THESIS

THE TRANSCALLOSAL HIGHWAY: THE IPSILATERAL SILENT PERIOD AS A NEURAL BIOMARKER FOR IMPAIRED CORPUS CALLOSUM COMMUNICATION AND GAIT ASYMMETRY IN PEOPLE WITH MULTIPLE SCLEROSIS

Submitted by

Jordan Acosta

Department of Health and Exercise Science

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Master's Committee:

Advisor: Brett Fling

Alan Rudolph
Augusto Miravalle
Arlene Schmid

ABSTRACT

THE TRANSCALLOSAL HIGHWAY: THE IPSILATERAL SILENT PERIOD AS A NEURAL BIOMARKER FOR IMPAIRED CORPUS CALLOSUM COMMUNICATION AND GAIT ASYMMETRY IN PEOPLE WITH MULTIPLE SCLEROSIS

Multiple sclerosis is a neurodegenerative disease that damages the myelin sheath within the central nervous system. Axonal demyelination, particularly in the corpus callosum, impacts communication between the brain's hemispheres in persons with multiple sclerosis (PwMS). Changes in transcallosal communication impairs the coordination of gait which requires constant communication across the corpus callosum to excite and inhibit specific muscle groups. To further evaluate the functional role of transcallosal communication in gait and mobility, this study assessed the ipsilateral silent period (iSP), an indirect marker of transcallosal inhibition in PwMS. This study utilizes transcranial magnetic stimulation (TMS) to assess the inhibitory capacity between the brain's hemispheres. There is a lack of research analyzing directionality data between the more and less affected hemisphere in PwMS. Therefore, we evaluated outcome metrics dependent upon the individual's more affected hemisphere calculated from the subject's more affected limb observed during walking assessments and self-report. We hypothesize that the iSP may serve as a neural biomarker for transcallosal impairments evaluated by directionality

differences between the hemispheres and highlight transcallosal inhibition as an underlying neural mechanism for gait asymmetries in PwMS. From twenty-nine PwMS, metrics such as depth iSP% average, duration, depth iSP% max, and onset latency were collected. No statistically significant differences were found between the two hemispheres. This suggests that PwMS may be able to preserve their interhemispheric inhibitory capacity irrespective of their more affected hemisphere. Additionally, another component of the study investigated gait coordination utilizing a split-belt treadmill training paradigm. Limb excursion asymmetry (LEA) measures, pre and post-training, were analyzed for spatial coordination and as a measurement of locomotor adaptability in PwMS. The relationship between LEA change and dSP% average highlighted a significant correlation ($r=0.46$, $p= 0.02$). Thus, showing that less interhemispheric inhibition corresponds with more spatial adaptability leading to a more symmetric gait. These findings may help determine the potential of iSPs as a neural biomarker to address gait asymmetries and stratify participants into mobility rehabilitation protocols.

ACKNOWLEDGEMENTS

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INTRODUCTION

Multiple sclerosis (MS) is the most common progressive neurological disease of young adults worldwide.[1] In 2017, approximately 1 million adults were living with MS in the United States and 2.8 million people worldwide.[2, 3] Furthermore, disease onset and diagnosis are most common between the ages of 20-40 years old and women are 3 times more affected than men.[2] Additionally, a recent report in 2019 documented the total economic burden of direct and indirect medical costs to be $85.4 billion.[4] The rising prevalence and various hardships people with MS (PwMS) experience are widespread and extensive. Importantly, PwMS endure higher rates of comorbidities in comparison with the general population.[5] There is a great need to improve the lives of PwMS, but first we need a deeper understanding of the neural mechanisms underlying observed gait asymmetries, which is the focal point of this study.

MS is a neurodegenerative disease that damages the myelin sheath within the central nervous system (CNS). It is often characterized by chronic inflammation resulting in microscopic plaques referred to as lesions within various locations of the CNS.[6] The plaques are indicative of white matter damage caused by an abnormal immune system attack response. Although white matter damage occurs in numerous regions of the CNS in PwMS, it is typically most pronounced in the corpus callosum.[12,37] The corpus callosum is the largest white matter fiber bundle in the human nervous system and connects the right and left cortical hemispheres.[7] Importantly, PwMS present with a broad range of symptoms demonstrating extreme heterogeneity within the disease. The severity and vast range of symptoms are reflective of lesion burden, location, and degree of tissue injury. However, symptoms are often not reflective of MRI evidence of active plaques, commonly termed the clinical-radiological paradox.[8]

Therefore, research analyzing behavioral metrics and neurophysiological biomarkers are pivotal for the development of individualized treatment and rehabilitation efforts.

The neurotypical organization of the brain saturates each primary motor cortex with projections of descending corticospinal tracts to the corresponding contralateral muscles; the left hemisphere controls muscles on the right side of the body and vice versa. However, for bimanual movements requiring precise spatial and temporal coordination (e.g. tying your shoes, unscrewing a jar), interhemispheric communication is necessary and significantly impaired in aging populations and clinical populations like MS.[10-12] A large body of literature highlights the corpus callosum as the structure responsible for interhemispheric communication and this structure regulates the inhibition and excitation of specific muscle groups.[9-13] Reduced structural connectivity of the corpus callosum, even with the absence of lesions, is common in PwMS [12,21] and is strongly associated with impairments in spatiotemporal control of bimanual movements.[9,11,14] Although previous literature has primarily focused on bimanual tasks when analyzing transcallosal communication, recent work suggests the structural and functional integrity of the corpus callosum plays a critical role in the coordination of bilateral movements such as walking.[9,15]

In addition to impaired bilateral control of the upper limbs, PwMS also experience substantial deficits in spatiotemporal control of the lower limbs, manifested as increased asymmetries during gait. The majority of PwMS report significant differences in the strength and function between their legs. This asymmetry results in impaired walking ability being a common comorbidity in PwMS, with over 50% requiring mobility assistance within 18 years of diagnosis.[16] Furthermore, reduced coordination during gait (i.e. increased gait asymmetry) is

associated with increased metabolic cost, postural instability, falls and reduced quality of life observed in those living with Parkinson's disease or following a stroke.[17-19] However, the negative consequences of reduced coordination have only been recently evaluated in PwMS.[9] Additionally, due to the heterogeneity and unique pathophysiology of the disease, the neural mechanisms underlying gait impairments in PwMS are poorly understood.

Complex bilateral movements, such as gait, require constant communication across the corpus callosum to excite and inhibit specific neuronal pools in the primary motor cortices that activate muscle groups to successfully accomplish the desired task. In the current study we utilize transcranial magnetic stimulation (TMS) to assess the inhibitory capacity between the brain's hemispheres. TMS is a non-invasive stimulation method to evaluate biochemical properties of the motor cortex that reflect excitation via glutamatergic activity as well as inhibition via gamma-aminobutyric acid (GABA).[28,32-34] The cortical ipsilateral silent period (iSP) is a common method of assessing GABA circuitry and emphasizes an interruption of ongoing voluntary muscle contraction created by a focalized TMS pulse. This is used to assess upper and lower motor neurons within the corticospinal tract stemming from the motor cortex region of interest.[30] Thus, the current study utilizes the iSP as an indirect marker of the magnitude for interhemispheric inhibitory capacity.[22-25] Prior work demonstrates that PwMS exhibit reduced interhemispheric inhibition between the primary motor cortices compared to age-matched controls.[22] However, there is a lack of research analyzing directionality differences in interhemispheric inhibition between the more and less affected hemisphere in PwMS. This is a critical gap in the literature considering the substantial differences in motor function between the two sides of the body in PwMS, potentially reflected by altered communication between the two hemispheres of the brain.

The purpose of this study was to evaluate directionality differences of interhemispheric inhibition between the more and less affected hemispheres in PwMS and correlate them with spatial metrics of gait asymmetries observed through locomotor adaptation. The overarching hypothesis is that transcallosal function is an integral neural mechanism underlying control of the lower limbs and callosal degradation is a key contributor to mobility declines. Specifically, we hypothesize that interhemispheric inhibition will be greater from the less affected to the more affected hemisphere, as compared to interhemispheric inhibition from the more affected to the less affected hemisphere. Further, we hypothesize that greater gait asymmetries will be correlated to reduced transcallosal communication (i.e. diminished iSP) from the more affected to the less affected hemisphere in PwMS. Taken together, the iSP may serve as a neural biomarker for transcallosal impairments originating from the more affected hemisphere and highlight an underlying mechanism for gait asymmetries in PwMS. Understanding the relationship between behavioral outcomes and underlying neural mechanisms may provide insight for individualized rehabilitation protocols and stratifying patients into the most appropriate neurorehabilitation paradigms.

Aim 1: Determine if there are directionality differences in transcallosal inhibition between the more and less affected hemisphere in PwMS.

Aim 2: Evaluate if interhemispheric inhibition is a neural biomarker that predicts spatial adaptability during split-belt treadmill training in PwMS.

METHODS

Participants

Recruitment of participants stemmed from previous studies conducted in our lab, events held by the National Multiple Sclerosis Society, local neurology clinics (e.g. Advanced Neurology of Colorado), and social media platforms such as Reddit. The inclusion criteria were specified to relapsing remitting form of MS, between the ages of 18-86, fully ambulatory without assistance or stoppage needed to walk three-tenths of a mile. Twenty-nine PwMS participated in the study; 19 females; age range 31-67 years; mean age 50.5 ± 11.1 years. Individuals were required to have no musculoskeletal injuries within the past six months that may confound with walking ability as well as no history of brain injury, or any balance impairments unrelated to MS. We also attempted to mitigate fatigue as a confounding variable by providing rest periods and exertion check-ins throughout the protocol. Exclusion criteria for the TMS protocol was determined from previous studies in our lab and in accordance with single-pulse standard practice. These exclusion characteristics included: history of migraines, epilepsy, stroke, seizures, metal or implanted stimulators, certain medications, pregnant women, and any previous complications with TMS or MRI (see appendix for screening forms). The explicit exclusion criteria were necessary to avoid confounding variables for gait analysis as well as safety requirements for the TMS visit. All subjects were screened over the phone, once eligibility was determined participants were scheduled for two separate visits within 14 days of each other.

The study was approved by the IRB, and following the phone screening and informed consent, various surveys were distributed utilizing REDCap software (NIH/NCRR Colorado CTSI Grant Number UL1 RR025780). We collected information regarding demographics, self-reported disease aspects, current medications, physical activity anthropometrics, the Expanded

Disabilities Status Scale (EDSS), Multiple Sclerosis Walking Score 12 (MSWS-12), Modified Fatigue Impact Scale (MFIS), Short Form 36 (RAND 36), Beck Depression Inventory (BDI-II), and the Montreal Cognitive Assessment (MOCA).

Visit 1: Split-belt Treadmill Adaptation Paradigm

Participants were outfitted with 6 inertial sensors (ADPM, Inc.) and 16 reflective markers for three-dimensional motion capture collection at 100 Hz. The reflective markers were placed on various locations of the lower limbs stemming from the anterior and posterior superior iliac spine to the distal phalanx of both toes. The 6 inertial sensors were placed as follows: one sensor centered just below the collar bones on the flat part of the sternum, one sensor at the lumbar spine centered at the base of the spine, one sensor for each wrist similar to a watch, and one sensor per ankle centered on the anterior aspect of the ankle. The participants then completed five different walking trials (Figure 1).

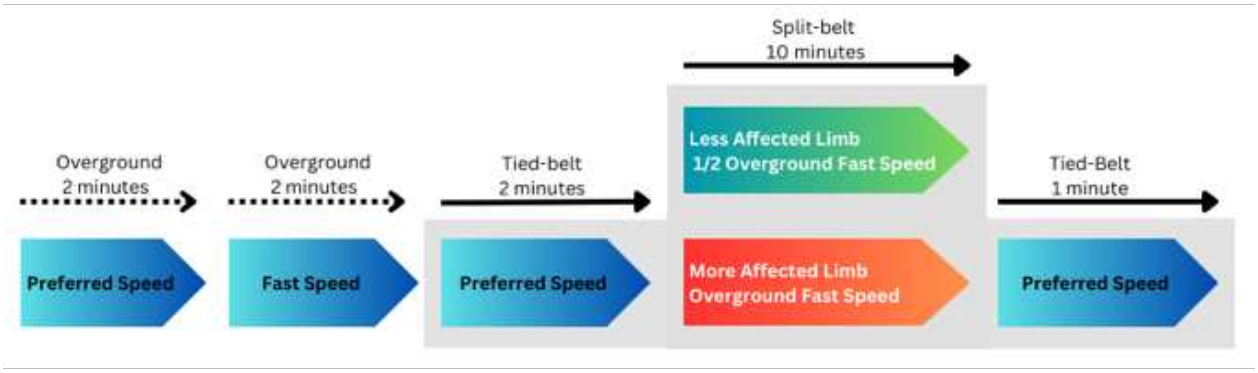

Figure 1: Treadmill training protocol. All participants completed two overground walks at a preferred and fast speed, then three treadmill trials followed. The shaded grey region indicates treadmill training with speed and timing given.

The first two walking trials were performed overground consisting of 2-minutes each and spanning a 30m walkway. The first trial was performed at the participants self-selected preferred walking speed and the second at the fastest speed each individual felt safe walking. Participants were instructed to walk back and forth down the hallway with normal upper limb movement and

gaze fixated straight ahead. No communication occurred during the walking trials, and any deviations or disruptions were documented. Quantitative analysis was performed from the data gathered by the inertial sensors to identify the leg with the shortest stride length (i.e. more affected limb) during over ground walking. Based on previous work, the limb with the shortest stride length should be trained on the faster belt to improve gait symmetry.[26] Therefore, the leg with the shorter stride length was assigned to the fast belt. The preferred and fast overground trials were necessary to determine the "tied" (both belts moving at the same speed) and "split" (belts moving at different speeds) configuration for the following treadmill trials.

The instrumented treadmill is custom-built with Bertec force platforms (Model 4060-10, Bertec Corp, Columbus, OH) beneath each belt. The treadmill consists of 2 separate belts, each with its own motor that permits the independent control of the speed of each belt (i.e., each leg). Following the overground walking trials, participants completed a 2-minute tied-belt trial set to their preferred overground speed. Afterwards, the 10-minute split-belt adaptation period began. The speed of the belts was unique for each participant and determined by the individual's overground fast walking speed. The "slow" belt speed was calculated by dividing their overground fast walking speed in half, and the "fast" belt speed equal to the overground fast walking speed. This split-belt treadmill training paradigm has been widely utilized in the post stroke population.[26.27] Finally, all participants were instructed to fixate their gaze straight ahead, engage in normal upper limb swinging, and refrain from looking down at the belts while walking to reduce the visual feedback regarding belt speeds whilst still keeping safety as a top priority. While walking on the treadmill, the subjects wore a ceiling-mounted safety harness around the upper chest and pelvis. The harness was applied to ensure that it did not support body weight nor interfere with participants' walking.

Data Collection

Gait analysis for spatio-temporal metrics was collected for both treadmill and overground walking. Three-dimensional trajectory and force data were processed using Vicon Nexus software (v2.14, Vicon Motion Systems, Oxford, UK). Trajectory positions were filtered using a Woltring filter. In combination with Vicon analyses, custom software written in MATLAB (MathWorks Inc, Natick, MA) was used for all subsequent analyses.

For this study, limb excursion asymmetry (LEA) is the primary spatial outcome metric and is a modified measurement of stride length. LEA is beneficial for treadmill analysis because participants are not translating while walking, instead, they are staying in place. If we were to utilize the conventional understanding of stride length (distance from heel strike to following ipsilateral heel strike) it would result in a net zero. Therefore, limb excursion quantifies the anterior-posterior distance covered by the limb from toe-off to ipsilateral heel strike (Figure 2).[29] LEA is calculated by subtracting limb excursion of the less affected limb from the more affected limb for each gait cycle.

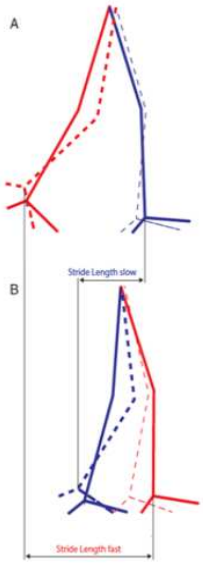

Figure 2: Limb Excursion Asymmetry (LEA) is a measurement of spatial coordination for stride length on a treadmill. A lower LEA indicates better spatial gait symmetry. Figure adopted from Hoogkamer (2014).

Visit 2: Transcranial Magnetic Stimulation

Each participant was comfortably seated in a semi-reclined modified dental chair with a pillow supporting their neck and hands on an adapted table with a connected force transducer (see Figure 3). We targeted the first dorsal interosseous (FDI) muscle due to its reliability in cortical location and reduction of contralateral involvement due to somatotopic organization of the motor cortex. TMS stimulations were collected using the MagPro x100 stimulator (MagVenture, Farum, Denmark) utilizing the C-B60 Butterfly Coil. The mapping of the 'hot spot' for the FDI was consistent across all participants meaning no unusual or inability to locate the muscle of interest. Muscle activity was recorded via bipolar electromyography (EMG) electrodes (Ag-AgCl, 8-mm diameter, 20-mm distance between electrodes, MVAP Medical Supplies Inc.) sampled at 1500 Hz and transmitted to a connected laboratory computer. The center of the head was measured by the halfway point from nasion to inion then from each tragus of the ear. Starting stimulation location for FDI was measured 5cm lateral and 1cm anterior to center of head marking.[31] The technique for stimulation was adopted from Wassermann et al. (1992).[30] The coil handle was sagittaly oriented with the handle pointing posteriorly and the figure-eight coil rested peripherally on the skull. This coil position provides the most precise form of stimulation and limits the amount of current spread to unintended M1 areas. Stimulation was delivered over each hemisphere separately, contralateral to the FDI of interest. The resting motor threshold (RMT) was analyzed in both hemispheres and defined as the lowest stimulus intensity that evoked a motor evoked potential (MEP) response of at least 50 μV on five out of ten trials.

Participants were then asked to complete maximal voluntary contractions (MVC) trials of each FDI against the force transducer. The individual's hands were resting on the adaptive table

with their forearms supported by the dental chair. A strap connected to the transducer was placed around the index finger resting between the middle and proximal phalanx. The table and placement of the secured force transducer were adjusted for each participant. Each participant completed 3 MVC trials for each index finger.

To elicit the iSP, participants were asked to maintain an isometric contraction at 50% of their MVC. Participants were given visual feedback on a screen directly in front of them, which gave a real-time force output number in red. The participants were asked to keep the red number at their documented 50% MVC and to maintain the force as steady as possible during the trial as well as avoid utilizing other muscles besides the FDI to compensate. Utilizing the previous scalp markings to ensure hot spot location, the TMS coil was placed on the ipsilateral hemisphere of the active FDI. Each trial was 1 min long and a focal TMS pulse was delivered at 120% of the RMT every 7–10s with a total number of stimulations averaging 10 per hemisphere for each trial. To ensure that we maintained the position of the coil on the hot spot, contralateral MEPs were recorded, although they were not the main outcome metric for this study. The metric of interest for this study is the result of the transcallosal signals through the posterior corpus callosum causing brief suppression of muscle activity (i.e. iSP capacity). The iSP was analyzed for both hemispheres and the order of FDI testing (i.e. right vs left) was randomized across participants. Participant's dominant hand and more affected side were documented to assess directionality. Figure 3A demonstrates the various physiological elements of the iSP protocol.[25] Each participant completed 3 trials for each hemisphere (~ 30 total iSP per side).[20,23] A minimum of 2 minutes of rest was given between each trial to avoid fatigue.

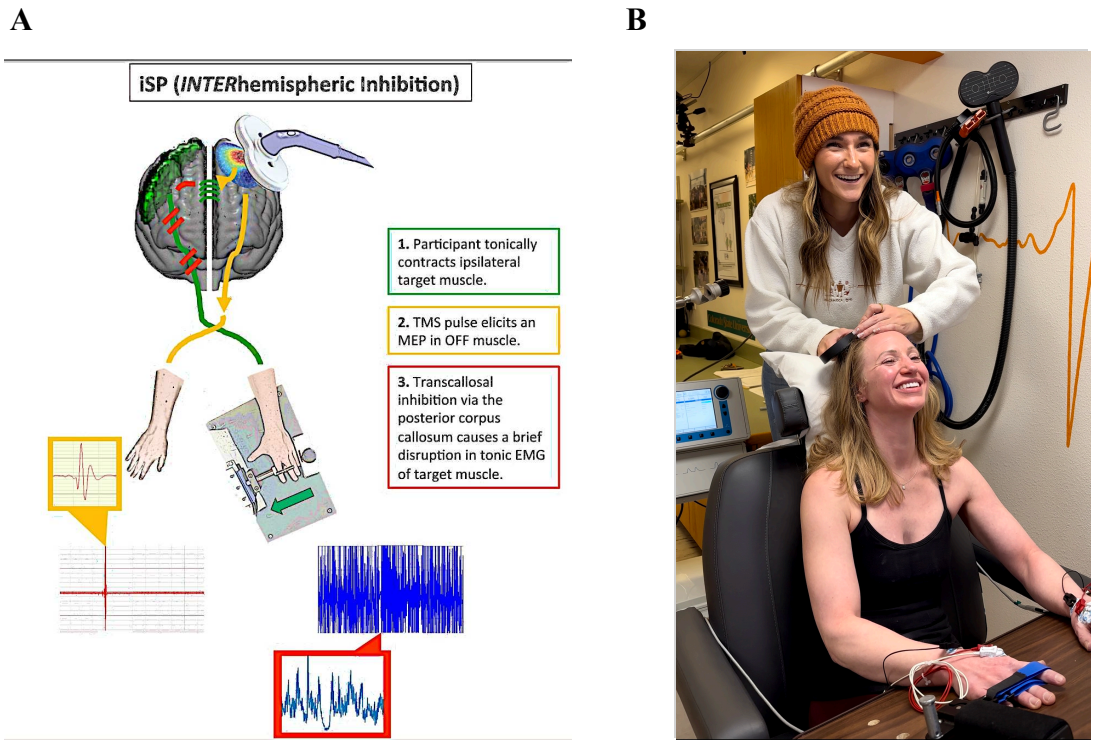

Figure 3: **A)** Adopted from Hupfeld et. al. (2020).[25] Participants were asked to push isometrically against a force transducer at 50% MVC. TMS pulse delivered to ipsilateral FDI hotspot producing contralateral MEP and potential iSP outcomes in voluntary contracting FDI. EMG suppression data used to analyze inhibitory capacity for each participant's more and less affected hemisphere. **B)** Actual image from consenting participant.

Data Collection

The primary TMS outcome metric for this study was depth of silent period percent average (dSP% avg). Previous literature found iSP depth to be more accurate and sensitive for delineating between various populations such as younger and older adults.[20] Therefore, dSP% avg was chosen for this study to highlight differences between the hemispheres in PwMS. EMG signals collected from the ipsilateral FDI muscles during each trial were filtered and rectified offline using AcqKnowledge software (Biopac Inc., Santa Barbara, CA) with a bandpass filter (10-1000Hz).[25,28] The filtered and rectified data was then imported into a custom MATLAB (MathWorks, Nantick, MA) script to identify and calculate individual iSPs. This script was adapted from previous work in our laboratory. [28] It provides several inhibitory metrics including

dSP% avg, iSP duration, max depth of the iSP%, transcallosal conduction time, and iSP onset latency to assess inhibitory capacity and comparison between the more and less affected hemisphere of each PwMS. The code for iSP analysis extracts the EMG signal of each FDI from 100ms prior to each stimulation to 350ms post-stimulation. Following stimulation, iSP onset was identified as the point when EMG activity dropped below 1.5 standard deviations of the pre-stimulus mean and ending of the iSP was defined as the time point when five consecutive data points were >1.5 standard deviations below the pre-stimulus EMG mean (Figure 4).[20, 28] Average iSP depth percent was calculated by taking the mean EMG signal for the entire iSP duration and normalizing this depth to the average pre-stimulus EMG level.[25,35,36]

Depth % Average = 100 – [(Mean EMGiSP / Mean EMGpre) x 100%]

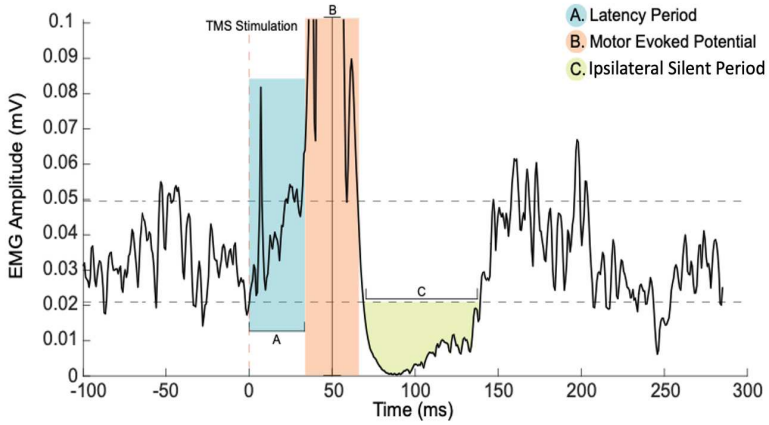

Figure 4: iSP magnitude quantified by the time and depth when the EMG trace dips below -1.5 standard deviations of the pre-stimulus EMG and ends when the EMG trace exceeds the -1.5 standards deviations for 5 consecutive data points. Figure adopted from Swanson & Fling (2018).[28]

Statistical Analysis

Multiple paired t-tests were performed comparing the more and less affected hemisphere within participants using R Statistical Software (v4.2.1; R Core Team, 2022) for the following iSP metrics: dSP% avg, duration, dSP% max, transcallosal conduction time, and onset latency. The reported p-values are from the pairwise comparisons. LEA was computed for the baseline tied-belt trial, which occurred prior to the 10-minute split-belt treadmill training. LEA was also

calculated for the catch trial tied-belt segment occurring post split-belt paradigm, and a difference value for the LEA was calculated to determine the extent of gait symmetry adaptation following split-belt treadmill training. Data for both of these components were continuous and normally distributed, thus we conducted a Pearson's correlation to determine the association between split-belt treadmill training and interhemispheric inhibition, assessed by dSP% avg. All data are presented as mean ± standard deviation (SD) unless otherwise noted and a p-value $\leq$ 0.05 was considered as statistically significant.

RESULTS

Participants

A total of 29 PwMS completed this study with a mean age of 50.55 ± 11.13 years and an average years since diagnosis of 15.07 ±9.37 (Table 1). This group of PwMS was very active compared to normative PwMS, exercising 4.21 ± 2.11 days per week, which is in alignment with this geographical location.[38] Additionally, 62% of PwMS reported their right side as more affected and every participant scored relatively low on MS disability scales with a mean EDSS of 3.44 ±1.14 and mean MSWS-12 of 21.97 ±12.03.

Table 1: Participant demographics, anthropometrics, physical activity information, and disability surveys. Values are means ± SD unless otherwise noted.

N	**29**
Sex (n, % female)	19 (65.51%)
Age (years)	50.55 ±11.13
Height (cm)	172.28 ±8.64
Mass (kg)	73.68 ±13.56
Affected side (n, % Right)	18 (62.06)
Activity frequency (days per week)	4.21 ±2.11
Years since diagnosis	15.07 ±9.37
Falls occurred the past 6 months	0.53 ±1.0
Expanded Disability Status Score (EDSS)	3.44 ±1.14
Modified Fatigue Impact Scale (MFIS)	30.93 ±14.84
Multiple Sclerosis Walking Score (MSWS-12)	21.97 ±12.03
Montreal Cognitive Assessment (MOCA)	27.52 ±2.28

Transcallosal Communication

The comparison of the more and less affected hemisphere for all inhibitory outcome metrics (dSP% avg, iSP duration, dSP% max, transcallosal conduction time, and iSP onset latency) indicated no statistically significant differences. Group averages are presented for each outcome and differences were assessed as less affected (LA) minus more affected (MA) hemisphere (Table 2).

Table 2: Group iSP outcome metrics. Positive difference values indicate a stronger iSP for the less affected hemisphere. No statistically significant differences found between more and less affected hemisphere for all inhibitory outcome metrics.

Outcome metric	LA Hemisphere	MA Hemisphere	Difference (LA-MA)	*p*-value
dSP % avg	59.18 ±9.74	57.42 ±12.67	1.76	0.58
iSP duration (ms)	39.07 ±19.72	40.21 ±17.69	-1.14	0.98
dSP % max	84.90 ±10.47	81.94 ±12.21	2.96	0.27
Transcallosal conduction time (ms)	16.11 ±3.92	16.34 ±4.50	-0.23	0.85
iSP latency (ms)	39.77 ±3.95	40.26 ±4.78	-0.49	0.87

A representative participant demonstrates the average iSP trial data comparing more and less affected hemisphere (Figure 5). The primary transcallosal communication metric (dSP% avg) is indicated by the average iSP depth calculated by taking the mean EMG signal for the entire iSP duration and normalizing this depth to the average pre-stimulus EMG level. The shaded regions highlight the area of interest for muscle suppression (i.e., inhibitory capacity). No significant differences were found between the individual's more and less affected hemisphere.

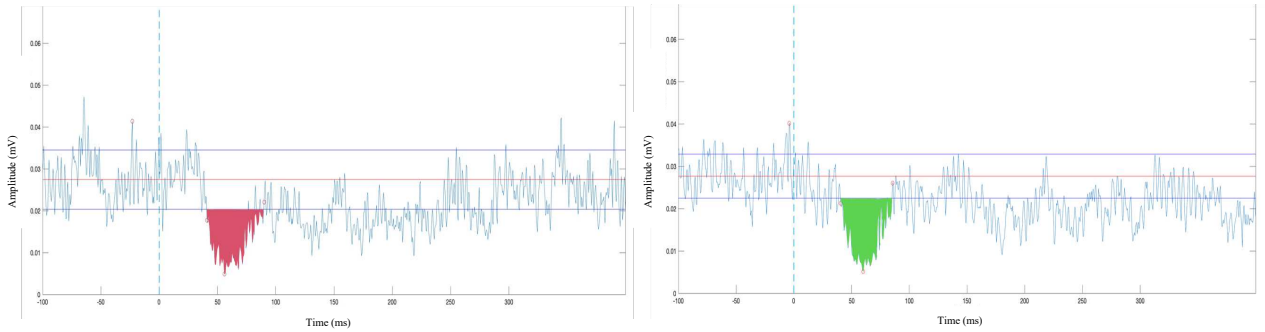

Figure 5: Representative participant's average iSP trial data showing the more affected hemisphere (left, red) and the less affected hemisphere (right, green). Shaded regions indicate dSP% avg. Time (ms) is displayed on the x-axis, whereas EMG amplitude(mV) is on the y-axis. No significant differences found.

Furthermore, group dSP% avg data is displayed for each individual's more and less affected hemisphere (Figure 6). Lower values of dSP% avg reflect less inhibitory capacity. Each data point represents an individual's average trial dSP% avg for the corresponding hemisphere. However, no differences were observed when analyzing average group dSP% avg and affected hemisphere. As stated previously, dSP% avg was chosen due to the metric's enhanced accuracy and sensitivity in aging and clinical populations.[20]

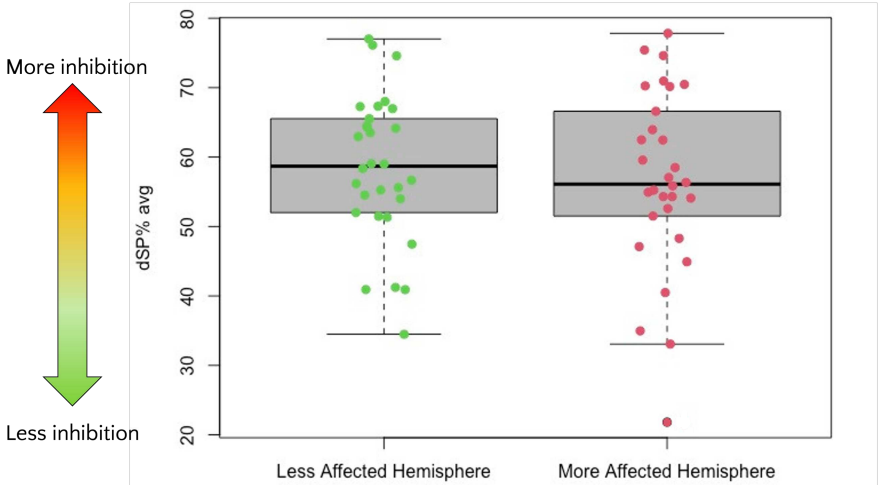

Figure 6: Group dSP% avg for each participant's more and less affected hemisphere. No significant differences observed.

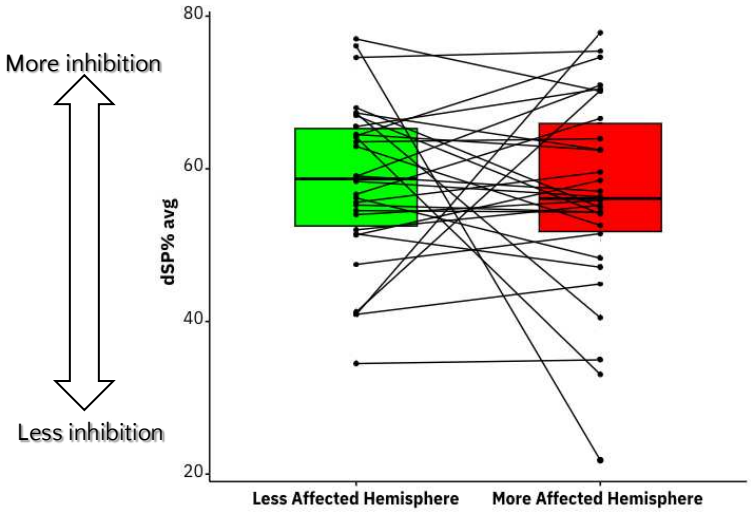

Figure 7: Paired data points representing the individual's dSP% avg for their more and less affected hemisphere. No significant differences observed.

Spatial Symmetry Gait Adaptation and Transcallosal Inhibition

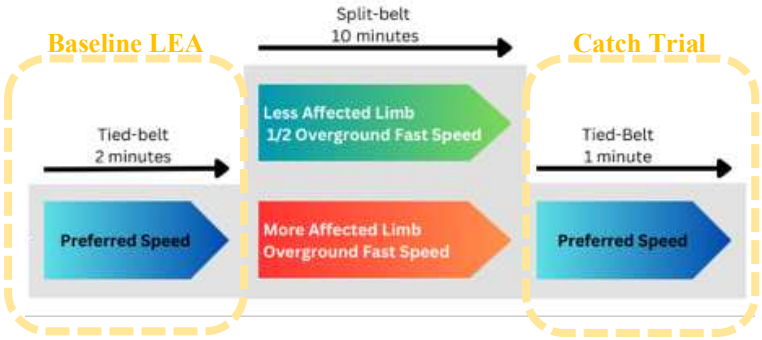

Figure 8: Split-belt treadmill paradigm with baseline and catch trial identified. LEA calculated by subtracting limb excursion of the less affected limb from the more affected limb for each gait cycle. Baseline LEA representing pre-split-belt treadmill training and catch trial LEA post protocol.

For the second aim of the project, a significant correlation was identified between the participant's transcallosal inhibition and spatial gait adaptation (Figure 9). The individual's dSP% avg value which was calculated from the less minus more affected hemisphere was correlated to their LEA change. The LEA change value is determined by the participant's catch trial LEA minus baseline LEA from the split-belt treadmill protocol to reflect spatial gait

adaptation ability. Negative LEA change values indicate better adaptation reflecting more spatially symmetric gait following the split-belt treadmill training protocol (Table 3).

Table 3: Group LEA outcome metrics. Negative values indicate an asymmetry. Change LEA calculated by Catch Trial LEA minus Baseline LEA to represent spatial adaptation following split-belt treadmill protocol.

Measurement	Group Average (mm)
Baseline LEA	5.66 ±38.71
Catch Trial LEA	14.58 ±50.12
Change LEA	**-8.93 ±20.52**

The correlation depicted a significant relationship between less interhemispheric inhibition from the less affected hemisphere in PwMS and better spatial adaptation of gait following the split-belt treadmill paradigm. In other words, those demonstrating less interhemispheric inhibition became more spatially symmetric with their walking following the split-belt adaptation protocol.

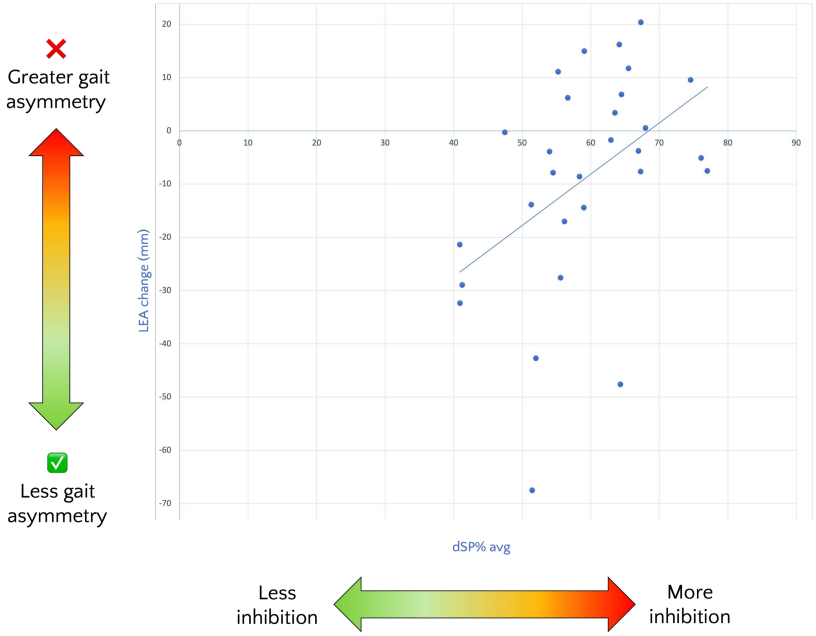

Figure 8: Significant correlation of less inhibition and greater gait symmetry (r = 0.46, p-value = 0.02). The individual's dSP% average on the x-axis maps inhibitory capacity while the LEA change value on the y-axis plots the spatial adaptation ability resulting from the split-belt treadmill protocol. Greater gait asymmetry was correlated with more transcallosal inhibition.

DISCUSSION

The aim of this study was to evaluate directionality differences of interhemispheric inhibition between the more and less affected hemispheres in PwMS and correlate them with spatial metrics of gait asymmetries observed through locomotor adaptation. Specifically, we hypothesized that interhemispheric inhibition would be greater from the less affected to the more affected hemisphere, as compared to interhemispheric inhibition from the more affected to the less affected hemisphere. This hypothesis was refuted as no statistically significant differences were observed between the more and less affected hemisphere in PwMS. Further, we hypothesized that greater gait asymmetries would be correlated to reduced transcallosal communication (i.e. diminished iSP) in PwMS. Although this original hypothesis was refuted, we observed a significant correlation between less interhemispheric inhibition and greater spatial gait adaptation following the split-belt treadmill protocol. Taken together, the iSP may serve as a neural biomarker for transcallosal impairments and highlight an underlying mechanism for gait asymmetries in PwMS.

Interhemispheric Inhibition in PwMS

A large body of literature highlights the corpus callosum as the structure responsible for interhemispheric communication and the regulation of inhibition and excitation of specific muscle groups.[9-13] Furthermore, interhemispheric connections between the motor cortices through the corpus callosum can be damaged by inflammatory or residual gliotic plaques in PwMS.[21] Reduced structural connectivity of the corpus callosum, even with the absence of lesions, is common.[12,21] However, analyzing directionality differences of interhemispheric inhibition originating from the more and less affected hemisphere in PwMS is largely

understudied. This is a critical gap in the literature considering the substantial differences in motor function between the two sides of the body in PwMS, potentially reflected by altered communication between the two hemispheres of the brain.

In the current study, we evaluated dSP% avg, dSP duration, max dSP%, transcallosal conduction time, and onset latency for iSPs from the more and less affected hemisphere in PwMS. Previous studies have reported increased values for each metric being representative of greater interhemispheric inhibition.[9,15,21,28] The primary iSP metric of interest was the dSP% avg due to the enhanced accuracy and sensitivity for various populations.[20] Additionally, although previous literature focuses primarily on iSP duration, recent studies have highlighted the inaccuracy of this evaluation due to an irregularly occurring second phase of inhibition in the FDI.[35,39] This may be due to the initiation of additional ipsilateral corticospinal pathway, but needs further evaluation.[40] Therefore, this study utilized the dSP% avg and measured the difference of each metric calculated by the less minus more affected hemisphere to assess directionality differences. Where positive values indicated a stronger iSP for the less affected hemisphere correlated to greater inhibition. However, no statistically significant group differences were identified for the various iSP outcome metrics (e.g. dSP% avg difference: 1.7625; *p*-value 0.5752).

Only one previous study has investigated interhemispheric inhibition directionality differences in PwMS, however the researchers merely evaluated onset latency, duration, and transcallosal conduction time.[41] In alignment with the results of this study, Jung et.al. reported no statistically significant iSP side differences in PwMS (onset latency: 3.6 ± 3.9; duration: 5.8 ± 6.4; transcallosal conduction time: 3.4 ± 3.4).[41] Results from the current study expanded

upon these findings with additional directionality metrics. No interhemispheric inhibition directionality differences between the more and less affected hemisphere was observed in PwMS. These findings highlight the potential of PwMS being able to preserve their inhibitory capacity regardless of their more affected hemisphere. Although it has been identified that PwMS have overall reduced interhemispheric inhibition when compared to healthy controls [21,22,41], our findings provide novel insight into the similarities of transcallosal communication capacity originating from each hemisphere. While the results of the study contradicted the original hypothesis (i.e., PwMS demonstrating reduced interhemispheric inhibition from their more affected hemisphere), the preservation of inhibitory capacity irrespective of affected hemisphere is beneficial for corpus callosum connectivity and potential conservation of disease progression. With the majority of PwMS documenting a more and less affected limb, particularly with regards to gait, our findings prompt the need for additional research to investigate alternative neural mechanisms responsible for the asymmetry.

A potential explanation for the lack of directionality differences in interhemispheric inhibition may be due to the white matter fibers being impaired regardless of the hemisphere of signal initiation. We recognize corpus callosum connectivity and structural integrity is impaired in PwMS.[12,13,21,37] Therefore, the hemisphere where signal initiation is produced may be irrelevant due to the tract impairment through the corpus callosum. In other words, imagine a car crossing a one-way bridge; the side on which the car starts is insignificant if the bridge is damaged in the middle because it will be slowed to the destination regardless. Therefore, the diminished structure of transcallosal fibers in PwMS may dictate no directionality differences being observed for interhemispheric inhibition irrespective of hemisphere.

Spatial Gait Adaptation

Complex bilateral movements, such as gait, require constant communication across the corpus callosum to excite and inhibit specific neuronal pools in the primary motor cortices that activate muscle groups to successfully accomplish the desired task. Therefore, disturbances in transcallosal communication may lead to gait impairments. In individuals diagnosed with stroke, previous studies identified alterations in interhemispheric inhibition correlated to increased motor overflow in both legs following stroke.[45] Their results suggest a bilateral mechanism contributing to motor overflow correlating to diminished coordination capability in stroke individuals. In the MS population, a recent study investigated spatiotemporal aspects of gait in PwMS and documented impaired bilateral coordination at self-selected walking speeds.[46] These findings formed the basis of investigation for our study correlating neurophysiology underpinnings with gait metrics in PwMS.

Previous studies within the stroke population have utilized split-belt treadmill training paradigms to address observed spatiotemporal gait asymmetry.[26,27] However, our novel study not only utilized the split-belt paradigm on PwMS, but also correlated spatial gait metrics with interhemispheric inhibition to evaluate potential underlying neural mechanism to describe gait irregularities. Our results identify a significant correlation ($r = 0.46$, p-value = 0.02) between reduced interhemispheric inhibition and better spatial adaptation following the split-belt treadmill paradigm in PwMS. Although this result challenges our original hypothesis (i.e. greater interhemispheric inhibition would correlate to better spatial adaptation), it is in alignment with previous stroke literature investigating transcallosal communication and motor performance.[47,48]

In a chronic stroke study, Murase et. al. identified deeper interhemispheric inhibitory metrics from intact to lesioned hemisphere resulted in slower performance in upper-limb motor

tasks.[47] Their results suggest a link between higher interhemispheric inhibition and poorer motor recovery in post stroke individuals. Expanding upon these findings, Madhavan et.al. demonstrated similar findings for lower limb coordination. Their study analyzed ipsilateral conductivity and motor conflict as a barrier to ankle tracking ability in post stroke individuals. Researchers found strong ipsilateral influence (i.e., interhemispheric inhibition) correlated to impaired limb control.[48] Together these two studies support our findings within the MS population where increased interhemispheric inhibition correlated to poorer behavioral outcomes, specifically gait asymmetry. Our novel results highlight the associations of reduced inhibition and better spatial adaptation following the split-belt treadmill training paradigm. Furthermore, a recent study from Boddington et. al., presented targeting interhemispheric inhibition for neuromodulatory therapy options. Researchers discovered that increased inhibition in the ipsilesional hemisphere may limit functional recovery and contribute to residual motor disability after stroke.[49]

Overall, the phenomenon of increased interhemispheric inhibition associated with poorer motor outcomes is repeatedly documented for the stroke population. Our study aligns with the stroke literature, but provides novel findings within PwMS. Therefore, although MS and stroke are different neurophysiologically, it is insightful to share similar inhibitory and behavioral correlated findings. The statistically significant finding from our study prompts potential for different neuromodulatory rehabilitation paradigms and emphasizes the benefit of lower levels of interhemispheric inhibition with better spatial gait adaptation in PwMS. More research is necessary to evaluate the neural mechanisms for the association and how to effectively target interhemispheric inhibition for rehabilitation.

Potential mechanisms to describe the association of reduced interhemispheric inhibition and better behavioral performance observed in the stroke population and our study with PwMS may be explained through neuromodulatory and compensatory mechanisms. The level of interhemispheric inhibition may be reliant upon the motor task and could be described as dynamic. Previous research has identified more cortical influence being involved with upper-limb motor coordination specifically through the corticospinal tract and additional cortico-motoneuronal connections.[50] Whereas motor control for lower limb coordination during locomotion is predominantly mediated by interneurons, and cervical and thoracolumbar propriospinal systems become coupled and coordinate arm and leg movements.[51] Therefore, our observed correlation between reduced interhemispheric inhibition and greater spatial gait adaptation may be reliant upon lower limb coordination recruiting various neural circuitries beyond solely cortical influenced as seen in upper limb tasks for PwMS. Stemming from this idea, an interesting proposal from stroke researchers Boddington et. al., suggest interhemispheric inhibition may be dynamically influenced by movement initiation.[49] An additional mechanism to describe our observed association may be due to compensatory sensorimotor areas that cope with the asymmetry and reduced corpus callosum connectivity to preserve lower limb coordination. A recent study from Brancaccio et. al. suggests that post stroke patients manage to cope with lesions by relying on the contralesional sensorimotor areas that compensate for the ipsilesional damaged ones.[52] Therefore, there may be potential for PwMS to utilize various compensatory mechanisms to explain the paradox of reduced interhemispheric inhibition correlating to better spatial gait adaptability observed in our study.

Limitations

PwMS are known to have cognitive deficits, and split-belt treadmill adaptation has been shown to increase cognitive load.[42] The specific influence of this novel task increasing cognitive load for the participants are unknown. Additionally, another predictor of spatial adaptation of gait while performing on the split-belt treadmill may have been visuospatial cognition, as shown in recent evidence.[43] The cognitive test (MOCA) is unlikely to be sensitive enough to detect this, and an assessment such as the Brief International Cognitive Assessment for MS [53] which is a more time-consuming test may add an important distinction between participants for their more affected limb documentation. Another limitation was that this sample of PwMS was relatively healthy and active, with majority of our participants having a low disability level, scoring with mild impairments. If this sample was more representative of the MS population, there may have been interhemispheric inhibition differences as well as more asymmetry of gait observed at baseline.

Future Directions

Due to no statistically significant differences being identified between the more and less affected hemisphere in PwMS, further investigation of neural mechanisms underlying gait asymmetry in PwMS is warranted. Additionally, the specific underpinning of the spatial locomotor adaptation correlating with reduced interhemispheric inhibition may provide insight into rehabilitation trainings paired with neurostimulation. Future directions may include combining split-belt treadmill training with functional near-infrared spectroscopy (fNIRS) to measure cortical activation during sensorimotor adaptation compared to neurotypical controls.[44] Split-belt treadmill adaptation coupled with fNIRS may allow for assessment of the influence of amplified sensory signaling on cortical activation to reduce asymmetry in PwMS. Understanding

the relationship between behavioral outcomes and underlying neural mechanisms may provide insight for individualized rehabilitation protocols and stratifying patients into the most appropriate neurorehabilitation paradigms.

CONCLUSION

Understanding the underlying neural mechanisms for observed gait asymmetries in PwMS is pivotal for neurorehabilitation opportunities as well as preservation of the individual's quality of life. The purpose of this study was to evaluate directionality differences in interhemispheric inhibition between the more and less affected hemisphere in PwMS as a potential biomarker for gait asymmetries. Additionally, we aimed to understand the relationship between interhemispheric inhibition and gait adaptability utilizing a split-belt treadmill paradigm. Our results identified no statistically significant differences in directionality between the more or less affected hemisphere for any interhemispheric inhibitory metrics. This finding may suggest PwMS preserve inhibitory capacity irrespective of the more affected hemisphere. We interpret this to suggest transcallosal communication is indicative of white matter fiber integrity rather than cortical hemisphere of signal initiation. Additionally, we identified a statistically significant correlation between reduced interhemispheric inhibition and better spatial gait adaptation with the split-belt treadmill protocol. Those who had a lesser dSP% avg inhibitory outcome metric responded better spatially after the split-belt treadmill resulting in a more symmetric gait. These findings are in alignment with several post stroke studies investigating reduced interhemispheric inhibition and greater motor performance.[47-49,52] Together these results emphasize the potential for more targeted rehabilitation protocols utilizing interhemispheric inhibition as a biomarker for spatial adaptation during gait.

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APPENDIX

Screening Form

Screening Details

Record ID	_____
Screener	_____
Create Subject	
Date Completed	_____
First name	_____
Last name	_____
Date of Birth	_____
Age at Screening	_____
Subject Type:	◯ Healthy Control ◯ Multiple Sclerosis
Gender:	◯ Female ◯ Male ◯ Unknown or Not Reported
Contact Information	
Primary phone number	_____
Email:	_____
Street Address	_____
City	_____
State	_____
Zipcode	_____

Mobility	
Do you have any difficulty with balance or falls?	◯ Yes ◯ No
If yes, details of balance difficulties.	_____
Number of falls in the last month	_____
Number of falls in the last 6 months	_____
Fall Details	_____
Are you able to stand for 10 min?	◯ Yes ◯ No
Specify details if unable to stand 10 min.	_____
Do you have any difficulty with walking?	◯ Yes ◯ No
Are you able to walk more than 3 tenths of a mile without stopping to rest. (This is a little farther than 5 football field lengths.)	◯ Yes ◯ No ((EXCLUSION IF NO))
Exclusion Comments:	_____
If yes, specify difficulty with walking.	_____
Difficulty walking or standing barefoot?	◯ Yes ◯ No
Do you use an assistive device?	◯ Yes ◯ No
If yes, what kind?	◯ Walker ◯ Cane
If yes, how often?	◯ Always ◯ Occasionally
Additional assistive device details	_____

Do you exercise?	◯ Yes ◯ No
If yes, how many hours per week?	_____
If yes, what type of exercise?	_____

Neurological

Head Injury	◯ Yes ◯ No
If yes, specify head injury details.	_____
History of Increased intracranial pressure	◯ Yes ◯ No ((EXCLUSION IF YES))
Exclusion Comments:	_____
Implanted Brain Stimulator?	◯ Yes ◯ No ((EXCLUSION IF YES))
Exclusion Comments:	_____
History of Stroke	◯ Yes ◯ No
If yes, specify stroke details.	_____
Do you have any aneurysm clips?	◯ Yes ◯ No ((EXCLUSION IF YES))
Exclusion Comments:	_____
History of seizure or convulsion	◯ Yes ◯ No ((EXCLUSION IF YES))

Exclusion Comments:	_____
History of Neuropathy/Numbness	◯ Yes ◯ No
If yes, specify neuropathy details.	_____
History of Migraines/Frequent headaches	◯ Yes ◯ No ((EXCLUSION IF YES TO MIGRAINE))
Exclusion Comments:	_____
Do you have any metal in your head aside from normal dental work?	◯ Yes ◯ No ((EXCLUSION IF YES))
Exclusion Comments:	_____
History of Multiple Sclerosis: Year diagnosed	_____
Type of Multiple Sclerosis	◯ Relapsing - Remitting MS ◯ Primary - Progressive MS ◯ Secondary - Progressive MS ◯ Progressive Relapsing MS (Exclusion if anything other than Relapsing)
Last Relapse Date:	_____ (A year is fine. If they don't know the exact date just input 1/1/YEAR.)

Orthopedic

Do you have any pain?	◯ Yes ◯ No
Back Pain	◯ Yes ◯ No
Neck Pain	◯ Yes ◯ No
Leg Pain	◯ Yes ◯ No
Arm Pain	◯ Yes ◯ No

Other Pain	◯ Yes ◯ No
If yes to pain, specify details.	_____
Do you have any arthritis	◯ Yes ◯ No
If yes, specify arthritis details.	_____
Have you had a Joint Replacement / Amputation	◯ Yes ◯ No
If yes, specify joint replacement details.	_____
Do you have any current fractures or a history of fractures?	◯ Yes ◯ No
If so how many?	_____
EXCLUSION IF YES: Open Head Fracture Lower limb musculoskeletal injury within 6 months unless symptoms have resolved (i.e. sprained ankle, broken leg, etc)	◯ Yes ◯ No
Fracture / Exclusion Comments:	_____
If yes, what was fractured?	_____
If yes, the year	_____ (Month and day don't matter, just the year so input 1/1/(year))
Additional fracture details	_____

Sensory Deficits	
Vision problems	◯ Yes ◯ No
History of Ocular Foreign body?	◯ Yes ◯ No ((EXCLUSION IF YES))
Exclusion Comments:	_____
Corrected with Glasses/Contacts	◯ Yes ◯ No
Specify details of vision problem.	_____
Do you have any dizziness	◯ Yes ◯ No
If yes, specify dizziness details.	_____
Any Vestibular (Inner Ear) problems?	◯ Yes ◯ No
If yes, specify details of vestibular issue.	_____
Cochlear Implants?	◯ Yes ◯ No ((EXCLUSION IF YES))
Exclusion Comments:	_____
Hearing problems	◯ Yes ◯ No
If yes, specify hearing problem details.	_____
Other sensory deficit problems	◯ Yes ◯ No
If yes, specify detail other sensory deficits.	_____

Health History	
History of Diabetes	◯ Yes ◯ No
If yes, specify diabetes details.	_____
Any recent surgeries?	◯ Yes ◯ No
Any surgeries that affect your balance?	◯ Yes ◯ No
Surgery details and dates	_____
Fainting or lightheaded when standing	◯ Yes ◯ No
If yes, specify details of fainting.	_____
History of Heart problems?	◯ Yes ◯ No
If yes, specify details of heart trouble.	_____
Circulation problems?	◯ Yes ◯ No
If yes, specify circulation issues.	_____
Current of past chemotherapy treatment?	◯ Yes ◯ No
If yes, specify chemotherapy details.	_____

Other	
Dominant Side	◯ Right ◯ Left
Highest Level of Education	◯ AA Degree ◯ BS/BA ◯ Doctorate ◯ High School graduate / GED ◯ Not graduated high school ◯ Masters ◯ Some College
Number of years post high school	_____
(If female) currently pregnant	◯ Yes ◯ No
Exclusion Comments:	_____
Medications Per Day	_____

Other Studies	
Are you in any other Research Studies?	◯ Yes ◯ No
Is this a long-term study (>1 visit)?	◯ Yes ◯ No
If yes, who is the study coordinator?	_____
Name of study or what it involves	_____

General Notes	
Notes	_____
FOR RA ONLY - General Impression	◯ Normal ◯ Questionable ◯ Impaired
Screen Fail?	◯ Yes ◯ No

TMS Screening Form

TMS Screening

Record ID _____

TMS Screening Form Upload

Screen date _____

TMS Participant Screening Form

	Yes	No
Have you ever had an adverse reaction to Transcranial Magnetic Stimulation?	○	○
Have you ever had a stroke?	○	○
Aneurysm clips or coils?	○	○
Cardiac pacemeaker or wires?	○	○
Internal cardioverter defibrillator (ICD)?	○	○
Carotid or cerebral stents?	○	○
Deep brain stimulator?	○	○
Metallic devices implated in your head?	○	○
Dental implants?	○	○
Cochlear implant/ear implant?	○	○
CSF (cerebrospinal fluid) shunt?	○	○
Cardiac stents, filters, or metallic valves?	○	○
Tattoo (facial i.e. permanent makeup)?	○	○
Medication patch/nicotine patch?	○	○
Have you ever had a seizure (epilepsy)?	○	○
Has anyone in your family been diagnoses with epilepsy?	○	○
Wearable carioverter defibrillator?	○	○
Implanted insulin pump?	○	○
Programable shunt or valve?	○	○
Hearing aid?	○	○
Blood vessel coil?	○	○
Sugical clips, staples, or sutures?	○	○

Wearable monitor (e.g. heart monitor)?	◯	◯
Shrapnel, bullets, pellets, BBs, or other metal fragments?	◯	◯
History of migraines?	◯	◯
Radioactive seeds?	◯	◯
Portable glucose monitor?	◯	◯
Other implanted metal or device? Please specifiy:	◯	◯
Have you ever been a machinist, welder, or metal worker?	◯	◯
Have you ever had a facial injury from metal and/or metal removed from your eyes?	◯	◯
Are you pregnant?	◯	◯
Have you ever had complications from an MRI?	◯	◯
Medicaitons?	◯	◯

Age: _____

Weight (lbs): _____

Height (in): _____

Medication Disclosure:

Medications to be aware of: [include a list of such medications that would be exclusionary for each grouping]

- Tricyclic anti-depressants (e.g. Amitriptyline, Amoxapine, Desipramine (Norpramin), Doxepin, Imipramine (Tofranil), Nortriptyline (Pamelor), Protriptyline (Vivactil), Trimipramine (Surmontil))

- Neuroleptic agents (e.g. Abilify (aripiprazole), Clozaril (clozapine), Geodon (ziprasidone), Latuda (lurasidone), Risperdal (risperidone), Saphris (asenapine), Seroquel (quetiapine), Zyprexa (olanzapine))

- Drugs that lower the seizure threshold will be excluded (e.g. Tramadol, Bupropion, Fluoxetine, Clozapine, Cocaine, Penicillin)

Must be able to abstain for 24 hours in advance of testing, if unable (EXCLUSION)
- Meclizine
- Scopolamine
- Benzodiazepines such as valium
- Sedatives such as Ambien
- Narcotic pain medications
- Antihistamines

Common MS Medication:

- Injectable medications: Avonex (interferon beta-1a), Betaseron (interferon beta-1b), Copaxone (glatiramer acetate), Extavia (interferon beta-1b), Glatiramer Acetate Injection (glatiramer acetate -- generic equivalent of Copaxone 20 mg and 40 mg doses), Glatopa (glatiramer acetate -- generic equivalent of Copaxone 20mg dose), Plegridy (peginterferon beta-1a), Rebif (interferon beta-1a), Zinbryta (daclizumab)

- Oral medications: Aubagio (teriflunomide), Gilenya (fingolimod), Tecfidera (dimethyl fumarate), Ampyra (dalfampridine)

- Infused medications: Lemtrada (alemtuzumab), Novantrone (mitoxantrone), Ocrevus (ocrelizumab), Tysabri (natalizumab)