Document:
Study Protocol and Statistical Analysis Plan

Official Study Title:
The Neural Mechanisms of Split-Belt Treadmill Adaptation in People with Multiple Sclerosis
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Principal Investigator: Brett Fling

Study Title: Neural Underpinnings and Sensory Feedback Augmentation During Split-belt Treadmill Training in People with Multiple Sclerosis

I. Aim and Hypotheses

A. Study Objectives, Aim and Hypotheses

1. Brief Intro: 93.7% of people with multiple sclerosis (PwMS) have impaired mobility and commonly report walking dysfunction as the largest factor affecting their quality of life and have a higher risk of falls and musculoskeletal injury. Split-belt treadmill training, where the speed of each leg is controlled independently, alters each leg's spatial and temporal stepping pattern, and improves gait symmetry in people with Parkinson's disease and stroke. Our recent work is the first to demonstrate strong evidence for similar gait improvements in PwMS. In addition, sensory stimulation produces improved gait performance and motor coordination within these populations. However, there is limited research investigating the neural mechanisms underlying these gait adaptations and whether sensory augmentation can elicit enhanced adaptation during split-belt treadmill training.
2. Study Objectives: The objective of this study is to investigate the neural mechanisms of gait adaptation and the effects of transcutaneous electrical nerve stimulation (TENS) on adaptability during split-belt treadmill training in PwMS. Functional near-infrared spectroscopy (fNIRS) will be used during a split-belt treadmill training paradigm to assess cortical activation during gait adaptation. Additionally, the effect of split-belt treadmill training coupled with TENS on gait adaptability in PwMS will be tested with active and inactive TENS during split-belt treadmill sessions. Cortical activation and the effect of TENS on gait adaptability will be compared between neurotypical adults and PwMS to assess differences that can be attributed to multiple sclerosis. Specifically, we hypothesize that the sensorimotor integration areas of the cortex including the supplementary motor areas (SMA), premotor cortex (PMC), somatosensory cortex (S1), and posterior parietal cortex (PPC) will have heightened activity during split-belt treadmill training, compared to when the belts are moving the same speed. Additionally, we hypothesize that the application of TENS during split-belt treadmill training will significantly increase gait symmetry improvements when compared to split-belt treadmill training alone and will also result in improved adaptation retention.

B. Background and Significance

1. Study Significance: Studies have consistently demonstrated that split-belt treadmill training can acutely improve gait symmetry, however there is minimal application of this paradigm toward enhancing or prolonging these improvements. The impact of this study is not only revealing the cortical mechanisms of sensorimotor adaptation and improving gait symmetry, but a necessary step towards translating these improvements out of the lab into a real-world environment thereby persistently improving mobility for not only PwMS but the tens of millions of individuals who experience gait dysfunction.

2. Our recent studies showed robust gait symmetry improvements in PwMS following split-belt treadmill training and additionally has demonstrated that TENS improves positional awareness and motor learning during a stepping task. The combination of these findings leads us to want to investigate the neural mechanisms of split-belt treadmill training and if TENS can enhance to effects of split-belt treadmill training.
3. Literature Review: Many studies have demonstrated that split-belt treadmill training can alter gait symmetry in people with stroke, Parkinson's disease, and most recently in PwMS. This split-belt treadmill training modality has over 20 years of investigation, yet the neural mechanisms of sensorimotor adaptation remain poorly understood, especially at the cortical level. Along with our preliminary work with TENS, Ng and colleagues found that TENS significantly increased gait speed and timed-up-and-go performance post-stroke and additionally, Almuklass et al. found that TENS generated immediate improvements of gait, balance, and motor skills for PwMS.
4. Background: We specifically hypothesize that the sensorimotor integration areas of the cortex (including SMA, PMC, S1, and PPC) will have heightened activity during split-belt treadmill training due to findings from studies utilizing positron emission tomography (PET) during split-belt treadmill walking, findings from studies measuring brain activity during dual task walking, and the necessity of these regions to process changing spatial information, such as one leg moving twice as fast during split-belt treadmill training. Additionally, through studies suggesting that TENS improves sensory signaling and gait performance we predict that this modality will also improve gait adaptability during split-belt treadmill training.
5. This is the first study to measure real-time cortical activation during split-belt treadmill training in any population. This proposal not only addresses the lack of research regarding the cortical mechanisms that accompany split-belt treadmill training but also evaluates a method to enhance adaptation by coupling the training with sensory augmentation via TENS.

II. Research Plan and Design

A. Study Type and Design: This is a crossover study with both PwMS and age and sex-matched neurotypical controls. 30 PwMS and 20 neurotypical controls be randomized into two groups and will participate in two split-belt treadmill training sessions while outfitted with cortical fNIRS and TENS devices on their bilateral tibialis anterior and rectus femoris. The first group will complete split-belt treadmill training with TENS OFF during the first visit and TENS ON during the second visit. The second group will complete split-belt treadmill training with TENS ON during the first visit and TENS OFF during the second visit to control for a learning effect. In between visits there will be a 4-week washout period. Participants will receive TENS only during the 10-minute split-belt adaptation period of the paradigm. During all other periods the TENS will be OFF.

B. Sample size, statistical methods, and power calculation

1. Total number of subjects who will be approached (including screen fails, controls and subject withdrawals) to reach enrollment numbers for the lifetime of the study for this investigator's site: 60
2. Total number of subjects to be *enrolled* at this site: 50

3. Brief Description/justification for the proposed sample size in *lay terms*: From our preliminary data we computed a large effect size of 1.01 for change in brain activity in the SMA from baseline walking to split-belt treadmill training. According to our power analysis, to maintain an alpha of 0.05 and a power of 0.8, at least 10 participants per group are needed to detect significant changes in brain activity.
4. Simple randomization will occur (TENS first or TENS second) for both groups (PwMS and neurotypical controls).
5. No masking will occur in this study.

C. Subject Criteria (See Vulnerable Populations appendix 1, if applicable): PwMS and neurotypical controls from ages 18-86. All races and genders are included.

1. Inclusion criteria:
    - A diagnosis of relapsing remitting multiple sclerosis OR a neurotypical adult (ages 18-86)
    - Not experiencing an active relapse
    - Able to stand and walk without an assistive device
    - Able to walk for three tenths of a mile without stopping to rest
    - MS participants must have a neurologist confirmed diagnosis of relapsing-remitting MS.
    - MS participants must be independently ambulatory in the community, expanded disability status scale (EDSS) score < 5 (clinical measure of MS severity).
    - Participants must be 18-85 years of age at the time of participation in the study.
    - Participants must be able to stand/walk on a firm surface for least 30 minutes.
    - Participants must be able to stand from a seated position on their own volition.
2. Exclusion criteria:
    - Unable to walk for 500 meters without assistance
    - Musculoskeletal injury in past 6 months
    - Lower extremity surgery in past 6 months
    - Unable to abstain from medications that impair balance
    - Currently pregnant
    - History of traumatic brain injury
    - History of vestibular disease
    - History of any other balance impairment unrelated to multiple sclerosis
    - MS participants must not have primary progressive MS
    - MS participants must not have on-going MS relapse
    - MS participants must not have other neurologic conditions besides MS, and concurrent conditions affecting ability to comply with the study procedures including musculoskeletal injury
    - MS participants must not have excessive fatigue to the extent that participants can not complete all testing required.
    - Participants must not have significant vision impairment
3. Withdrawal/Termination criteria: Participants will be withdrawn if they are upon baseline walking assessment the researcher determines that they do not have enough mobility to complete the split-belt treadmill training paradigm.

4. Participants are allowed to be concurrently enrolled in another research study, unless this study causes them to fall within the exclusion criteria.

III. Subject Participation

A. Recruitment:

1. Subjects will be recruited from the local community. We typically recruit from the North Central Colorado Urban Area. This 13-county Urban Area comprises the four contiguous Metropolitan Statistical Areas of north central Colorado including the Denver-Aurora-Broomfield, Fort Collins-Loveland, Boulder and Greeley.
2. Multiple sclerosis affects all races, all genders, and all ages, but is most prevalent in women of Northern European descent. This study will recruit individuals with multiple sclerosis, with no restrictions for race or gender. We therefore expect the study population to reflect the general demographics of multiple sclerosis. Our lab group is well versed in the nuances of minority population enrollment to facilitate equity recruitment and in clinical trial participation. Our inclusion criteria of 18-86 years were chosen for comparison of clinical and biological differences between individuals with multiple sclerosis and neurotypical controls across a variety of age groups. These age groups will allow us to detect meaningful differences between individuals with multiple sclerosis and neurotypical controls across the lifespan. Additionally, we will be poised to identify changes in function as a result of multiple sclerosis and will be able to avoid age as a confounding factor due to age and sex matching. Our lab group has previously studied multiple sclerosis among this age range for 10+ years and have the necessary accommodations (e.g., bodyweight harness, handrails, wheelchairs) for all levels of disability status.
3. Over 100,000 people within the Colorado-Wyoming region are afflicted with MS and participant recruitment will be determined in consultation with Dr. Augusto Miravalle from the Department of Neurology / Rocky Mountain MS Center at the University of Colorado where over 8,000 patient visits are conducted annually. Participants will be recruited from the UC Health Regional Neuroscience Center at Poudre Valley Hospital and the Multiple Sclerosis Center of the Rockies in Fort Collins as well as the Local National MS Society and other local MS and general Neurology clinics. Potential participants will be identified through word of mouth, clinician support, or flyers. Interested participants will be contacted via email or telephone where they will be informed in greater detail what the study involves. Following an explanation of the study potential participants will be asked if this particular study is of interest to them and if so a screening for eligibility will be scheduled.
4. In Kuali, we have attached a copy of a flyer that will be distributed via email to people at the Brain Health Center of the Rockies, the Center for Healthy Aging, and the Colorado-Wyoming chapter of the National Multiple Sclerosis Society.
5. An email template used to introduce participants to the research study is also included and attached in the recruitment folder in Kuali.

B. Screening Procedures or Interview/questionnaire: Demographic information, health history (screening), and general physical activity questions will be done prior to beginning the testing session and is recorded REDCap via private survey's sent to the participant's private email. All questions asked to participants are attached in the attachment section. They are

meant to provide the research staff an idea as to whether or not the participant would qualify for the study. These various forms give a better picture of the participants unique symptoms and their mobility status to be sure that participation is safe for them as well as make associations between collected data and reported status. An important confounder to consider when walking is cognitive status. The BDI and MOCA will provide additional information about cognitive and emotional status that will allow us to have a better full picture of the participant when analyzing their data. Only trained graduate students will be allowed to perform screening and determine eligibility. Additionally, if the graduate student is unsure due of eligibility due to symptom characteristics, the PI, Brett Fling, will be contacted to confirm. The Pi, Brett Fling will review inclusion and exclusion criteria of participants weekly to confirm eligibility.

C. Informed consent process and timing of obtaining of consent

1 Trained graduate students will be the only personnel allowed to deliver the informed consent. Andrew Hagen will be the sole consenter for this study.

2 The consent process will include a written and signed document along with a trained graduate student verbally describing the consent form. The participant will initial and date each page of the consent form, along with signing the last page. The trained graduate student will describe the study in lay terms in person and the participant will be allowed to ask questions. The consent process will be before any of the study procedures to confirm the participant is completely informed and consented to all portions of the study before enrolled.

3 Trained graduate students and the principal investigator will determine whether the subject is able to give consent. People with cognitive impairments and minors will be excluded from this study due failing to meet the inclusion/exclusion criteria.

D. Specific procedures and techniques used throughout the study

1. Laboratory tests
   - **Gait Adaptation.** This will be assessed using inertial sensors and 3-D motion capture. Both use noninvasive markers that are placed on the lower limbs of the participants. Our outcome measures assessing gait adaptation will be step length asymmetry (SLA). Step length is quantified by the anterior-posterior distance between heel markers at leading limb heel strike. Step length asymmetry will be calculated by subtracting the step length of the less affected limb from the step length of the more affected limb for each consecutive step and normalized to participant stride lengths and will be assessed continuously throughout each gait trial. We will use step length asymmetry to identify 1) the adaptation rate during the split-belt adaptation period to assess speed of adaptation between conditions and groups, and 2) adaptation savings, or the difference in adaptation rate between visit 2 and visit 1, to quantify adaptation retention between conditions and groups.

- **Cortical Activation via FNIRS**. All fNIRS data will be acquired using a mobile NIRSport2 device (NIRx Medical Technologies, nirx.net), which will be secured to participants' backs using backpack-like straps and buckles. This head probe was designed to measure regions of interest (ROI) that were established a priori. The Juelich atlas within the fNIRS Optodes Location Decider (fOLD) toolbox in MATLAB was used to design our optode montage which identifies channel locations above superficial components of the frontal, motor, and sensory regions. Our fNIRS head probe will include 16 sources and 15 detectors which will create 48 channels over the designated ROIs (Figure 1). Additionally, eight short-separator detectors will be used to measure scalp perfusion. All fNIRS data will be acquired and streamed to a laptop using Aurora software v.2021.9 (NIRx Medical Technologies, nirx.net/aurora). Raw fNIRS data will be processed using a commercial software tool, Satori v.1.8 by Brain Innovation (NIRx Medical Technologies, nirx.net/satori) to perform pre-processing steps, such as motion artifact removal, physiological noise detection, and channel selection and removal. Following, the raw light intensity data will be converted to optical density values and then converted to oxygenated hemoglobin (HbO), deoxygenated hemoglobin and total hemoglobin values using the Modified Beer-Lambert Law automatically through Satori software. The HbO beta values calculated from general linear models will be used as a proxy for cortical activity, and we will measure changes in HbO beta between each protocol period described in Figure 2B.

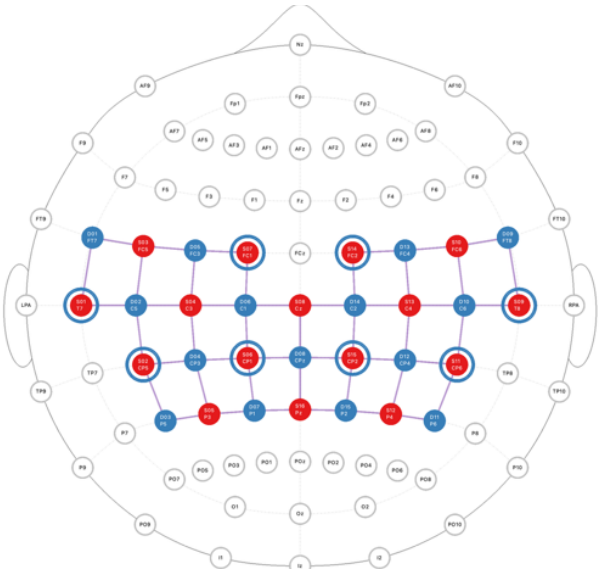

Figure 1. Optode montage (16x15) created using Brodmann atlas within fOLD to allow measurement of cortical hemodynamics for premotor, sensorimotor, and posterior parietal regions. Each red circle indicates a source optode, each blue circle represents a detector optode, and each purple connecting line represents a unique channel.

2. Study Procedures:
   - **Split-belt Treadmill Training Paradigm.** The participants will be outfitted with 6 APDM Opal inertial sensors (APDM Inc, Portland, OR) as well as 16 retroreflective markers for collection of three-dimensional motion capture data during a 10-minute split-belt treadmill training paradigm (Figure 2). For the overground walking portion, participants will be asked to walk back and forth down a 30m hallway for 2 minutes while APDM Mobility Lab is collecting inertial sensor gait cycle parameters. For the treadmill portion, participants will be asked to walk on a custom-built instrumented,

split-belt treadmill with two Bertec force platforms (Model 4060-10, Bertec Corp, Columbus, OH) consisting of two separate belts, each with its own motor that permits the speed of each belt to be controlled independently and collects ground reaction forces at 1000 HZ. The speed at which a participant is waking will be individualized to their overground preferred walk speed and fast walk speed. Specifically, during the tied-belt walking period, the belts will be set to the overground preferred walking speed. During split-belt treadmill adaptation period, the "fast belt" speed will be equal to the participant's fast walk speed while the "slow belt" speed will be one half of the participants fast walk speed (2:1 ratio). Prior

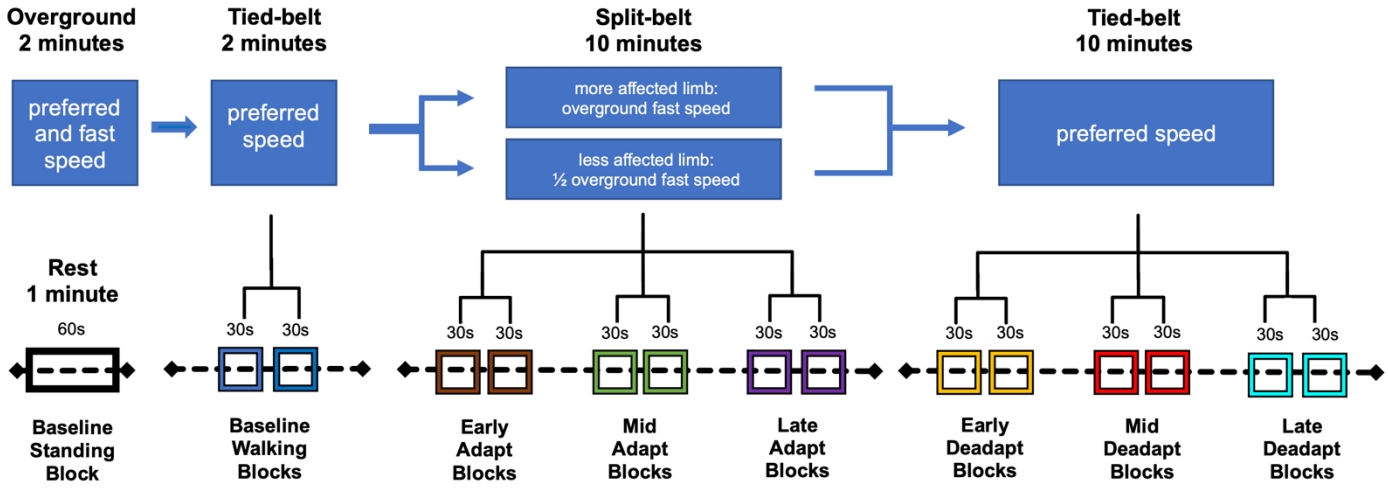

Figure 2. **(Top)** Time course for the split- belt treadmill training paradigm depicting the stages of baseline, adaptation, and deadaptation **(Top)** fNIRS block design collection protocol. Following a 1-minute baseline rest period, fNIRS will be collected during tied-belt walking, split-belt treadmill training, and deadaptation utilizing an average of two 30-second blocks for each period of the paradigm to assess differences in activation between each period.

research has determined that the "fast belt" speed should be set for the more affected leg to improve gait asymmetry. Following the adaptation period, a 10-minute deadaptation period with the belts tied at the preferred speed will occur to monitor gait symmetry changes during deadaptation between groups.

- **TENS Protocol.** 30 PwMS and 30 age- and sex- matched controls will be randomized into two groups and complete two split-belt treadmill training sessions identical to Aim 1, while outfitted with cortical fNIRS and TENS devices on their bilateral tibialis anterior and rectus femoris. The first group will complete split-belt treadmill training with TENS OFF during the first visit (these are the participants in Aim 1) and TENS ON during the second visit. The second group will complete split-belt treadmill training with TENS ON during the first visit and TENS OFF during the second visit to control for a learning effect. In between visits there will be a 4-week washout period (Figure 3). Participants will receive TENS only during the 10-minute split-belt adaptation period of the paradigm. During all other periods the TENS will be OFF. We will use an FDA-approved clinical TENS device, the LG-TECELITE Therapy System (LGMedSupply, Cherry Hill, NJ). Stimulation will consist of bursting biphasic pulses delivered with electrode pairs (2 in. × 4 in. pads) placed on the skin over the

stimulated muscles. The stimulus frequency will be set at 5 Hz with a seven pulses of 0.15 ms based on recent evidence suggesting that bursting TENS is most effective for improving walking performance and limits nerve accommodation to the stimulation. The amplitude of the TENS stimulation will be individualized for each participant in accordance with their specific motor threshold. The TENS amplitude will be slowly increased at 1 mA increments on each individual muscle until non-voluntary muscle contractions can be seen or felt by the investigator, then the amplitude will be set to 2 mA below the motor threshold for each muscle and limb during the split-belt adaptation period. Additionally, we are choosing to outfit TENS on the tibialis anterior and rectus femoris due to previous studies successfully improving gait performance in PwMS using these stimulation locations (Figure 3).

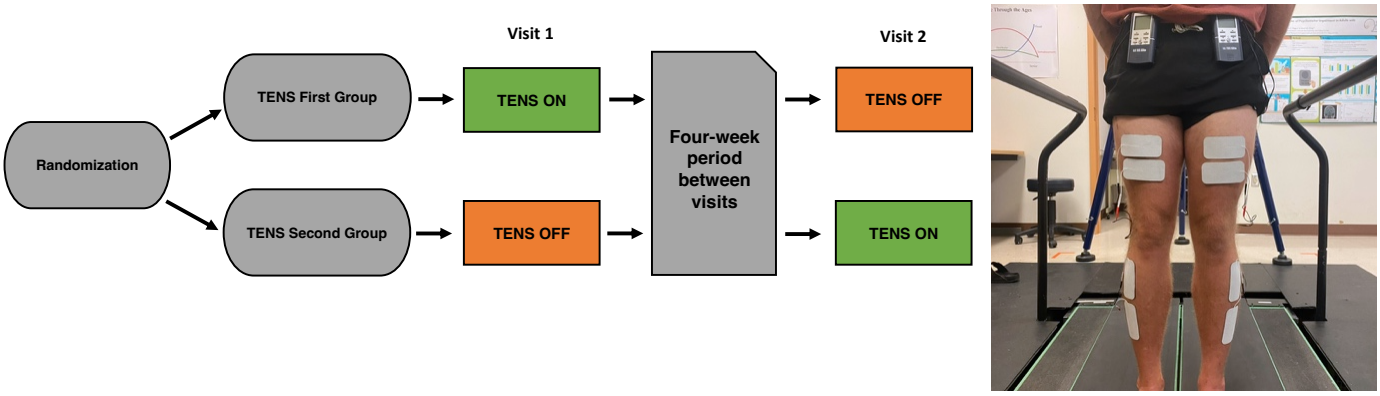

Figure 3. *(***Left)** Study design schematic including randomization and TENS condition at each visit. **(Right)** Example of TENS electrode placement on the bilateral tibialis anterior and rectus femoris.

- **Clinical Assesments**
  During the first visit participants will complete clinical testing to gain a more holistic view of their symtpoms. The first test a vibration sensory threshold assessment on the left and right hallux. This threshold testing involves the use of Rydel-Seiffer vibrating tuning fork. This tuning forks provides a visual measurement of vibration, allowing for a quantifiable measurement of vibration perception across participants. It has also been shown in recent work that lower limb sensory perception is associated with split-belt treadmill adaptation. Additionally, we will include the modified Clinical Test of Sensory Interaction for Balance (mCTSIB) using the BTrackS system. This is a very commonly used clinical assessment for balance that is noninvasive and easy to implement. This test can distinguish the function of different domains of balance individually, including vision, proprioception, and vestibular function. An assessment of each balance domain will additionally allow us to better quantify the factors that contribute to successful adaptation on the split-belt treadmill. Both of these assessments will be performed by a postdoctoral fellow in our lab, Dr. Kristin Johnson, who is a board-certified doctor of physical therapy (DPT) and will take around five minutes in total.

- **Photo/Video Use**

Additonally, photos or videos may be taken for gait analysis purposes, and qualitative assesment of gait adaptation. Participants may choose to op in or opt out of this via the informed consent form.

3. All Procedures are for research purposes only and are not a part of standard therapy.
4. No biospecimens are used in this study.

E. Costs to Subjects: The only cost to participants is transportation to our lab and their time (approximately 4-6 hours over two sessions).

F. Payment, including a prorated plan for payment: Participants will be compensated via cash. Participants will receive a total of $100.00 for completing the study. If a participant only finishes the first day of the study that participant will receive $50.00. Upon completion of the second day that participant will receive an additional $50.00. If a participant drops out or withdrawals from the study partway through a session that participant will still be compensated $50.00.

G. Research-related injury: The Colorado Governmental Immunity Act determines and will limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury.

H. Risk/benefit assessment: Greater than minimal risk

1. Physical risk: Yes.
   i. Musculoskeletal injury. There is a low risk of joint, tendon, or muscle pain, inflammation, or swelling during or after a testing session of your gait, and balance. This risk is reduced through the use of well-trained assistants and also by the mild nature of the gait and balance testing.
   ii. Falls. The walking tasks may cause participants to lose their balance and fall. However, during the overground walking trials the research team is trained to walk alongside participants at all times; if a participant happens to lose their balance, research staff will be there to assist and prevent a fall. For the treadmill walking trials, participants will be wearing a harness attached to a secure beam to ensure safety and security while walking. All safety measures will be taken to ensure a secure and comfortable environment. Participants will be allowed to take a break from walking tasks whenever necessary. It is not likely that a participant will fall.
   iii. Headache. Although rare, a potential side effect of our brain measurement is headache due to pressure on the scalp. However, we have many adjustments available in our cap to make the participant as comfortable as possible and mitigate this risk.
   iv. Skin Irritation. The electrical impulses that a TENS unit produces may cause a buzzing, tingling, or prickling sensation, which some people may find uncomfortable. Some people may be allergic to the adhesive pads. In rare cases, patients have reported burns at the sites where the electrodes are placed. However, we use a very mild level of stimulation which greatly reduces this discomfort. A TENS unit consists of a battery-powered device that delivers electrical impulses through electrodes (or sticky pads) placed on the surface of your skin. Overall, for the vast majority of people, TENS is believed to be safe and well-tolerated with little to no side effects. However,

manufacturers of TENS units universally warn individuals with pacemakers, epilepsy, or are pregnant that these conditions are contraindications to use as they can lead to potential complications. We are only placing TENS on the legs, so these warnings will not be relevant to this study.

2. Psychological risk: Yes.
    i. Breach of confidentiality: Although robust protocols and procedures are in place to keep your Protected Health Information private and secure, there is a chance of a breach of confidentiality.
    ii. Emotional distress. Some of the questions asked may be personal or embarrassing. Participants may refuse to answer any potentially uncomfortable question. Also, participants may learn information about their balance and walking that could be potentially upsetting.
3. Social risk: No
4. Economic risk: No
5. Potential benefit of participating in the study
    a. None
    b. This study will further elucidate differences in neurological activity and the potential of TENS to be a rehabilitation strategy for PwMS.
    c. The results of this study will help determine the connections between walking asymmetry and brain activity, and the potential of TENS which will be helpful when working with diseased populations.

I. Location where study will be performed: All portions of the study will be performed in the Sensorimotor Neuroimaging Laboratory at Colorado State University.

J. Collaboration (with another institution, if applicable): N/A

K. Single IRB Review for a Multi-site study (if applicable): N/A

L. Community-Based Participatory Research (if applicable): N/A

M. Personnel who will conduct the study, including:

1. Primary responsibility for the following activities, for example:
    a. Determining eligibility: Andrew Hagen
    b. Obtaining informed consent: Andrew Hagen
    c. Maintaining participant's research records: Andrew Hagen
    d. Drawing / collecting laboratory specimens: Andrew Hagen
    e. Performing / conducting tests, procedures, interventions, questionnaires: Andrew Hagen

N. Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

1. Please note that any study proposal with more than minimal risk must include a data and safety monitoring plan. Elements of the plan should include the following:
    a. Persons/groups who will review the data (study team; independent safety monitor, data monitoring committee or formal DSMB). This study is a registered clinical trial on clinicaltrials.gov (NCT05878873) where Dr. Brett Fling is the lead investigator and will be responsible for trial monitoring. Confidentiality will be strictly maintained to minimize psychological and economical risks. Participant identities will be kept confidential via participant identification numbers (i.e. SBNT_000). The computer

that will store the project database will be protected by current network security. An institutional computer account and password will be required to access the computer storing the recruitment database and the REDCap database will be password protected, and only project personnel approved by the PI will be given access. Only those individuals listed on the IRB consent form and within the Kuali IRB protocol will have access to the data.

b. Data/events that will be reviewed: Objective, de-identified data will be reviewed in a rolling fashion throughout the course of this project to allow for dissemination of preliminary results and for the use of preparing future grants based upon results. Any adverse events that occur will be documented, reported and reviewed immediately.
c. Frequency of review: Data will be reviewed monthly by our team.
d. Types of analyses to be performed: Analyses include objective outcomes from the split belt treadmill training protocol. This includes gait kinematics and kinetics, as well as cortical activation as assessed by fNIRS data.
e. Safety-related triggers that would cause the PI to alter the study/remove a participant: If an individual was unable to safely ambulate either on their own within the hallway, or on our treadmill in a safe and efficacious manner, participation would be halted immediately. Triggers that would identify an individual as a potential risk include repeated stumbling, tripping, or falls where our body weight support harness catches the individual.

2. Describe how adverse events (anticipated and unanticipated) and unanticipated problems will be ascertained and handled. Explain exactly which type of problems will be considered serious and reported to the IRB. The reporting timeframe should also be detailed. The primary adverse event that could result from this protocol is an unintended fall by a participant. To mitigate any risks that would occur as a result of a fall we implement a number of safety measures including the use of a "gait belt" while walking overground and a body weight support harness while walking on our treadmill. These are standard of care practices and the PI of this project has conducted similar mobility-based research for nearly two decades and has yet to report an adverse event as a result of an accidental fall. If an individual were to fall during this project, we would immediately contact the medical director of the HPCRL, Dr. David Thompson for acute assessment. Following this, we would report to the IRB within 2 business days, as we believe any unintentional fall is an adverse event and requires reporting.
3. Explain exactly what will happen if a patient experiences an adverse event or other problem (for example, will discontinue study participation). As describe above, immediate attention would be provided by our in-house physician and medical director, Dr. David Thomson and further steps will be conducted under his advisement. If an individual were to experience an accidental fall, we would discontinue study participation at that point.

IV. Data Collection and Protection

A. Data Management and Security:

1. All data collected, including identifiable information or personal health information will be stored either electronically on secured Colorado State University server or through a REDCap database with access restricted to study personnel. Any data not in electronic form

will be stored in a locked file cabinet with access limited to study personnel. The Sensorimotor Neuroimaging Laboratory is located in Moby room 154 within the Department of Health and Exercise Science. All hard copy data will be stored in the Sensorimotor Neuroimaging Laboratory, with access being restricted to authorized key holders and key study personnel.

2. Brett Fling, Kristin Johnson, and Andrew Hagen will have access to the study data.
3. All consent information will be stored electronically on a secure server through Colorado State University accessed only by our designated research team. Information collected electronically will be stored on a secure Colorado State University network server to prevent any breaches in confidentiality. Additionally, all questionnaires, demographics, and screening information will be stored in a REDCap database controlled by CCTSI and only approved study personnel will have access this database. Data we stored on the R:/Drive which is a secure server or on REDCap. Data will be kept for a minimum of 3 years after the completion of the study by Dr. Brett Fling.
4. People will be deidentified and all information will be used with a subject code (e.g., SBNT_000, with the key to this code available only as a physical copy stored in of locked lab.
5. Andrew Hagen and Brett Fling will have access to and maintain the key to the code.
6. Data will only be linked to a participant via a participant code.
7. All data collected, including identifiable information or personal health information will be stored either electronically on secured Colorado State University server or through a REDCap database with access restricted to study personnel. Any data not in electronic form will be stored in a locked file cabinet with access limited to study personnel. The Sensorimotor Neuroimaging Laboratory is located in Moby room 154 within the Department of Health and Exercise Science. All hard copy data will be stored in the Sensorimotor Neuroimaging Laboratory, with access being restricted to authorized key holders and key study personnel.
8. No mobile devices will be used for collection or storage of data.
9. N/A. No identifiable data will be sent outside of CSU.

B. Sample / Specimen Collection: N/A

C. Procedures to protect subject confidentiality: Individual participant data will be de-identified with given subject numbers, a master list will be stored separately on the secure server. Access to the data will be limited to study personnel. Subject ID example (SBNT_001). If we are collecting data on multiple people during a single day, we will leave ample time between sessions so that participants will not run into each other. All consent information will be stored electronically on a secure server through Colorado State University accessed only by our designated research team. Information collected electronically will be stored on a secure Colorado State University network server to prevent any breaches in confidentiality. Additionally, all questionnaires, demographics, and screening information will be stored in a REDCap database controlled by CCTSI and only approved study personnel will have access this database.

V.Reporting

A. Study results to participants: Upon completion of the study, all enrolled participants will receive a page summarizing group results in lay-terms. This will not mention any identifiable data and only group results will be present. This page will also include the meaning of the results, the clinical application, and the future directions of our work.

B. Dissemination Plan: Brett Fling will ensure that the clinical trial registered with this study has up to date information and that results are submitted to ClinicalTrials.gov as outlined in the policy and according to the specific timelines stated in the policy. The results of this study will be created into at least three manuscripts and submitted to for publication in peer reviewed journals. Additionally, both Brett Fling and Andrew Hagen will present the results of the study in the form of workshops, podium presentations, and poster presentations at local, regional, national, and international conferences within 2 years from the start of the study.

C. Statistical Analysis Plan: In this crossover design, PwMS and healthy control participants will be pseudo-randomized to begin in either the TENS First or TENS Second condition. Randomization will be performed using a random number generator and will be counterbalanced across age, sex, and fast limb to ensure group balance. A predefined list of participant numbers with corresponding randomized assignments will be generated prior to enrollment. Linear mixed-effects models will be used to identify differences in adaptation and fNIRS outcomes, with group (PwMS vs. healthy controls), visit (Visit 1 vs. Visit 2), and TENS condition (TENS ON vs. TENS OFF), along with their interactions, as fixed effects, and participant number as a random effect. Assumptions of linearity, normality of residuals, and homoscedasticity will be tested for each model. Following model creation, a repeated measures ANOVA will be performed for each adaptation outcome (adaptation rate and adaptation savings) and for cortical activation (HbO beta). Type III ANOVAs with Satterthwaite’s method for degrees of freedom estimation will be used for all analyses. Post–hoc comparisons will be conducted using estimated marginal means (EMM) to further investigate significant main effects and interactions. P–values will be corrected using the Benjamini–Hochberg false discovery rate within each outcome and group when applicable. Cohen's d will be calculated to quantify effect sizes. Separate regression models will be conducted to assess the influence of demographic covariates on primary outcome models. All statistical analyses will be conducted using the lme4 and emmeans packages within R and were two-tailed with an alpha threshold set at 0.05.