated whether turning deficits are driven by deficits to all systems or if one particular balance system is driving turn related deficits. Theoretically identifying the underlying impairment(s) (i.e., en-bloc, vision, somatosensory, vestibular) may be advantageous for specific rehabilitation approaches, with the objective of improving turning performance and reducing fall risk in the MS population. In fact, a couple of preliminary investigations have reported that poor gaze stability is related to reduced dynamic balance and furthermore the utility wireless inertial sensors placed on the head are able to track the progression of gaze rehabilitation and head control (Garg, Dibble et al. 2018, Loyd, Fangman et al. 2019, Loyd, Saviers-Steiger et al. 2020).

NEUROPHYSIOLOGY IN MULTIPLE SCLEROSIS

Although neurophysiology can be assessed in a variety of different ways, the future directions portion pertaining to this document will only discuss non-invasive brain stimulation (NIBS) techniques including – single pulse TMS (spTMS), paired pulse TMS (ppTMS), and repetitive TMS (rTMS). NIBS has proven to be a useful tool in elucidating various biochemical processes related to motor impairment in MS; although, to date research utilizing spTMS has primarily investigated upper limb related measures of excitability and the associations between disability severity in MS. However, disability severity heavily weighs measures of lower limb impairments via an individual's ambulatory ability. Given that the upper and lower limbs interact with(in) the environment in vastly different ways, neurophysiological control of both is possibly different. Therefore, substantially more research is needed for the investigation of neurophysiological control of the lower limbs in PwMS. Given that the current study in Chapter 3 is the second study

(to date) measuring the cortical silent period associated with the leg region of the motor cortex, and the first to incorporate inhibitory amplitude measures, there remains a lack of conclusive literature and plenty of opportunity for future studies.

Aside from the study performed in Chapter 3, the evaluation of inhibitory activity using spTMS for lower limb motor impairments remains vastly inconclusive. While the spTMS related healthy aging literature also remains inconclusive, significant interactions between inhibitory activity and various objective linear gait measures (i.e., gait coordination and variability) often impaired in PwMS have been observed (Swanson and Fling 2018, Richmond, Swanson et al. 2020). As such, studies specifically associating objective lower limb impairments such as muscle weakness, spasticity, postural stability, and mobility to excitability and inhibitory outcomes requires further investigation in PwMS.

Moreover, ppTMS techniques such as short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), or long-interval intracortical inhibition (LICI) have demonstrated promising results and associations with levels of impairment in the upper limbs of PwMS (Mori, Kusayanagi et al. 2013, Nantes, Zhong et al. 2016, Nantes, Zhong et al. 2016, Neva, Lakhani et al. 2016), although they have yet to be applied to the lower limbs. Together with spTMS measures, the addition of ppTMS protocols will provide greater delineation of GABAergic receptor subtype ($GABA_A$ and $GABA_B$) and cortical vs. corticospinal excitability, thus providing greater detail when studying the pathophysiology of lower limb motor impairment in MS.

While sp- and ppTMS provide in-vivo measures of excitatory and inhibitory neurophysiological activity, the use of rTMS offers the ability to modulate corticospinal excitability (i.e., the balance of inhibition and excitation). Studies performing rTMS, have

been able to identify associations between these neuromodulatory changes and observed behavioral adaptations. For instance, the current MS literature has primarily focused on ameliorating symptoms of lower limb spasticity, but smaller studies have presented promising preliminary results for the effects of rTMS on postural stability and walking impairments (Centonze, Koch et al. 2007, Mori, Codeca et al. 2010, Mori, Ljoka et al. 2011, Leocani, Nuara et al. 2012, Boutiere, Rey et al. 2017). Given the promising preliminary results, additional research needs to be performed to fully understand the potential effect rTMS may have on PwMS demonstrating mobility limitations. If the results from these preliminary investigations are confirmed by larger and more rigorous research protocols, the clinical utility of rTMS may prove beneficial, and furthermore effective in partially ameliorating mobility related dysfunction in PwMS.

NEUROANATOMICAL STRUCTURE IN MULTIPLE SCLEROSIS

The study in Chapter 4, demonstrates clusters of cortical thinning in PwMS, and furthermore associations between left sensorimotor cortical thickness and turning performance in PwMS. While there are a variety of MRI based neuroimaging techniques and analyses capable of assessing neuroanatomical structure (i.e., diffusion tensor imaging and graph theory approaches, respectively) the future directions discussed here will focus on cortical thickness assessments in particular cortical and subcortical locations. While the sensorimotor cortex is vitally important for integrating and regulating descending motor commands, prior research has identified a number of cortical and subcortical locations also known to influence mobility. As such, we propose the investigation of subcortical structures including the basal ganglia and thalamus, and cortical structures such as the posterior parietal cortex.

The thalamus and basal ganglia which is a group of subcortical nuclei consisting of the caudate nucleus, putamen, globus pallidus, subthalamic nucleus, and substantia nigra are important structures mediating and regulating various functions (i.e., cognition, memory, emotion, etc.) including motor control and locomotion (Allali, Blumen et al. 2018). In MS, atrophy of the thalamus, putamen, caudate, and globus pallidus have been linked to ambulatory performance in MS via the Timed 25-Foot Walk (T25FW) test (Motl, Hubbard et al. 2015, Onu, Aroceanu et al. 2015, Motl, Zivadinov et al. 2016). While these studies have shown reduced structural volume in MS to be associated with worse ambulatory performance (i.e., longer time to complete the T25FW), no studies have associated objective spatiotemporal measures of locomotion to localized levels of atrophy. Interestingly, a recent study identified increased structural volume of the left pallidum after a 12-week aerobic exercise program, however, as the authors note, this study was not sufficiently powered and requires a larger sample size to confirm their results (Feys, Moumdjian et al. 2019). Regardless, these initial findings are compelling and possibly provide a distinct anatomical structure associated with locomotion, capable of beneficial adaptation.

Another anatomical location worth investigating is the posterior parietal cortex which is involved in the integration of visually guided movement planning of locomotion (Drew and Marigold 2015). Specifically, animal studies have shown that cell clusters within the posterior parietal cortex demonstrate substantial changes in neuronal discharge prior to and following gait modifications that require visual guidance (Andujar, Lajoie et al. 2010, Marigold, Andujar et al. 2011). Further, human studies incorporating electroencephalography (EEG) have shown increased neuronal activity in the posterior

parietal cortex during a visually guided gait adaptation task in virtual reality, suggesting higher cortical activation may reflect greater motor planning and visuomotor processing (Wagner, Solis-Escalante et al. 2014). Similar results have also been reported in an MS pilot study assessing posterior parietal cortex activation during a bout of walking, although the authors did not report differences in activation between PwMS and neurotypical controls (Hoxha, Glassen et al. 2019). Although the associations between the posterior parietal cortex and visually guided locomotion have been observed using different imaging techniques, the MS literature has identified the posterior parietal cortex commonly experiences cortical thinning in MS (Nygaard, Walhovd et al. 2015, Preziosa, Pagani et al. 2017). Therefore, assessing the associations between cortical thinning of the posterior parietal cortex and objective measures of locomotion may provide additional context as to mobility disability in PwMS.

LIMITATIONS

This series of studies is not without particular limitations. Given that the same cohort of individuals was included in each study there are some collective limitations that apply. A collective limitation is the inclusion of only individuals with RRMS with an average EDSS of 3.5. Therefore, these results should be interpreted responsibly when associating them with other MS phenotypes or severity levels. Additionally, while this study was sufficiently powered to detect between group differences, the sample size remains quite small and future studies should incorporate similar measures in larger cohorts of participants. While the prior two limitations are collective for the series of studies presented, there are study specific limitations.

For the turning study (Chapter 2), we only assessed two distinct turn angles (180˚ and 360˚) which does not fully represent the degree of turns performed on a daily basis (Leach, Mellone et al. 2018). Therefore, it remains to be evaluated whether lesser turn angles demonstrate similarities or differences between PwMS and neurotypical controls. As such, the interpretation of these results can only suggest that PwMS present with reduced turning performance for 360˚ self-selected natural and fast pace turns. Additionally, we only assessed broad measures of turning (i.e., duration, velocity, angle, etc.), which do not account for the various idiosyncrasies likely associated with the measures of turning we evaluated. However, that was not an aim of the current study, and as such future studies should aim to evaluate the kinematics of axial segmentation of turning.

The assessment of neurophysiological function in PwMS (Chapter 3) was also not without limitations. Given that the leg region of the motor cortex (i.e., paracentral gyrus) lies within the longitudinal fissure and was the site for TMS simulation, there was a slight possibility of stimulation over-flow to the homologous paracentral gyrus. However, during hot spot detection MEPs were collected simultaneously from both legs, and special care was taken to elicit MEPs from the targeted hemisphere and corresponding TA only. Additionally, this study incorporates two additional measures of intrahemispheric inhibitory activity which have yet to be reported in the literature. While these measures (%dcSP Ave and %dcSP Max) are novel to the cSP literature they have been incorporated extensively in the upper limb ipsilateral silent period (iSP) literature (Fling and Seidler 2012, Davidson and Tremblay 2013, Coppi, Houdayer et al. 2014, Chieffo, Straffi et al. 2019). Moreover, these measures have demonstrated success in

differentiating between groups (Hupfeld, Swanson et al. 2020), and furthermore have demonstrated greater reliability than the traditionally reported silent period duration for the iSP (Fleming and Newham 2017). Although reliability was not evaluated in the current study, we note the standard deviations for each measure and group were quite small, thus we suspect %dcSP Ave and %dcSP Max are likely reliable measures to use for the evaluation of intrahemispheric inhibition.

Lastly, some limitations apply to Chapter 4; the assessment of cortical thickness differences between neurotypical controls and PwMS. One limitation of the study was that we did not include specific MRI sequences suitable for the detection of GM lesions, such as Double Inversion Recovery (Abidi, Faeghi et al. 2017). However, the analysis performed for this particular study integrated T1 + T2-FLAIR data which has been shown to significantly improve cortical segmentation and the identification of cortical atrophy, as opposed to analyzing only one data type (Lindroth, Nair et al. 2019). Additionally, despite the vertex-wise analysis, we cannot rule out that white matter atrophy could have influenced the results. While FreeSurfer segmentations were rigorously assessed for errors, differences could exist between subjects with or without sulci deformation (i.e., widening) due to white matter atrophy. However, it remains unclear whether this would preferentially affect the results of particular cortical regions. Lastly, there remains limited information as to the effects of juxtacortical lesions on cortical atrophy (Klaver, Popescu et al. 2015, van de Pavert, Muhlert et al. 2016), however, it cannot be ruled out entirely that juxtacortical lesions did not have some influence on the results (Mistry, Abdel-Fahim et al. 2014).

CONCLUSION

The effects of MS are multifaceted and heterogeneous, although certain similarities exist in people diagnosed with the disease. For instance, over the course of the disease, the vast majority of PwMS present with diminished mobility, neuropathophysiological dysregulation, and non-random cortical atrophic patterns (Steenwijk, Geurts et al. 2016, Cameron and Nilsagard 2018, Peterson and Fling 2018). Together these decrements have been associated with a variety of indicators including, but not limited to, increased incidence of falls, disease severity, and reduced quality of life. This project adds to the growing body of literature in four distinct ways, 1) by characterizing turning differences between neurotypical controls and PwMS, 2) supporting lower limb related excitatory and inhibitory neurophysiological differences between neurotypical controls and PwMS, 3) supporting cortical atrophic patterns and lateralization in PwMS, and 4) identifying associations between turning performance and inhibitory activity and regional sensorimotor atrophy in PwMS.

Together, the collective results from this series of studies demonstrate that in PwMS neuroanatomical structure and neurophysiological function are associated, and furthermore they are independently related to turning performance. Upon closer inspection, the associations between inhibitory activity and turning performance are characteristically stronger associations than those between sensorimotor regional atrophy and turning performance in PwMS. Therefore, these results may suggest that inhibitory influence is more associated with dynamic lower limb movements compared to grey matter thickness in PwMS, although a mediation analysis would provide greater clarity as to the degree of influence on turning performance. Additionally, we interpret from these results that neurotypical controls perform turns better without large inhibitory

influence whereas, PwMS require greater inhibitory influence to perform turns similarly to their neurotypical counterparts. Finally, these results indicate that PwMS may utilize higher order neural mechanisms to perform dynamic movements typically associated with fall risk.

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