DISSERTATION

LOCOMOTOR ADAPTATION IN PEOPLE WITH MULTIPLE SCLEROSIS: MECHANISMS AND NEUROMODULATION

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ABSTRACT

LOCOMOTOR ADAPTATION IN PEOPLE WITH MULTIPLE SCLEROSIS: MECHANISMS AND NEUROMODULATION

Locomotor adaptation on a split-belt treadmill is a popular motor learning technique where two independent treadmill belts move at different speeds, generating adaptation of stepping over time. Much is understood about the dynamics and neural control of this adaptation, but large questions remain about its long-term retention and applicability beyond controlled laboratory settings. In this dissertation, locomotor adaptability was assessed in people with multiple sclerosis (PwMS), a population with pronounced sensory impairments. This investigation was among the first to show that despite disrupted neural communication, PwMS maintained the ability to adapt their stepping in space and in time in response to the split-belt treadmill. Following, a biomechanical assessment found that for PwMS, increased propulsive force was the largest kinetic contributor to adaptation and was strongly linked to decreased dorsiflexion, indicating that ankle joint dynamics drive much of the observed stepping changes. To address sensory impairments in PwMS, the next study evaluated the use of transcutaneous electrical nerve stimulation (TENS), a neuromodulation method that increases afferent excitability and has been used to improve motor coordination. However, TENS has yet to be investigated in the context of motor learning. This work demonstrated that TENS improved the retention of locomotor adaptation in PwMS after four weeks and decreased cortical activation in both PwMS and healthy controls. These findings suggest that TENS facilitates the recall of motor memories and promotes the automaticity of motor learning, giving it potential as an additional tool to enhance rehabilitation effectiveness in PwMS.

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CHAPTER 1 – LOCOMOTOR ADAPTATION ON A SPLIT-BELT TREADMILL: MECHANISMS, MODULATION, AND CLINICAL UTILITY

Introduction

Human locomotion is incredibly flexible, allowing individuals to adjust their stepping behavior in response to various environmental demands, such as different terrain, weather conditions, or even different shoes. When presented with musculoskeletal injury or neurological damage, locomotion also adapts in compensatory ways that are often maladaptive. Identifying the mechanisms underlying locomotor adaptation is critical for enhancing motor learning and designing effective rehabilitation strategies that promote long-term gait improvements. An important experimental tool that has advanced our understanding of locomotor adaptation is the split-belt treadmill, which independently controls the speed of each limb with separate belts. By creating a speed asymmetry between limbs, this paradigm induces adaptive changes in gait, providing a controlled perturbation to examine how the nervous system recalibrates movement.

Beyond its role in studying motor learning, the split-belt treadmill has potential clinical relevance as a tool to reduce gait asymmetry. Gait asymmetry leads to an increased risk of falls, higher metabolic cost, and impaired balance control,[1–4] making it a pressing mobility concern in many neurological conditions such as stroke, Parkinson's disease, multiple sclerosis, and spinal cord injury. Accordingly, many studies have explored the potential of locomotor adaptation, induced through split-belt walking, to restore gait symmetry.[5] There is strong evidence to suggest that only some gait parameters adapt and that certain neural substrates, particularly the cerebellum, control and store the new locomotor pattern. Additionally, studies have investigated different ways that adaptation can be modulated to enhance its effectiveness, including the magnitude of stepping

errors, the sensory environment, supplemental feedback, neuromodulation, and cognitive demands. However, despite decades of split-belt treadmill research, considerable questions remain. The limited transfer of the adaptation on the treadmill to overground walking has received markedly less attention, yet it is the most relevant aspect for understanding how to achieve lasting gait symmetry improvements in clinical settings. This review summarizes the neural mechanisms underlying locomotor adaptation, examines the various modulators that influence adaptation dynamics, and discusses the challenges of overground transfer and its implications for rehabilitation.

Locomotor Adaptation Dynamics

What Adapts and What Does Not?

When walking on a split-belt treadmill, many studies have investigated which gait parameters are affected by this perturbation and how they are influenced. The earliest studies examined the immediate effects of walking with asymmetric belt speeds in cats with transected spinal cords and in human infants. In the spinalized cats, sudden changes were observed in the support time between limbs, with the 'slow limb', or the limb on the slower belt, showing a longer stance time and shorter swing time compared to the 'fast limb', or the limb on the faster belt.[6] In infants, this pattern of altering stance time between limbs to maintain alternating rhythmic stepping occurs even before the development of voluntary walking ability.[7,8] Other work demonstrated this immediate behavior in adults,[9,10] but Reisman et al.[11] were the first to establish distinct time courses for how different gait parameters change when exposed to a split-belt treadmill over an extended period. They suggest the presence of two separate processes: rapid, reactive feedback-driven adjustments and slower, predictive feedforward modulation. Intralimb gait parameters, including stance time and stride length, were exclusively regulated by reactive feedback and

immediately adjusted within a few steps of the perturbation. In contrast, interlimb gait parameters, including step length asymmetry and double support ratio, were primarily governed by predictive feedforward modulation. This process led to gradual adaptation over time, and the presence of aftereffects once the belts returned to equal speeds, which is a key feature of predictive learning and storage.[11,12] Twenty years later, these findings of immediate intralimb adjustments alongside gradual interlimb adaptations have been strongly supported by subsequent locomotor adaptation studies in various populations, including people with cerebellar ataxia[13,14] stroke,[5] traumatic brain injury,[15] Parkinson's disease,[16,17] multiple sclerosis,[18] and both younger and older populations,[19,20] among others. Interestingly, recent evidence suggests that the limb on the fast belt may play a predominant role in driving these interlimb adaptive changes.[21–23]

In locomotor adaptation studies using a split-belt treadmill, the most common measures of interlimb coordination include step length asymmetry, step time asymmetry, double support asymmetry, limb angle phasing, and center of oscillation, while primary measures of intralimb coordination are stance time, and stride length (or, more accurately, limb excursion).[24] Importantly, each of these measures reflect either spatial or temporal control of gait. Focusing on interlimb adaptive parameters, step length asymmetry and center of oscillation are indicative of spatial adaptation, whereas step time asymmetry, double support asymmetry, and limb angle phasing are indicative of temporal adaptation. However, step length asymmetry is a hybrid measure that can be dissociated into spatial, temporal, and perturbation components.[25]

The adaptation of spatial and temporal parameters seems to operate largely independently, with evidence suggesting that clamping adaptation of spatial parameters (i.e., preventing them from adapting) does not interfere with adaptation of temporal parameters.[26] Additionally, spatial and temporal adaptations are uncorrelated, and spatial and temporal baseline asymmetries are

predictive of aftereffects only within their respective domain.[18,27] Temporal control appears to be prioritized and have greater regulation: children develop the ability to adapt temporal parameters sooner,[19,28,29] temporal adaptation occurs more rapidly than spatial adaptation,[26,27] and temporal adaptation is more resilient to interference.[30,31] Other complementary components also adapt robustly during split-belt walking, likely contributing to the overall system of dynamic gait control. These include anterior and posterior ground reaction forces,[22,23,32,33] lower limb joint dynamics (most prominently the ankle joint),[34–37] foot placement,[38–40] and even the Hoffmann reflex.[41] For instance, ankle dynamics adapt during the stance phase, with ankle plantarflexion beginning earlier in the gait cycle to generate the necessary increase in propulsion for the fast limb.[23,34,42]

Phases of Locomotor Adaptation

Locomotor adaptation on a split-belt treadmill follows a stereotypical time course in which gait parameters change across different phases of the paradigm. Reactive parameters, such as stance time asymmetry, exhibit immediate changes upon perturbation but do not adapt over time. While stance time asymmetry is mathematically a ratio between limbs, it remains an intralimb parameter because stance time itself is determined independently for each limb, unlike step length, which involves interlimb coordination. After a baseline tied-belt period where the belts move the same speed, the belts are split into different speeds and stance time immediately increases for the slow limb and decreases for the fast limb, with no further changes throughout the training period. Upon returning to tied-belt walking, stance time asymmetry is immediately corrected, returning to baseline without aftereffects (Figure 1.1A).[43] Conversely, adaptive parameters, such as step length asymmetry, undergo gradual recalibration. During split-belt walking, there is an immediate perturbation of asymmetry, with the slow limb taking larger steps, but this gradually adapts to restore symmetry over time.

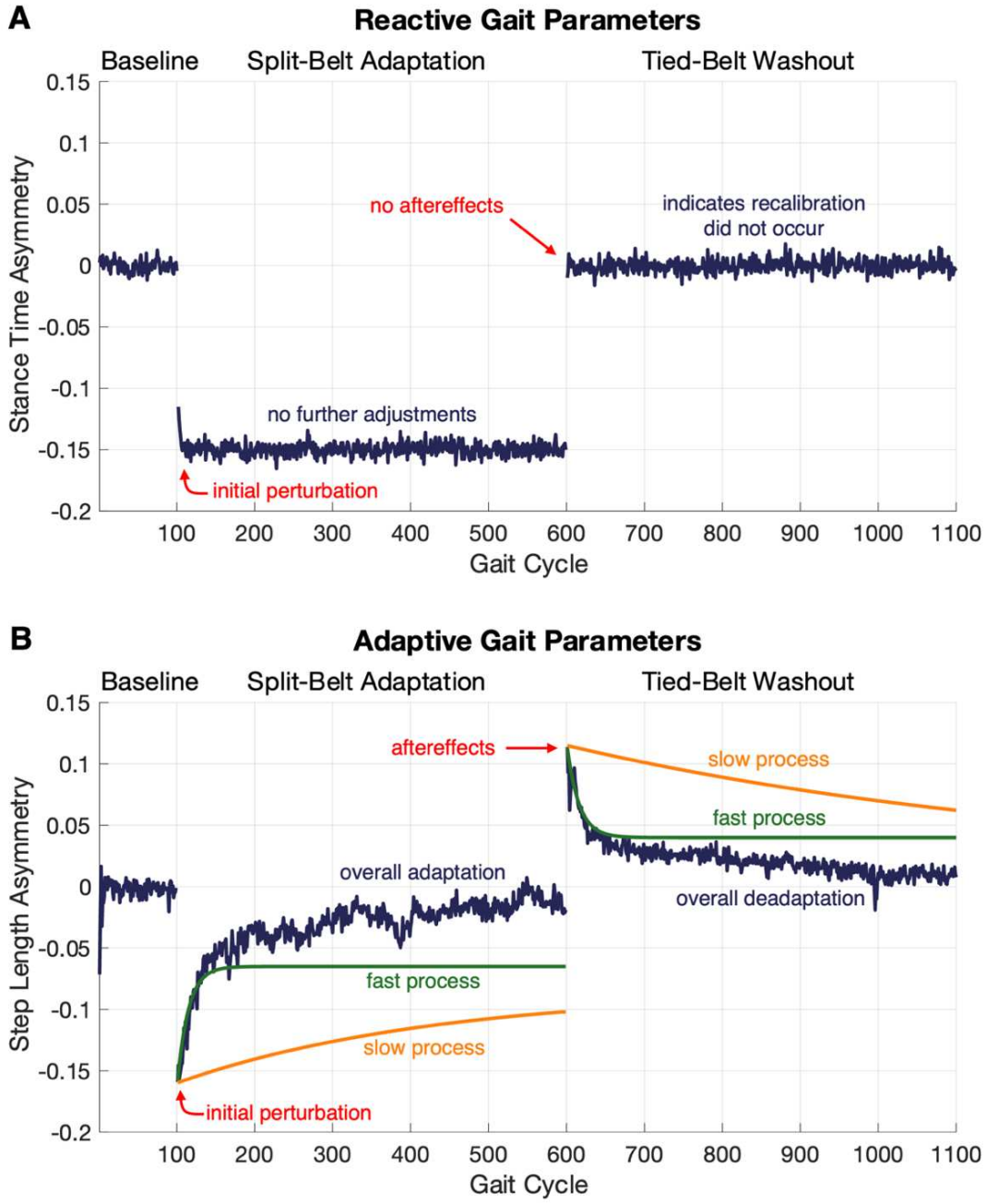

Figure 1.1. Reactive and adaptive responses to split-belt walking. **(A)** Stance time asymmetry as an example reactive parameter. Following adjustments to initial perturbation, no further changes appear. Additionally, there is no deviation from baseline once returning to tied-belt walking (no aftereffects), indicating recalibration did not occur. **(B)** Step length asymmetry as an example adaptive parameter. After initial perturbation, gradual adaptation occurs, represented by the combination of slow and fast processes (two-rate state-space model). Upon returning to tied-belt walking aftereffects emerge, indicative of recalibration that gradually washes out over time.

It has been proposed that this adaptation relies on a two-rate state-space model, consisting of a fast process that adapts quickly but is not well retained, and a slow process that adapts gradually and is retained more persistently.[32,44–46] The initial period of step length asymmetry adaptation reflects the fast process, whereas the early and steady-state periods primarily reflect the slow process. After returning to tied-belt walking, negative aftereffects emerge, demonstrating asymmetry in the opposite direction compared to during adaptation, though these effects are transient (Figure 1.1B).[47] Some paradigms investigate the ability of this adaptation to transfer to overground walking. In such cases, a catch trial is often implemented during the adaptation phase, where the belts are briefly tied (typically around 10 seconds) before resuming the remaining split-belt walking. This design allows for the comparison between treadmill aftereffects and overground aftereffects, which are markedly smaller.[48–50] Additionally, when participants are readapted on the split-belt treadmill after prior exposure, they exhibit a smaller initial perturbation followed by a faster rate of adaptation, which is indicative of adaptation savings.[51–53]

Neural Mechanisms of Locomotor Adaptation

Strong evidence indicates that reactive adjustments and adaptive learning are governed by distinct neural mechanisms. Early work demonstrated that spinalized cats exhibit reactive adjustments in stance and swing time when walking on a split-belt treadmill, indicating that this response is mediated primarily at the spinal cord level, likely through central pattern generators.[6] Similarly, human infants, despite lacking voluntary walking ability, maintain the ability to use sensory feedback and central pattern generators to maintain rhythmic stepping on a split-belt treadmill, further supporting spinal contributions to reactive control.[7,8,54] In contrast, adaptive learning is dependent on supraspinal substrates, particularly the cerebellum. Observations in decerebrate cats, where the cerebral cortex is disconnected but the cerebellum remains intact, have

shown that adaptive behavior persists at the cerebellar level and leads to increased climbing fiber discharge.[55,56] Moreover, when disrupting cerebellar function by injecting the vermis with nitric oxide, this adaptive ability is diminished, reinforcing the cerebellum's influence.[57] Extensive work has since demonstrated the dominant theory that feedforward predictive control underlies adaptive changes through recalibration of forward models,[12,44,58] though this is not without criticism.[59] Importantly, this recalibration is dependent on sensory prediction errors rather than task or target errors.[60] For each motor command, an efference copy is sent to the cerebellum, where a forward model predicts the sensory consequences of the command. Discrepancies between the predicted and actual sensory feedback, known as sensory prediction errors, drive the adaptation of forward models, refining feedforward control to improve stepping symmetry over time.[58,61] Further implicating the cerebellum in this process, research has demonstrated that cerebellar-brain inhibition is proportional to adaptation magnitude, and that adaptation rate can be modulated using cerebellar transcranial direct current stimulation.[62–64]

When considering adaptation as a two-rate system, the initial rapid portion likely relies more on feedback control and is typically associated with explicit processes.[65] Importantly, in locomotor adaptation on a split-belt treadmill specifically, some evidence suggests that explicit strategies may have less influence compared to other sensorimotor adaptation pardigms,[66,67] including visuomotor rotations and reaching force fields. This is likely due to walking being a highly autonomous and tightly controlled motor behavior.[68] In contrast, extensive evidence demonstrates that that slow process is more dominantly driven by implicit forward model recalibration.[12,67,69,70] The aftereffects that emerge following adaptation directly indicate forward model recalibration, and this occurs not only in the motor domain but also in the perception of leg speed, further indicating the role of sensory prediction and implicit recalibration in locomotor adaptation.[71–73]

While many supraspinal regions are involved in the processing, adaptation, and execution of the forward model, the cortico-cerebellar loop is theorized to be the primary pathway for updating and integrating the forward model into motor output.[74–76] A set of studies provided compelling evidence supporting this notion, specifically in the context of locomotor adaptation. Notably, diffusion tensor imaging identified a strong association between adaptation magnitude and radial diffusivity of the inferior cerebellar peduncle, implicating incoming sensory input from the periphery to the cerebellum in locomotor adaptation.[77] Additionally, this group demonstrated that recall and faster relearning during re-exposure to the split-belt treadmill have distinct functional substrates, with recall being associated with thalamic-cortical connectivity and relearning with cerebellar-thalamic-cortical connectivity.[78] Within this cortico-cerebellar loop, processing of sensory prediction errors is thought to occur in Purkinje cells, which integrate signals of expected and actual sensory consequences from climbing fibers (via the inferior olive) and parallel fibers (via mossy fibers). Purkinje cells then generate inhibitory output to the deep cerebellar nuclei. This updated forward model is transmitted via the superior cerebellar peduncle to the ventrolateral thalamus (and additionally the red nucleus). The signal is then relayed to cortical regions, including the premotor cortex, primary motor and sensory cortices, and posterior parietal cortex, where it is integrated into a refined motor command and executed via the corticospinal tract primarily. This iterative process continually reduces discrepancies between expected and actual sensory feedback to minimize errors and optimize motor performance.

Adaptation Insights from Different Populations

Cerebellar Ataxia

Foundational knowledge of the neural mechanisms underlying locomotor adaptation has originated from studies examining how specific brain lesions disrupt different components of

adaptation. By assessing which aspects of learning and error correction are impaired, these studies help clarify the distinct contributions of various brain regions. The first of these studies investigated people with cerebellar ataxia. Interestingly, while reactive adjustments were intact, recalibration was markedly impaired and these participants did not experience aftereffects, indicating that forward model recalibration did not occur.[13] This finding has since been validated in other studies, though lesion location and severity seem to be an important factor influencing adaptation impairment.[14,60,73,79] Overall, these findings contribute strong support for the involvement of the cerebellum in adaptation, but not in reactive adjustments.

Cerebral Damage

Adaptability has also been assessed in individuals with a variety of cerebral damage, including stroke, Parkinson's disease, traumatic brain injury, cerebral palsy, and hemispherectomy. Individuals post-stroke still retain the ability to adapt and generate aftereffects,[5,80] but prior work has indicated that both the fast and slow components of adaptation often occur at a reduced rate.[43,81–83] Additionally, savings with repeated split-belt treadmill exposure appears to be particularly impaired.[84] Conversely, one study has suggested that stroke may primarily disrupt the execution of motor commands rather than forward model recalibration.[85] Similarly, individuals with Parkinson's disease exhibit decreased adaptation rates compared to controls,[17,86] with adaptation being further impaired in those with freezing of gait or when off of dopaminergic medication.[16,87,88] In individuals with traumatic brain injury, cerebral palsy and hemispherectomy, reactive feedback remains intact, but feedforward adaptability is similarly slowed.[15,89,90] Interestingly, children with hemispherectomy exhibit normative spatial adaptation but significantly impaired temporal adaptation, suggesting that the cerebrum or interhemispheric communication may have greater regulation over temporal aspects of adaptation.[90] While findings

vary across studies, a common pattern shows reactive control remains intact, adaptation is slowed, sometimes in spatial domains and sometimes in temporal domains, but still occurs. Interestingly, overground transfer seems to be increased compared to controls, which may implicate the cerebrum in the ability to switch between different walking contexts.[48] However, the lesion locations across studies are widespread and variable in severity, limiting the ability to identify a specific region responsible for adaptation impairments. Instead, disrupting cerebral circuits in general does not completely impair, but slows adaptation efficiency.

Development and Aging

Both development and aging affect the capacity for locomotor adaptation. In children without disability, temporal adaptability is developed early (by the age of 3), but adaptation in the spatial domain is often slowed or even absent.[19,28,91] Despite this, young children display greater overground transfer, which decreases as they develop, suggesting differences in their ability to switch between walking contexts.[92] Spatial adaptability gradually improves throughout childhood and adolescence, similar to the delayed developmental trajectory of the cerebellum and prefrontal cortex.[93–95] In older adults, magnitude of adaptation is largely unaffected, while findings on adaptation rate are mixed.[96] Some studies report slowed adaptation in older adults,[20,97] with even further decreases in cases of cognitive impairment,[98] while others find no significant differences compared to younger adults.[17,99–101] Importantly, studies showing preserved adaptation rates often involve older adults closer to middle age,[96,99] suggesting that adaptation rate may decline progressively with age. Similar to young children, older adults exhibit greater overground transfer than young adults, with this effect being directly linked to impaired cognitive function.[97,102] Relatedly, aging is associated with degeneration and increased compensatory recruitment of the prefrontal cortex during walking.[103] However, adapting at a self-selected walking speed may also

enhance overground transfer, which is an important consideration when comparing young and older adults.[102] Together, the findings of increased overground transfer in young children and older adults indicate that there is a clear cognitive component underling the ability to switch between different walking contexts.

Ways to Modulate Locomotor Adaptation

Error Size

One common way locomotor adaptation dynamics have been probed is by manipulating belt speed ratios during the adaptation phase. Implementing these different error sizes selectively enhances distinct aspects of learning. Larger belt speed ratios (i.e., 4:1 versus 2:1) generate faster adaptation rates and larger aftereffects upon returning to tied-belt walking.[53,104,105] While larger errors also enhance savings, unlike other forms of motor learning, consolidation time does not appear to influence savings, rather overall exposure to errors drives this effect.[53] Error size can also be modulated by altering propulsion demands instead of belt speed ratios. When walking on an incline compared to flat ground, adaptation rate and aftereffects are also increased in a similar manner.[22,106] Importantly, exposure to a greater proportion of large errors while at the same belt speed ratio also drives improved learning. Repeatedly switching between split-belt walking and tied-belt walking and thereby spending more time in early adaptation when errors are largest, generates greater adaptation savings after washout the following day compared continuous split-belt walking. [51] Notably, the savings in this 'switching' group were nearly as large as in the group who experienced no washout between days. The mechanism underlying these effects likely involves increased exposure to larger sensory prediction errors, driving greater magnitude adjustments step-by-step. Additionally, it has been proposed that multiple exposures to the

perturbation leads to participants 'learning to learn',[107] as savings magnitude is similar even if the direction of the perturbation is switched between legs.[51]

The structure of how errors are introduced also influences learning outcomes. While variable errors during adaptation (i.e., changing belt speed ratios) do not affect adaptation rate or savings,[108] they may increase aftereffects, suggesting some strengthening of learning or heightened sensitivity to errors.[49] Studies have also investigated the effect of gradual errors by increasing the belt speed ratio gradually through the adaptation period, rather than abruptly at the beginning. Gradual errors lead to slower adaptation, reduced aftereffects, and no amount of savings.[49,52,109] However, gradual errors do lead to significantly greater overground transfer,[49,109] and reduce cognitive demand during adaptation.[110] This improvement may be driven by the process of credit assignment. Compared to abrupt errors, gradual errors are less strongly associated with the perturbation itself and more with the body's natural dynamics. As a result, the nervous system attributes the errors to general gait control rather than the specific task, promoting learning that transfers more effectively across different walking contexts.[50,111]

Sensory Environment

During treadmill walking a visual-proprioceptive mismatch occurs, where proprioceptive signals indicate forward motion, but visual information does not detect any displacement. A very insightful study by Torres-Oviedo et al.[112] revealed that the sensory environment in which adaptation occurs greatly affects adaptation dynamics. They demonstrated that simply closing one's eyes during the adaptation paradigm increases aftereffects and enhances overground transfer. Further results showed that spatial parameters exhibit similarly increased overground transfer even when vision was only occluded during overground transfer, whereas temporal parameters were more sensitive to the visual occlusion during adaptation, but not during overground transfer.[112]

Similar to removing vision, visual distraction (i.e., watching a video) increases the amount of overground transfer.[113] These results may be linked to sensory reweighting, where increased reliance on proprioception enhances transfer to new contexts. Additionally, reducing visual input during adaptation may decrease explicit contributions toward learning, which are known to be more context-dependent than implicit contributions.[114] Another method to mitigate the visual-proprioceptive mismatch during treadmill walking is by providing auxiliary optic flow that simulates natural walking using virtual reality. One study saw no effect when optic flow speed was matched to the slow belt speed during adaptation,[115] but another study suggests that the timing of optic flow is crucial. Specifically, optic flow increased adaptation rate, especially for spatial parameters, but only when the optic flow speed matches the speed of the next step, not the current step.[116]

Sensory weighting has also been augmented by applying vibration to the plantar surface of the foot during adaptation. While this does not influence adaptation or aftereffects, it may improve overground transfer,[117] likely by increasing proprioceptive gain in the sensory system as previously noted. Additional evidence suggests that cutaneous afferent signaling affects adaptability. In a force field perturbation walking task, an electrical stimulus disrupting afferent input at the ankle joint and additionally anesthetizing the ankle joint impaired adaptation magnitude.[118] Other studies have investigated the effect of holding on to handrails or using a walker during adaptation and have shown that using handrails reduces initial perturbation, adaptation rate, and aftereffects.[119–121] This indicates that balance demands impact adaptability, aligning with earlier findings suggesting that decreased body loading, and therefore decreased balance demand and afferent input, also diminishes aftereffects.[10] Collectively, this body of work highlights that the sensory

conditions during locomotor adaptation significantly alter adaptation dynamics, especially the amount of transfer to overground walking.

Visual Feedback

Several studies have incorporated visual feedback, such as projecting live step length errors on a screen, to supplement locomotor adaptation. In traditional motor learning literature, external feedback is known to enhance learning rates and retention of learning.[122] Earlier locomotor adaptation research has suggested that supplemental visual feedback of step lengths during learning may improve adaptation rate,[31] however other evidence suggests that the effects of feedback are more nuanced. Roemmich et al.[69] examined the effect of providing feedback for only the first minute during adaptation, then removing feedback for the remainder of the paradigm. While feedback initially improved the rate of stepping symmetry improvements, stepping performance immediately decreased after it was removed, matching the performance of those who never received feedback. This finding of similar motor performance after removal of initial feedback has also been substantiated in people post-stroke.[81] Importantly, there were no changes in aftereffects suggesting that feedback can accelerate motor performance improvements of step length asymmetry but does not enhance learning or forward model recalibration.[69] This distinction highlights that voluntary corrections (explicit strategies) and forward model recalibration (implicit strategies) occur concurrently to improve stepping performance, but that these processes are dissociable.[69] These results provide a strong explanation for why visual feedback can increase the rate of stepping symmetry improvements, but does not influence aftereffects[31,123] or savings,[124] indicating that no additional forward model recalibration has occurred. Further supporting the separation of explicit and implicit control during adaptation, evidence in multiple adaptation paradigms suggests that implicit forward model recalibration cannot be enhanced or disrupted with

differing explicit strategies, even with feedback that is incongruent with or attempts to block implicit strategies.[67,125]

Beyond supplementing locomotor adaptation, studies have investigated whether visual feedback of asymmetric step lengths alone can generate adaptation, yielding mixed results. Long et al.[67] suggested that visual feedback alone can alter stepping symmetry performance, but does not produce aftereffects, indicating reliance on explicit performance strategies rather than learning. Similarly, binary reinforcement alone may not generate learning, but when performed sequentially after locomotor adaptation it may prolong overground transfer.[126] Conversely, Sato et al.[127] demonstrated that explicit feedback of stepping alone can drive learning of asymmetric step lengths, and that feedback involving punishment can generate savings. Other work has also shown aftereffects and savings following explicit reinforcement of asymmetrical step lengths.[128,129] The combination of these findings suggests that alternative motor learning strategies, including reinforcement and use-dependent plasticity, must be specific but may contribute to learning and storage of a new walking pattern, though through different mechanisms than adaptation.

Neuromodulation

There is relatively limited research on the effects of neuromodulation on locomotor adaptation, with most work using transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). It has been established using TMS that reduced cerebellar-brain inhibition (CBI), or the inhibitory influence of the cerebellum on the primary motor cortex, after learning is strongly correlated with adaptation magnitude ($r = 0.78$).[62] This implies that adaptation may be regulated by long-term depression of Purkinje cells. Additionally, suprathreshold repetitive TMS on the primary motor cortex decreases adaptability in a force field locomotor task,[130] possibly by disrupting the inhibitory balance between the cerebellum and primary motor cortex. Prior

evidence demonstrates that cathodal tDCS decreases CBI while anodal tDCS increases CBI, likely through altering excitability of Purkinje cells and therefore the influence of the cerebellum on motor outpt.[64] In line with this, Jayaram et al.[63] investigated the effects of cerebellar tDCS on locomotor adaptation and found that, compared to a sham, anodal tDCS increased adaptation rate as hypothesized, while cathodal tDCS decreased it. Notably, tDCS only improved adaptability when applied over the cerebellar hemisphere ipsilateral to the fast limb,[63] which aligns other evidence suggesting the fast limb primarily drives the adaptation of stepping symmetry.[21–23] Importantly, aftereffects were unaffected by any tDCS condition, raising the question of whether tDCS-related improvements are a result of motor performance alone, rather than learning or forward model recalibration.

Few studies have followed up on the promising results of improved locomotor adaptation with cerebellar tDCS. One study conducted three days of locomotor adaptation with cerebellar anodal tDCS and generally found no significant changes in adaptability or savings across days.[131] However, there was a potential effect of tDCS reducing the rate of aftereffect decay only during a follow-up session without tDCS. Other work investigated the effect of anodal cerebellar tDCS on locomotor adaptation in people with multiple sclerosis and found no changes in adaptability or savings.[132] However, tDCS was delivered offline, applied only during a 15-minute rest period immediately following adaptation. Additionally, a study applying tDCS on the posterior parietal cortex observed that cathodal tDCS contralateral to the fast limb decreased adaptation rate and increased aftereffects, indicating some level of posterior parietal involvement.[133] Importantly, tDCS in this study was only applied offline for 20 minutes before the locomotor adaptation session. Overall, more research on the effects of tDCS on locomotor adaptability is needed to determine its clinical utility. However, anodal cerebellar tDCS ipsilateral to the fast limb concurrently during

adaptation, not before or after, appears promising in accelerating adaptation rates, yet it may not modify aftereffects.

A dearth of studies have investigated the effects of peripheral neural stimulation on locomotor adaptation. Plantar tactile vibration has been explored as a way to promote increased sensory weighting toward proprioception, but results indicate no effects on adaptation rate or magnitude, though a potential increase in overground transfer following adaptation was observed.[117] Additionally, a disruptive electrical stimulus applied to the ankle during locomotor adaptation decreased adaptation magnitude, with no changes in aftereffects.[118] A potentially promising result suggests that transcutaneous electrical nerve stimulation (TENS) to the tibialis anterior and rectus femoris during locomotor adaptation does not increase adaptation rate or aftereffects but significantly enhances adaptation savings when TENS is applied only during a follow-up visit (see Chapter 4 of this dissertation). In this study, TENS was primarily beneficial for people with multiple sclerosis who have widespread sensory impairments but demonstrated an insignificant moderate effect on savings in healthy controls as well. This finding suggests that augmentation of sensory input into the central nervous system via TENS may be most effective in populations with sensory impairments, such as those with multiple sclerosis.

Excitability of motor learning neural circuits is also influenced by brain-derived neurotrophic factor (BDNF), which is thought to enhance synaptic plasticity via long-term potentiation, promoting motor learning.[134] Previous work has suggested that BDNF gene polymorphisms influence locomotor adaptability,[135] and consequently studies have attempted to increase circulating BDNF to improve learning outcomes with mixed success. High-intensity exercise is known to increase circulating BDNF,[136] and studies tested whether this also improves locomotor adaptability. While high-intensity exercise prior to locomotor adaptation did increase

serum BDNF levels, these increases did not result in significantly improved adaptation rate or savings, though a trend toward increased savings in the high-intensity exercise group was observed.[137,138] Acute intermittent hypoxia (AIH) is another method to increase circulating BDNF.[139] Evidence supports that AIH may increase the adaptation magnitude and savings for temporal gait parameters and decrease net metabolic power.[140] Additionally, these changes in temporal adaptation and net metabolic power are associated with AIH-induced increases corticospinal excitability.[141] While BDNF has been implicated in motor learning, interventions aimed at increasing BDNF during locomotor adaptation produced mixed outcomes, with AIH showing the most promising effects.

Cognitive Demands

Cognitive load has been shown to impact locomotor adaptation dynamics,[110] often investigated through dual-task paradigms where participants are required to walk on a split-belt treadmill while performing a cognitive task simultaneously. When engaging in a secondary cognitive task, strong evidence demonstrates that these dual-task conditions reduce adaptation rates and increase variability. Numerous studies have confirmed this effect using tasks such as word counting during a television show,[31,99] auditory n-back tasks,[142,143] and, with mixed results, auditory Stroop tasks.[21,144] However, when performing a gradual adaptation, dual-task conditions did not alter adaptation rate,[113] presumably due to gradual adaptation constraining rate of change, but it has also been suggested that it may reduce cognitive demands, leaving more cognitive resources available.[110] Dual-task effects seem to be more prominent during early adaptation, likely because this phase relies more on explicit contributions, making it more susceptible to interference.[145] Notably, there is some evidence to suggest that the reduced adaptation rates during dual-task conditions occur for spatial parameters, but not temporal parameters.[31,145] The dual-task

influence on aftereffects is less consistent, with some work reporting that aftereffects are retained longer if dual-tasks were present during adaptation,[31,142] while others do not show any differences,[99,143,145] or even find diminished aftereffects.[21,113] Part of the dual-task influence on locomotor adaptation is attributable to aging with older adults exhibiting more susceptibility to cognitive-motor interference. Some studies have also found older adults prioritize locomotor adaptation over cognitive performance.[21,143] Interestingly, only one study has examined the dual-task effects on overground transfer, with evidence indicating greater overground transfer following adaptation under dual-task conditions.[113] This finding of reduced adaptation rate and subsequently more overground transfer aligns with findings of gradual adaptation increasing overground transfer,[49] indicating that transfer effects are in part mediated by adaptation rate.

Table 1.1. Modulators of locomotor adaptation. List of investigated locomotor adaptation modulators and their effects on adaptation dynamics, including adaptation rate, aftereffects, savings, and overground transfer. ↑: increase, ↓: decrease, ↔: no effect,↑↓: mixed results, ?: unexplored.

Modulator	Adaptation Rate	Aftereffects	Savings	Overground Transfer
Error Size				
large errors	↑	↑	↑	?
abrupt errors	↑	↑	↑	↓
gradual errors	↓	↓	↓	↑
variable errors	↔	↑	↔	?
Sensory Environment				
eyes closed	?	↑	?	↑

video distraction	↔[a]	↓[b]	?	↑
optic flow	↑[c]	↔	?	?
plantar vibration	↔	↔	?	↑[d]
holding handrails	↓	↓	?	?
Visual Feedback				
live step length feedback	↑↓[e]	↔	↔	?
feedback alone without split-belt perturbation[f]	↑	↑↓	↑	?[g]
Neuromodulation				
cerebellar anodal tDCS	↑↓	↔	↔	?
cerebellar cathodal tDCS	↓	↔	?	?
Posterior parietal cathodal tDCS[h]	↓	↑	?	?
disruptive ankle stimulation	↓	↔	?	?
lower-limb TENS	↔	↔	↑[i]	?
high intensity exercise	↔	?	↔	?
acute intermittent hypoxia[j]	↑	↔	↑	?
Cognitive Demands				
dual task	↓	↑↓	↔	↑

Notes: a. This was tested with a gradual adaptation paradigm, where adaptation rate may be clamped. b. Testing occurred following an overground washout, not during a catch trial. c. Only when the optic flow speed matches the speed of the next step, not the current step. d. Overground transfer was only quantified using stance time symmetry, a metric known to be reactive rather than adaptive,[11] which limits the validity of this result. e. When feedback is removed during adaptation, symmetry becomes equal to those who never had feedback, suggesting it improves performance but not learning. f. In this row, ↑ indicates if the corresponding adaptation dynamic is possible, rather than increases. g. There was a case where feedback performed sequentially after split-belt walking increased overground transfer. h. tDCS was only applied for 20 minutes before adaptation, not during it. i. This was only significant for people with multiple sclerosis but had a moderate trend in healthy controls. j. Only adaptation magnitude was measured, not rate, and effects were only present in temporal parameters.

Long-Term Storage and Overground Transfer: The Major Clinical Gap

Despite numerous studies exploring locomotor adaptation dynamics across various populations, a relatively small percentage have focused on adaptation savings, and even more critically, overground transfer. The split-belt treadmill is a valuable tool to probe locomotor learning capacity, but to be clinically feasible for driving long-term gait symmetry improvements in real-world environments, much more work is needed. Savings and overground transfer are the most clinically relevant aspects of adaptation, as they determine whether learned gait changes persist and generalize beyond the laboratory. Table 1.1 outlines various approaches researchers have taken to modulate adaptation to change dynamics, highlighting that savings and overground transfer remain the least explored. It is well-established that with repeated exposure to the split-belt treadmill, both the initial perturbation magnitude and adaptation rate are improved for each subsequent exposure, demonstrating savings and motor memories of this novel walking pattern.[146] The amount and rate of savings appears to be more influenced by exposure of large errors, such as during the early phase of adaptation, than by total time spent on the split-belt treadmill or consolidation time.[51,53] Additionally, these motor memories are relatively permanent, with evidence indicating that savings remains for at least three weeks and likely much longer,[147] though this needs to be confirmed in future studies. Compared to savings, the dynamics of overground transfer are less known, especially in the long-term and across different populations.

The most well-known modulator influencing overground transfer is error size, with gradual errors, and consequently a slower adaptation rate, leading to more overground transfer.[109] It is hypothesized that the sensory experience is an important factor affecting overground transfer,[112] and that gradual errors more closely resemble the natural errors experienced during overground walking, thereby facilitating greater transfer of adaptation across contexts.[49] Additionally, one study found that age-appropriate or preferred belt speeds during adaptation also enhance overground transfer,[102] further suggesting that errors resembling those experienced during typical walking contribute to greater generalizability. Other evidence indicates that distraction or simply having closed eyes during adaptation also increases overground transfer, likely through a similar mechanism.[31,112,113] When participants are less focused on the split-belt perturbation, and therefore less consciously aware of the sensory conditions leading to stepping errors, this again facilitates adaptation that is less context-specific and allows greater overground transfer. Interestingly, overground transfer seems to be enhanced in older adults, clinical populations, and young children, compared to healthy young adults.[48,92,97] Some have postulated that this increase in overground transfer is attributable to reduced cognitive or sensory capacity that results in impaired abilities to switch between different walking contexts (i.e., split-belt walking and overground walking).

Importantly, very few studies have investigated the effects of multiple locomotor sessions on long-term overground transfer. One group found some promising results in a case study and a cohort of people post-stroke. In both studies, participants completed 12 adaptation sessions over four weeks, with each session lasting 30 minutes. In the case study, the patient improved their step length asymmetry by 17% when tested one month following treatment.[148] In the cohort study, seven people were classified as responders while five were nonresponders to this treatment. At the whole group level, there was a significant decrease in step length asymmetry following the 12

adaptation sessions, but this decrease was no longer significant at the 1-month and 3-month follow-ups.[149] Recently, Wang et al.[150] showed that a 4-week locomotor adaptation intervention (20 total sessions) can improve overground spatial symmetry in people post-stroke more than traditional treadmill training, with effects maintained at a 4-week follow-up. Notably, improvements in spatial symmetry were associated with concurrent gains in Timed Up and Go performance, indicating that the intervention may also influenced longer-term changes in functional outcomes. While these studies have some encouraging results, it is also unknown whether these effects would be similar in healthy young adults or other populations, as people post-stroke are known to have enhanced overground transfer.[48] Future research is needed to further our understanding of overground transfer after repeated sessions of locomotor adaptation, as there could be important interactions between adaptation and use-dependent plasticity to generate lasting improvements in gait.[129]

The challenge remains: even when combining locomotor adaptation and use-dependent plasticity (i.e., repeated practice) to improve stepping symmetry, participants may only be improving their ability to task-switch between the treadmill and overground environments. Strong evidence indicates that with each additional exposure to the split-belt treadmill, both aftereffects and overground transfer decrease.[53,72,102] This pattern raises concerns about whether the sensory differences between these environments can be overcome, or whether the nervous system too apt to recognize these contexts and create separate forward models for split-belt and overground walking. These issues remain central for researchers and clinicians when evaluating the clinical utility of locomotor adaptation for long-term gait symmetry and functional improvements.

One explanation to these findings is a task-switching hypothesis, which suggests that with repeated adaptation bouts, participants are becoming better at switching between contexts rather than generalizing this adaptation across contexts. However, an alternative to task-switching is the

credit assignment hypothesis, which posits that the nervous system attributes errors to be either externally generated (i.e., specific to the treadmill), or self-generated (i.e., relevant across contexts). This mechanism has been more strongly supported in other learning paradigms,[111,151,152] but only recently was investigated empirically in the context of locomotor adaptation. Rossi et al.[50] tested both hypotheses by having participants undergo typical locomotor adaptation followed by alternating periods of overground walking and a treadmill speed-matching task. The speed match task required participants to adjust belt speeds until they perceived their walking as symmetrical. Due to established perceptual aftereffects following locomotor adaptation,[71,72] participants actually walked with asymmetrical belt speeds during the speed match task while perceiving them as equal. This unique manipulation allowed for split-belt walking to occur without sensory prediction errors during the task, preventing further recalibration. According to the task-switching hypothesis, each transition from the speed match task to overground walking should reintroduce overground aftereffects, as participants repeatedly switched between environments. However, the results showed that after the first overground walking bout, no further overground aftereffects were observed when alternating between split-belt and overground walking. [50] This finding supports the credit assignment hypothesis, suggesting that the nervous system maintains both a 'shared' forward model for split-belt and overground walking (associated with self-generated errors) and separate forward models for each environment (associated with externally generated errors), which explains why adaptation only partially transfers overground. The shared model washes out during the first overground walk, while the recalibrated treadmill-specific model, reflected by perceptual aftereffects, persists. This conclusion was further validated by comparing perceptual bias in the speed match task across groups, defined as the difference in belt speeds when participants perceive their walking as symmetrical. Participants who performed additional overground walking showed

the same rate of perceptual decay, or reduced perceptual bias across speed-match bouts, as those who did not, indicating that overground walking did not influence perceptual aftereffects, reinforcing that adaptation recalibrates a treadmill-specific forward model and a shared forward model separately.[50]

A credit assignment perspective also provides a strong framework to explain previous findings. Gradual adaptation, age-appropriate belt speeds, and distraction all likely increase the degree of shared recalibration by creating a learning environment more similar to overground walking, making the nervous system more apt to assign these errors as self-generated. In older adults and clinical populations, the increased overground transfer may be the result of an impaired ability to distinguish sensory differences between contexts and assign credit to errors appropriately, which researchers may be able to exploit. Additionally, locomotor adaptation paradigms that are more ecologically valid than split-belt walking, such as hip exoskeletons or motorized and mechanical shoe perturbations,[153–155] show promise in facilitating greater overground transfer by assigning more credit to a shared forward model. While much work remains in this area, the credit assignment framework is encouraging for clinical utility as it provides a mechanism that can be manipulated. Namely, how can we trick the nervous system into perceiving that more errors are self-generated and should be assigned to a shared forward model of walking that will transfer across contexts? To truly determine if long-term improvements in gait symmetry using locomotor adaptation are feasible, future directions of research should approach overground transfer with this perspective and the sensory environment in mind.

Conclusion

This review provides a summary of the locomotor adaptation dynamics when walking on a split-belt treadmill, the neural mechanisms enabling adaptation, different modulators and how

they impact adaptation dynamics, and discusses the clinical utility of locomotor adaptation moving forward. A substantial body of evidence indicates locomotor adaptation is cerebellum-dependent, relying on sensory prediction errors to drive forward model recalibration and generate aftereffects. While many studies have investigated ways to modulate adaptation, few have focused on savings and overground transfer. However, recent work demonstrates that a credit assignment framework best explains why the sensory environment during learning greatly impacts overground transfer. Although clinical feasibility remains distant, achieving lasting gait symmetry improvements with locomotor adaptation will require a stronger focus on enhancing overground transfer and a credit assignment perspective that prioritizes the sensory environment during adaptation.

This dissertation builds on the current understanding of locomotor adaptation, particularly in people with multiple sclerosis (PwMS), a population that often experiences unilateral motor impairments that result in gait asymmetries.[156] Despite the prevalence of these impairments, motor learning in PwMS remains understudied, with no prior research examining locomotor adaptation dynamics or potential strategies to enhance adaptation. Sensory deficits are common in PwMS,[157] which may disrupt motor learning in adaptation paradigms that rely on sensory prediction errors. Given this, modulators targeting sensory function could be a viable approach to improve adaptation effectiveness in this population and promote greater retention. This dissertation aims to characterize the mechanisms of locomotor adaptation in PwMS and identify neuromodulatory interventions that enhance adaptation, with the potential to influence long-term gait symmetry.

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CHAPTER 2 – LOCOMOTOR ADAPTATION IMPROVES SPATIAL AND TEMPORAL GAIT SYMMETRY IN PEOPLE WITH MULTIPLE SCLEROSIS[1]

Introduction

Multiple sclerosis (MS) is a neurodegenerative disease afflicting more than two million people worldwide[1] and has an average annual economic burden of USD 65,612 to each person with MS in the United States.[2] This chronic condition generally onsets between the ages of 20 and 50 and affects women three times more than men.[1] MS is characterized by the degradation of the myelin sheath, an insulating layer of lipids and proteins that increases the velocity of electrical impulse propagation along a nerve. This results in impaired neural communication to and from the brain. Typically, people with MS (PwMS) have more severe sensory and motor impairments on one side of their body.[3] This results in significant spatial and temporal gait asymmetries which subsequently leads to falls, musculoskeletal injuries, diminished engagement in daily life activities, and decreased quality of life.[4] Additionally, it has been shown that an asymmetrical gait results in a higher metabolic cost[5,6] and cognitive demand.[7,8] Accordingly, rehabilitation focused on minimizing gait asymmetries is exceedingly beneficial for PwMS to improve balance and mobility.[9]

Every day, people encounter obstacles such as stairs, curbs, and different surfaces that interrupt normal gait patterns. Thus, people must adjust spatial and temporal components of their walking in order to successfully navigate these obstacles. If either of these components is not

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corrected for, or if a person has a pathologically derived gait asymmetry such as in PwMS,[10] potentially injurious or even lethal falls may ensue.

A requisite for effective physical rehabilitation is an individual's ability to learn and adapt. Sensorimotor adaptation relies on externally imposed perturbations from the environment that induce a trial-and-error method of adjusting movements to new demands.[11,12] One potential therapeutic intervention based on sensorimotor adaptation is a split-belt treadmill paradigm which promotes one leg to move faster than the other leg. This process creates aftereffects that alter gait symmetry via feedforward storage of a new walking pattern.[13] The importance of focusing on locomotor adaptation as a potential rehabilitation strategy is accentuated by the need for non-pharmacological and individualized solutions for functional motor recovery in the many populations who experience gait dysfunction.[14]

Previous studies have demonstrated that people who have suffered a stroke and people with Parkinson's disease, who experience similar sensorimotor difficulties and gait asymmetries as PwMS, are able to adapt their gait to perturbations on a split-belt treadmill.[15–18] Reisman et al. showed that repeated split-belt treadmill walking improved post-stroke step length asymmetry,[19] while Hulzinga et al. found that split-belt treadmill walking moderately improved locomotor adaptability in people with Parkinson's disease.[20] Additional studies on PwMS showed that despite diminished motor function, PwMS still have preserved motor learning abilities.[21] These alterations in gait pattern, though not permanent, are indicative of neuroplastic adaptive capabilities and provide encouraging results for potential treatment strategies. In this study, the aim was to determine if spatial and temporal gait parameters are adaptable in PwMS during locomotor adaptation on a split-belt treadmill and if this can lead to a decrease in gait asymmetry. I maintained a two-part hypothesis. First, it is possible to adapt and improve spatiotemporal gait symmetry in

PwMS using a split-belt treadmill paradigm. Second, participants with poorer baseline symmetry, compared to averages reported in the literature for PwMS,[22] would experience greater symmetry improvements following split-belt treadmill adaptation.

Materials and Methods

Participants

A convenience sample of participants across northern Colorado was recruited. Inclusion criteria included people with relapsing-remitting MS from ages 18 to 86 that were fully ambulatory without an assistive device and could walk three-tenths of a mile without stopping to rest. This was to ensure participants could perform the split-belt treadmill adaptation paradigm safely and to avoid the effects of fatigue. Exclusion criteria included musculoskeletal injury within the previous six months, a history of brain injury, or any history of balance impairments unrelated to MS. The exclusion criteria were designed to target MS related balance and gait impairments and avoid other confounding factors. This study was approved by the Colorado State University Biomedical Institutional Review Board (protocol code 1664).

Following screening and informed consent, demographics and patient reported disease characteristics were collected using REDCap survey software (v. 13.1.32. Surveys collected include 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).

Split-Belt Treadmill Adaptation Paradigm

The participants completed five different walking trials (Figure 2.1). The first two trials were the overground baseline period. This consisted of two separate two-minute walk tests at their preferred walking speed and at a faster walking speed they felt they could maintain for 15 minutes.

The preferred and fast walking speeds determined tied-belt and split-belt treadmill speeds. Participants completed a two-minute walk on the treadmill in the tied-belt configuration set to their preferred walk speed. Immediately following, the adaptation period began. The belts were put into split configuration with the fast belt moving at participant's baseline fast walk speed, while the slow belt was set to half of the fast walk speed. After 10 minutes, the treadmill was set back to the tied-belt configuration for a one-minute post-adaptation trial at the preferred speed.

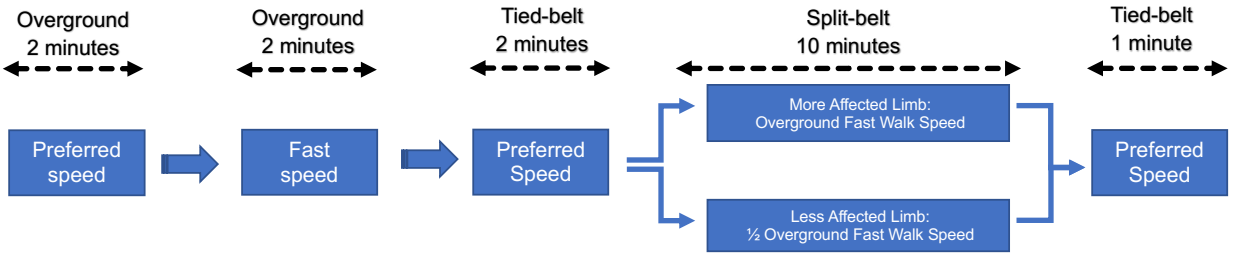

Figure 2.1. Participants completed five independent walking trials including baseline overground walking, baseline tied-belt treadmill walking, split-belt treadmill walking, and post-adaptation tied-belt walking.

Gait Analysis

Gait cycle parameters were measured during both overground and treadmill walking trials. The participants were outfitted with six APDM Opal inertial sensors (APDM Wearable Technologies, a Clario Company, Portland, OR, USA) along with 16 retroreflective markers for collection of three-dimensional motion capture data at 100 Hz. During overground walking, participants walked back and forth down a 30 m hallway, turning at each end, while APDM Mobility Lab collected inertial sensor gait cycle parameters. While treadmill walking, participants walked on a custom-built split-belt treadmill instrumented with Bertec force platforms (Model 4060-10, Bertec Corp, Columbus, OH). This treadmill consisted of two separate belts, each with its own motor that permitted the speed of each belt to be controlled independently and collected ground reaction forces at 1000 Hz. The speed at which a participant walked was individualized to

their overground preferred walk speed and fast walk speed. During split-belt treadmill adaptation, the fast belt speed was equal to the participant's overground fast walk speed while the slow belt speed was half of the participants fast walk speed (2:1 ratio). Prior research has determined that the fast belt should be under the more affected limb to improve gait symmetry.[23] The more affected limb was determined by participant self-reporting and investigator observation while overground walking.

Data Processing

Three-dimensional trajectory and force data were processed using Vicon Nexus software (v. 2.14, Vicon Motion Systems, Oxford, UK). Trajectory positions were filtered using a Woltring filter. Following, gait cycle events were identified from ground reaction forces using custom MATLAB software (v. 9.13.0, MathWorks Inc. Natick, MA, USA). Joint kinematics were calculated using the Vicon Plug-In Gait modeling pipeline. From a combination of trajectory and force data, gait cycle parameters and asymmetry metrics were calculated using custom MATLAB software. All gait cycle parameter means were calculated using only the second half of gait cycles from each trial to ensure gait stabilization.

For this study, the primary outcome variable to represent spatial symmetry was step length asymmetry (SLA). Step length was calculated by taking the anterior-posterior distance between heel markers at leading limb heel strike. A body centered model of heel location was used to mitigate the confounding effects of participant translation on the treadmill.[24] SLA was calculated by subtracting the step length of the less affected limb from the step length of the more affected limb for each consecutive step. Another spatial measure used was limb excursion asymmetry (LEA). This is a modified measure of stride length. When walking on a treadmill, participants were not translating while walking; rather, they were staying in place. Due to this, the conventional

understanding of stride length (distance from heel strike to following ipsilateral heel strike) resulted in a net zero stride length. A clearer term, limb excursion, quantifies the anterior-posterior distance traveled by the limb from toe-off to ipsilateral heel strike.[25] LEA was calculated by subtracting the limb excursion of the less affected limb from the limb excursion of the more affected limb for each gait cycle.

The primary outcome variable to represent temporal symmetry was phase coordination index (PCI). PCI is a measure of stepping accuracy and consistency in relation to anti-phased stepping during walking.[26] In perfectly timed gait, each step time is exactly half of the gait cycle duration. The PCI calculation is the summation of two measures representing the relative timing of contralateral heel strikes which determines phase, represented as phi (Φ). Phi was calculated through the normalization of step time with respect to stride time of the contralateral limb (i.e., Φ =180° for each step is ideal interlimb coordination). Once Φ was determined, the absolute error (ABS) of Φ from 180° and the covariation (CoV) of Φ were summed to give PCI, with a lower value equating to better phase coordination. These calculations provided a quantification of both absolute accuracy and relative consistency of stepping. This measure generated an index that can compare walking quality among participants, monitoring potential changes pre- and post-adaptation.

$$\varphi ABS = \frac{mean(|\varphi - 180°|)}{180°} \times 100 \quad \varphi CoV = \frac{stdev(\varphi)}{mean(\varphi)} \times 100 \quad PCI(\%) = \varphi ABS + \varphi CoV \quad (1)$$

Statistical Methods

Following baseline overground walking, participants were grouped as either responders or nonresponders. It was hypothesized that participants with a low baseline PCI or SLA (better symmetry) would experience minimal symmetry improvements following split-belt treadmill adaptation. In this analysis, participants were grouped as responders or nonresponders based on

their baseline PCI value as above or below the total sample median of 5.19%, and based on their baseline SLA value as above or below the total sample median of 25.48 mm. It was predicted that participants with a baseline PCI above 5.19% or a baseline SLA above 25.48 mm would have a greater response to split-belt treadmill adaptation. For analysis of overall adaptability in this sample, see Appendix A and Table A1.

Statistical processing and figure creation were completed using R Statistical Software (v. 4.2.1; R Core Team, Vienna, Austria), with analysis of variance (ANOVA) calculations using the rstatix package.[27] A 2 × 2 repeated measures ANOVA was used to assess differences in PCI and SLA, with group as a between-subjects factor and timepoint (baseline versus post-adaptation) as a within-subject factor. The Bonferroni adjustment was used to account for multiple comparisons when applicable. Residuals versus fitted plots along with quantile-quantile plots were used to confirm normality, and Mauchly's test was used to confirm sphericity. A significant two-way interaction was found between group and timepoint, therefore simple main effect and pairwise post-hoc analyses were conducted. The reported *p*-values were from pairwise comparisons. Effect sizes were calculated using Cohen's *d*.[28] After the creation of a general linear model, Pearson's product-moment correlation coefficients were calculated between changes in PCI and SLA.

Results

Participants

A total of 35 PwMS completed this study with a mean age of 51.66 (12.02) and a mean of 13.85 (0.73) years since diagnosis (Table 2.1). This sample was quite active compared to normative PwMS,[29] exercising 289.4 (266.8) minutes per week, which is typical for this geographical location. A total of 85% of participants reported symptoms of neuropathy.

Additionally, this cohort scored relatively low on MS disability scales,[30] with a mean EDSS of 3.57 (1.03) and mean MSWS-12 of 21.89 (12.08).

Table 2.1 Participant Characteristics. Reported mean and standard deviation (SD) of selected demographics, symptoms, and test scores. Overall, this cohort of PwMS was quite active and had mild symptoms compared other cohorts with similar years since diagnosis.

Characteristic	Mean	SD
N	35	
Age (years)	51.66	12.02
Sex	61% Female	
BMI (kg/m²)	25.37	4.19
Activity (min per week)	289.4	266.8
Years since diagnosis	13.85	10.73
Falls in last 6 months	0.65	1.02
Reported neuropathy	85%	
EDSS	3.57	1.03
MFIS	31.39	14.98
MSWS-12	21.89	12.08
BDI	7.63	7.06
MOCA	27.25	2.30

Spatial Symmetry

For this cohort of PwMS, there were 18 participants in the predicted responders group and 17 participants in the predicted nonresponders group, which were determined by their baseline spatial symmetry as above (responders) or below (nonresponders) an SLA of 25.48 mm. Those in the responders group experienced significant improvements in spatial symmetry ($p = 0.050$) following one session of split-belt treadmill adaptation, while the nonresponders experienced worsened symmetry ($p < 0.001$). This is represented by change in SLA from baseline to post-

adaptation, with a negative value indicating an improvement in spatial symmetry. The mean change in the responders group was –21.99 mm (SE = 12.41 mm) while the change in the nonresponders group was 38.43 mm (SE = 7.70 mm). There was also a significant difference in SLA change from baseline to post-adaptation between responders and nonresponders *(*$p < 0.001$) with an effect size of 1.40 as shown in Figure 2.2A. Along with SLA, LEA also demonstrated robust improvements in the responders group *(*$p < 0.001$) with a mean change of –21.29 mm (SE = 5.06 mm) compared to a mean change of 4.75 mm (SE = 2.08 mm) in the nonresponders group. A stride-by-stride analysis of SLA for a single participant (Figure 2.2B) showed baseline spatial asymmetry between limbs, followed by improved spatial symmetry during the post-adaptation aftereffects.

It was hypothesized that the mechanism of spatial adaptation would be increased step length of the affected limb since the affected limb was forced to take steps on a belt moving two times as fast as the less affected limb. However, there were no observed group changes from baseline to post split-belt adaptation for step length of the affected limb when grouped together or separated into responders and nonresponders *(*$p = 0.74$, $d = –0.11$) as shown in Figure 2.3. This demonstrates that participants used different strategies to successfully reduce spatial asymmetry.

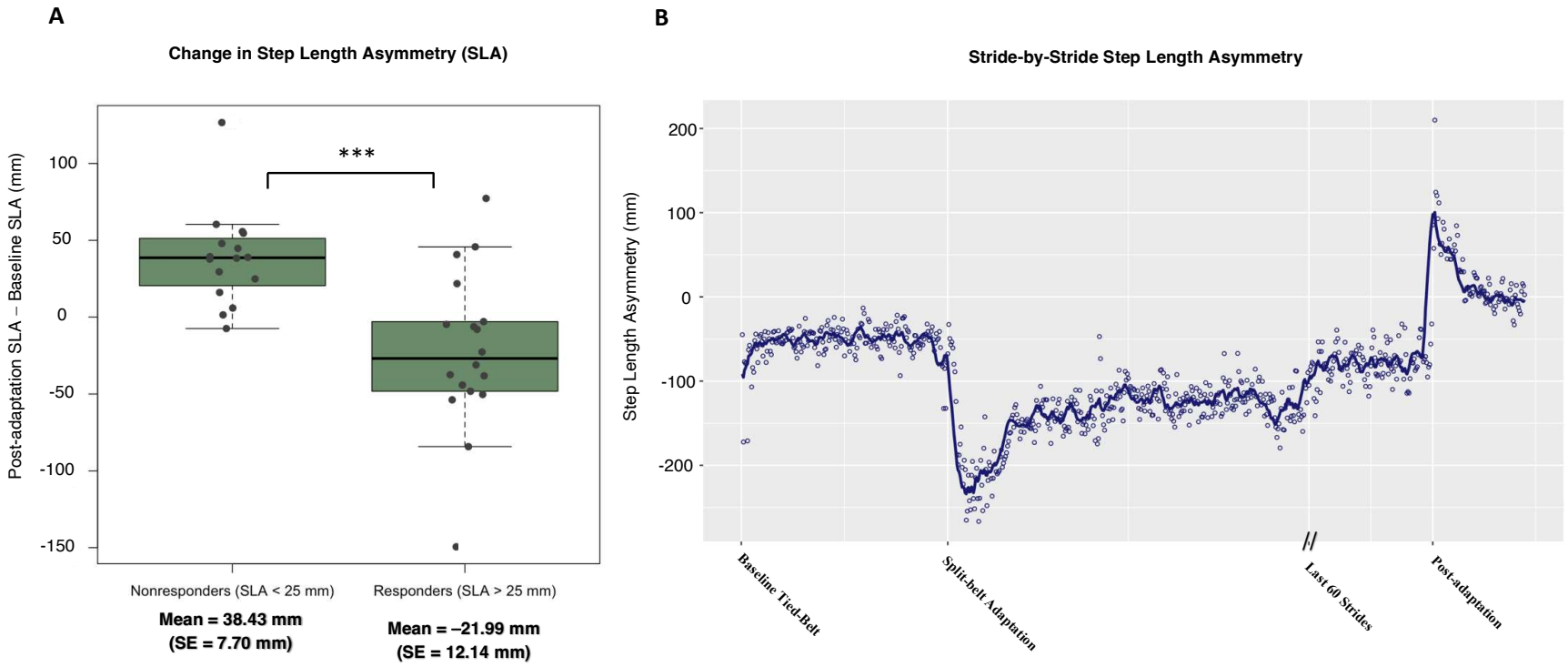

Figure 2.2. **(A)** Change in step length asymmetry (SLA) from baseline to post split-belt treadmill adaptation. It was hypothesized that participants with a low baseline SLA (better spatial symmetry) would experience minimal symmetry improvement following split-belt treadmill adaptation. Here, participants are grouped based on their baseline SLA as above or below the total sample median of 25.48 mm, with a significant difference between responders and nonresponders *(*$p < 0.001$, $d = 1.40$). **(B)** Stride-by-stride representation of SLA for a single participant. At baseline, the step length of the more affected limb is less than the step length of the less affected limb (spatial asymmetry). Following split-belt adaptation, the aftereffects demonstrate an improvement in spatial symmetry represented by a smaller SLA. • = individual participant change, *** = $p < 0.001$.

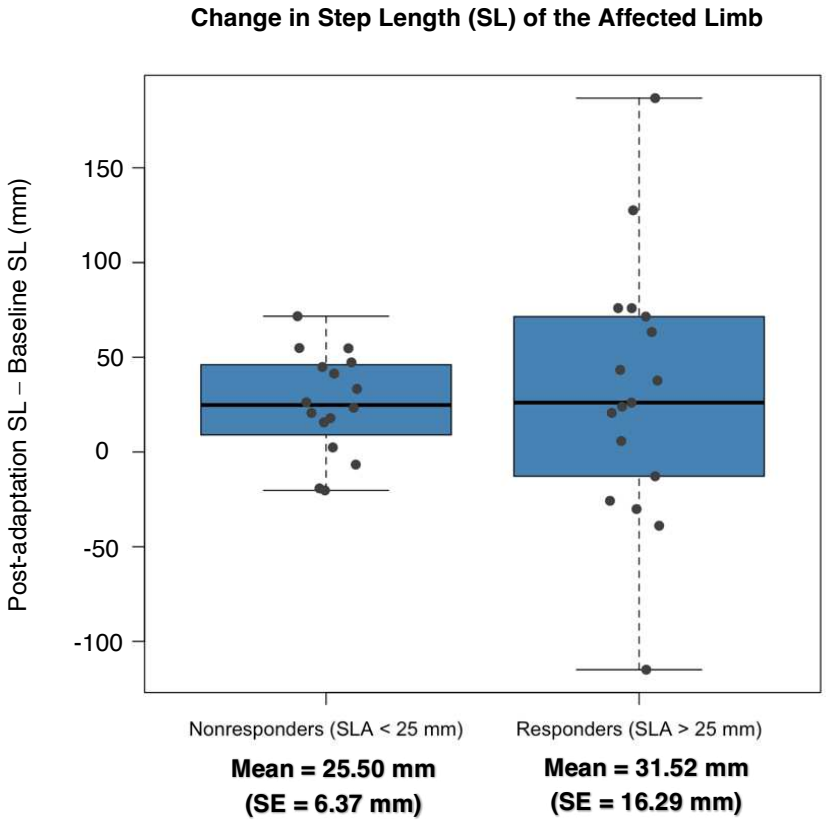

Figure 2.3. Change in step length of the affected limb from baseline to post split-belt treadmill adaptation. Participants are grouped using the same method from Figure 2.2. Contrary to the hypothesis, there was no change in step length of the affected limb following split-belt adaptation *(p* = 0.74, *d* = –0.11). • = individual participant change.

Temporal Symmetry

Temporal symmetry, represented by PCI, was also significantly improved following split-belt treadmill adaptation for the responders group. There were 17 participants in the predicted responders group and 17 participants in the predicted nonresponders group, with one participant excluded due to equipment error. A participant was assigned to this responders group if their baseline PCI was above the total sample median PCI of 5.19% (worse temporal symmetry). It is important to note that the responders group for PCI is not the same cohort as the responders group for SLA. Those in the responders group had significant temporal symmetry improvements during the post-adaptation trial with a mean change of –1.59% (SE = 0.51%, p = 0.007), while those in

the nonresponders group had a slight worsening of symmetry (mean = 0.71%, SE = 0.31%, p = 0.03). Similar to spatial symmetry outcomes, there was also a significant difference in PCI change from baseline to post-adaptation between the responders and nonresponders groups *(*$p < 0.001$), with an effect size of 1.33 as shown in Figure 2.4A. A stride-by-stride analysis of the Φ component of PCI for a single participant (Figure 2.4B) shows baseline temporal asymmetry between limbs with a high amount of variability, followed by improved temporal consistency during the post-adaptation trial aftereffects.

Correlation of Spatial Change and Temporal Change

In accordance with the second hypothesis, it was found that baseline symmetry values were predictors for symmetry improvement following adaptation, for both change in SLA *(*$r = –0.65$, $p < 0.001$) and for change in PCI ($r = –0.54$, $p = 0.0012$). However, individual participant spatial and temporal symmetry improvements were not correlated *(*$r = –0.12$, $p = 0.49$) as shown in Figure 2.5. This suggests that temporal gait parameters and spatial gait parameters may adapt independently.

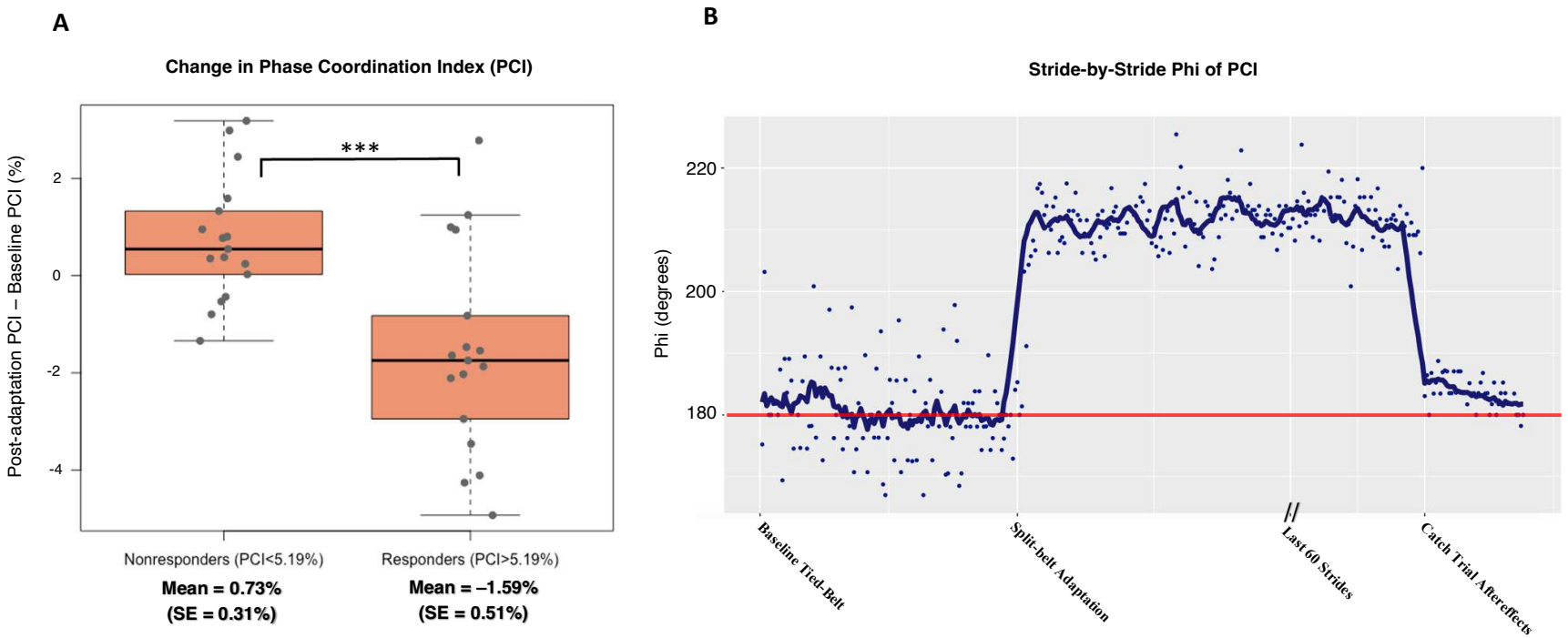

Figure 2.4. (A) Change in phase coordination index (PCI) from baseline to post split-belt treadmill adaptation. It was hypothesized that participants with a low baseline PCI (better temporal symmetry) would experience minimal symmetry improvement following split-belt treadmill adaptation. Here, participants are grouped based on their baseline PCI value as above or below the total sample median of 5.19% with a significant difference between responders and nonresponders *(p* < 0.001, d = 1.33). **(B)** Stride-by-stride representation of the phi component of PCI for a single participant with 180°, or the red line, depicting perfect temporal symmetry between limbs. At baseline, phi has large variability around 180°. During split-belt adaptation, phi is increased, due to forced temporal misalignment. Following, the aftereffects demonstrate less variation in phi, which indicated more ideal temporal coordination. • = individual participant change, *** = *p* < 0.001.

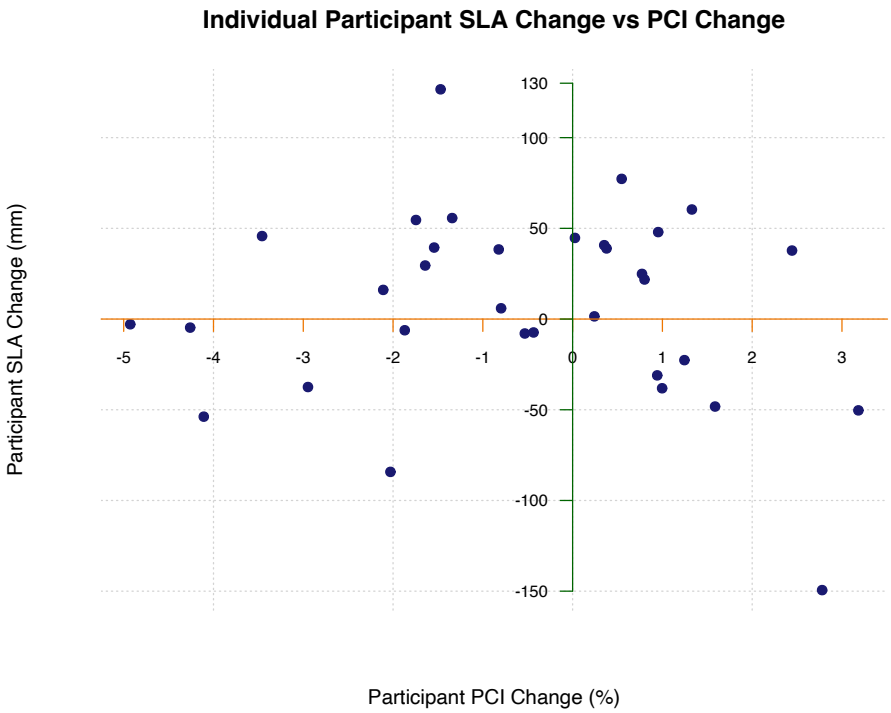

Figure 2.5. Change in step length asymmetry (SLA) plotted against change in phase coordination index (PCI). Pearson's correlation coefficient showed no correlation between SLA change and PCI change *(r* = –0.12, *p* = 0.49), suggesting that spatial and temporal gait parameters adapt independently of each other. • = individual participant change.

Discussion

Similar to previous work demonstrating the retention of postural motor adaptation in PwMS,[31] the current study indicates that PwMS also maintain a substantial capacity for locomotor adaptation. While there were responders and nonresponders in terms of symmetry improvements, nearly all participants adapted their gait, whether this improved or worsened overall symmetry (see Appendix A for additional data). This highlights the need for individuality in sensorimotor adaptation paradigms. Likely, each person with MS needs a different speed ratio that can be derived from their baseline symmetry, to optimize symmetry improvements on the treadmill. Additionally, baseline asymmetry predicted post-adaptation symmetry improvements both spatially and temporally. However, spatial and temporal changes were not correlated with each

other, suggesting that spatial and temporal gait parameters error-correct independently. These findings are in line with split-belt treadmill training outcomes in differing neurological populations. Specifically, the maintained ability for sensorimotor adaptation[11] and the lack of correlation between spatial and temporal changes.[32]

Baseline Asymmetry as a Predictor for Symmetry Improvements

It is imperative to consider the sensory system for PwMS as it has implications on motor performance. Sensory disturbances and abnormalities are observed in 80% of patients with MS,[33] reducing not only neuronal signaling, but also quality of life. The importance of sensory mechanisms for balance and gait has been largely studied over a variety of populations.[34] Specifically, proprioceptive feedback is relied upon heavily for locomotor coordination and spatiotemporal planning of precise movements.[35] Ongoing research is investigating populations where sensory mechanisms are impaired and subsequently motor performance is affected.[36] Further, a growing body of literature has investigated motor adaptability despite sensory dysfunction.[37]

Previous research utilizing a split-belt treadmill paradigm to evaluate adaptability showed improvements in both spatial and temporal asymmetries following stroke.[23] While long-term retention of sensorimotor adaptation is poorly understood, some studies found that repeated split-belt treadmill adaptation allowed for short-term adaptations and may even lead to longer-term improvements in interlimb symmetry post-stroke.[19] These findings demonstrate the capability of modulating gait cycle parameters despite cerebral damage. Therefore, this study expanded upon split-belt treadmill adaptation to PwMS who have white matter tract damage to further understand the mechanisms of sensorimotor adaptation, and the populations it may benefit.

These results highlight not only the adaptation potential for PwMS, but also that capacity for improvement is dependent upon baseline gait asymmetry. There was observed locomotor flexibility and reduced spatial and temporal asymmetries providing a more coordinated gait pattern following acute split-belt treadmill adaptation. Additionally, these findings highlight that individuals with greater asymmetry at baseline experienced the largest improvements in spatial and temporal gait symmetries following split-belt adaptation. Specifically, those who had worse than average spatial and temporal asymmetries at baseline (responders) saw significantly increased improvements compared to those who were more symmetrical at baseline (nonresponders). Furthermore, this evidence illuminates locomotor adaptability in PwMS despite axonal damage and sensory deficits. Corresponding with previous findings,[15] these improvements in symmetry are promising as it points to the potential of gait asymmetry being remediated with individualized adaptation paradigms.

Spatial and Temporal Independence

At baseline, participants had differing levels of gait asymmetry, with some having more severe spatial deficits and some having more severe temporal deficits. The majority of PwMS experience walking difficulties and develop compensatory strategies to continue walking, even if it is inefficient. Some compensation methods include taking smaller steps with their affected limb or increasing swing time with their affected limb in order to spend more time in stance with their less affected limb. These different compensation strategies create different types of asymmetries. Along with this, some participants greatly improve their spatial symmetry following split-belt treadmill adaptation, while experiencing negligible changes in temporal symmetry and vice versa. Participants' spatial improvements and temporal improvements were not correlated *(r = –0.12, p = 0.49)*. This indicates that split-belt treadmill adaptation affects spatial and temporal gait cycle

parameters independently for each participant, likely having different neural mechanisms for modulation. This finding is congruent with other studies[32] and is an important consideration when designing rehabilitation paradigms for PwMS. It is likely that either spatial or temporal coordination is more affected and should be prioritized during rehabilitation.

Neural Mechanisms of Adaptation in PwMS

One surprising finding was that the step length of the affected limb following split-belt treadmill adaptation was unchanged at the group level, while SLA had robust improvements in the responders group. Because the asymmetry measurement was affected step length minus less affected step length, this means that PwMS have different spatial adaptation strategies to successfully account for the belts going different speeds, rather than simply increasing the step length of the leg under the fast belt. This indicates that there is likely more nuanced supraspinal control involved during error-correction of stepping that contributes to feedforward adaptation for PwMS.

Split-belt treadmill adaptation relies on both reactive feedback control and predictive feedforward modulation. It has been demonstrated through lesion studies[38] that immediate gait parameter adjustments use feedback mechanisms that are not dependent on supraspinal control, with modulation happening at the spinal cord level, likely through central pattern generators. To maintain the effects of split-belt treadmill adaptation and experience feedforward aftereffects, cerebellar influence is essential.[39] In the cerebellum, the midline vermis and fastigial nuclei have been suggested to modulate posture and locomotion.[40] Along with this, Purkinje cell firing rates have been shown to increase robustly during split-belt treadmill walking in decerebrate cats which demonstrates the involvement of the cerebellum during locomotor adaptation.[41] For successful phasic bilateral coordination, the cerebrum, specifically the sensorimotor integration areas,

coordinate various afferent information to optimize motor output for successful motor planning. The interaction between the cerebellum and cerebrum is also necessary for adaptation, with studies finding that cerebellar brain inhibition is proportional to motor learning outcomes.[42] This observation that spatial and temporal adaptations are independent suggests that there may be different neural mechanisms for spatial and temporal modulation, which is in line with other findings.[32] Choi and colleagues found that in individuals with hemispherectomy, their spatial feedforward adaptability was unaffected while their temporal feedforward adaptability was significantly impaired, which implies that temporal coordination may rely more heavily on the cerebrum while spatial coordination may rely more heavily on the cerebellum.[43]

Communication of afferent information, primarily proprioception, during gait via the dorsal spinocerebellar tract and the posterior column-medial lemniscus are essential for accurate motor output, and therefore necessary for successfully updating forward models. It has been recently established that while PwMS have motor impairments, it is likely the mechanism of these motor impairments originates from inaccurate sensory signaling due to damaged myelin in the spinal cord and brain regions that coordinate bilateral movements.[22] The majority of split-belt treadmill adaptation rehabilitation research focuses on stroke or Parkinson's disease, where grey matter or cerebral injury likely causes impaired motor output. While the cerebellum is the primary region for sensorimotor adaptation,[44] due to the pathology of MS there may be a unique combination of cerebral and cerebellar contributions to motor learning to compensate for poor spinocerebellar signaling,[34] such as enhanced cerebral influence.

Future Directions

With the unique pathology of sensory impairment in MS, further investigation of neural mechanisms of feedforward adaptation in this population is warranted. Future phases of this study

include coupling split-belt treadmill adaptation with functional near-infrared spectroscopy (fNIRS) to measure cortical activation during sensorimotor adaptation compared to neurotypical peers.[45] While split-belt treadmill adaptation has shown acute decreases in gait asymmetry, few studies have successfully implemented this paradigm to improve gait coordination in the long term. Some studies have combined multiple sessions of split-belt treadmill training with cerebellar stimulation with varying success.[46,47] For PwMS, the impairment that leads to gait asymmetry likely lies in sensorimotor white matter transduction,[36] thus amplifying sensory signaling may be beneficial to further enhance feedforward storage of new walking patterns. One mechanism that has successfully improved sensory signaling is transcutaneous electrical nerve stimulation (TENS) in the periphery, which has been shown to increase excitability of peripheral afferent neurons and consequently improve motor coordination.[48] Pairing lower limb TENS with split-belt treadmill adaptation coupled with fNIRS allows for assessment of the influence of amplified sensory signaling on cortical activation and allows for investigation of amplified sensory signaling to improve feedforward sensorimotor adaptation. This would further inform clinicians on the utility of adaptation paradigms, such as split-belt treadmill training, coupled with enhanced afferent signaling as long-term rehabilitation strategies for PwMS.

Limitations

PwMS are known to have cognitive deficits, and split-belt treadmill adaptation has been shown to increase cognitive load.[49] Another predictor of responders and nonresponders may have been visuospatial cognition, as shown in recent evidence.[50] The cognitive test in this study (MOCA) was not sensitive enough to detect this, and an assessment such as the Trail Making Test could add an important distinction between participants, potentially being a predictor for the magnitude of aftereffects following split-belt treadmill adaptation. Another limitation was that this

sample of PwMS was relatively healthy, with the majority of participants having a lower disability level than typical when considering years since diagnosis. If this sample was more representative of the MS population, there may have been a greater number of responders to split-belt treadmill adaptation. However, an inherent limitation of split-belt treadmill adaptation is the required capability to complete the demanding walking task, which unfortunately excludes many individuals with a higher disability level and limits generalizability. Another beneficial addition to this study would be a longer tied-belt walking session following split-belt treadmill adaptation. This would allow for the recording of the rate of deadaptation following the aftereffects and be informative of group differences during deadaptation.

Conclusion

This is one of the first studies to investigate sensorimotor adaptability of locomotion in PwMS, a population that has pronounced sensory impairments.[36] These data demonstrated that PwMS maintain the ability to adapt their gait cycle parameters to improve symmetry, with those experiencing the greatest asymmetry at baseline having the greatest magnitude of symmetry improvements following split-belt treadmill adaptation. Additionally, spatial and temporal gait cycle parameters adapted independently, suggesting that there are separate neural mechanisms for feedforward adjustment of these parameters. Further investigation into enhancing neuroplastic change and understanding the neural mechanisms of adaptation in PwMS will be informative to rehabilitation strategies to further improve locomotion in PwMS and the many other populations who experience gait dysfunction.

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CHAPTER 3 – PROPULSIVE FORCE MODULATION DRIVES LOCOMOTOR ADAPTATION IN PEOPLE WITH MULTIPLE SCLEROSIS[2]

Introduction

Multiple sclerosis (MS) is the most common neurodegenerative disease affecting young adults, with the average onset occurring at 31 years of age.[1] As a result, most people with MS (PwMS) contend with the effects of the disease for more than half of their lifespan. MS is characterized by the degradation of the myelin sheath, a protective layer consisting of lipids and proteins that enhances the velocity of electrical impulse propagation along a nerve. Consequently, this degradation creates diminished neural communication throughout the central nervous system. Along with common symptoms of fatigue, neuropathy, and instability,[2] 93.7% of PwMS report having significant mobility impairments,[3] and that gait dysfunction is the leading contributor to decreased quality of life.[4] Specifically, PwMS typically have one side of the body that is more affected (MA) and one side that is less affected (LA) in strength and function. This leads to an asymmetrical gait pattern, one of the greatest risk factors for falls.[5]

Successful locomotion in diverse environments requires dynamic locomotor adjustments to ensure stability, efficiency, and safety. Perturbations during walking, such as variations in surfaces, and encounters with stairs, curbs, or entrances, demand real-time adjustments to navigate these challenges. Sensorimotor adaptation is a fundamental aspect of these locomotor adjustments, particularly when individuals encounter novel or unexpected conditions.[6,7] A common laboratory method for evaluating locomotor adaptability involves the use of a split-belt treadmill, where the

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speed of each leg is controlled independently on two separate belts.[8] When exposed to a split-belt treadmill, individuals experience a discrepancy between the movement of their two legs, creating an externally imposed perturbation. To walk successfully on the split-belt treadmill, the nervous system engages in an error correction process, continuously adjusting the timing and coordination of each limb.[9] This adaptive mechanism enables individuals to adjust their stepping and maintain balance despite the treadmill's asymmetric speed. The adaptation process results in aftereffects, where individuals exhibit changes in their locomotor pattern even after returning to normal walking conditions.[10] These aftereffects suggest that the nervous system stores the adapted walking pattern using feedforward predictive modulation.[11]

Understanding locomotor adaptability is essential not only for studying typical locomotion but also for developing effective rehabilitation strategies for dysfunctional gait. Previous research has demonstrated that individuals with various neurodegenerative conditions, such as stroke,[12,13] Parkinson's disease,[14,15] and more recently, PwMS,[16] maintain the ability to adapt while walking on a split-belt treadmill. While many studies have explored broader assessments of locomotor adaptability, such as step length asymmetry,[17] few have assessed the specific biomechanical changes that contribute to these broader adaptations. The aim of this study was to elucidate the specific kinematic and ground reaction force (GRF) changes that occur in PwMS during adaptation on a split-belt treadmill. The hypothesis was that PwMS would exhibit significant changes in propulsive force generation and ankle kinematics during this adaptive process due to PwMS commonly experiencing reduced propulsion and ankle joint moments and powers during typical walking.[18,19] Investigating these specific biomechanical changes provides a more nuanced understanding of locomotor adaptability in PwMS and can contribute valuable insights to the development of targeted rehabilitation interventions for this population.

Materials and Methods

Participants

A convenience sample of participants in and around northern Colorado was recruited. Inclusion criteria required participants to have relapsing-remitting MS, be between the ages of 18 and 86, be fully ambulatory without an assistive device, and be capable of walking three-tenths of a mile (~500 m) without stopping to rest. The criteria related to MS severity required individuals to score between 2.0 and 5.5 on the Expanded Disabilities Status Scale (EDSS). This ensured participants had at least minimal disability but could perform the split-belt treadmill adaptation paradigm safely and with minimal fatigue. Exclusion criteria precluded participants from having a history of balance impairments or brain injury unrelated to MS, or any musculoskeletal injury or related surgical procedure which impacted gait in the previous six months. This study was approved by the Colorado State University Biomedical Institutional Review Board (protocol code 1664).

Following screening and informed consent, demographics and participant-reported disease characteristics were collected using REDCap survey software (v. 13.1.32). Surveys collected included the 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).

Split-Belt Treadmill Adaptation Paradigm

Participants completed five different walking trials (Figure 3.1). The first two overground trials consisted of two separate two-minute walk tests: one at the participant's preferred walking speed, and the other at a faster walking speed they felt they could maintain for 15 minutes. Participants then completed a two-minute walk on the treadmill in the tied-belt configuration to

set their preferred walking speed. Immediately following this, the adaptation period was initiated, and the treadmill was put into the split-belt configuration: the fast belt was set to the participants fast walk speed, while the slow belt was set to half the speed of the fast belt (2:1 ratio). The adaptation period concluded after 10 minutes, at which point the treadmill was set back to the tied-belt configuration at the participant's preferred walking speed for a one-minute post-adaptation trial.

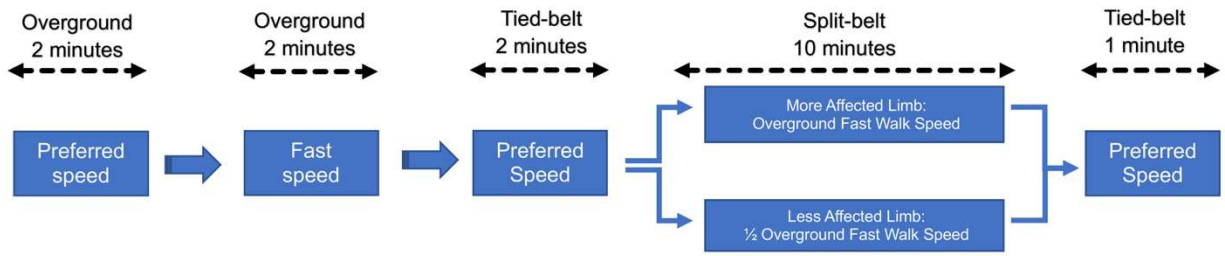

Figure 3.1. Participants completed five independent walking trials including baseline overground walking, fast overground walking, baseline tied-belt treadmill walking, split-belt treadmill walking, and post-adaptation tied-belt treadmill walking.

Gait Analysis

Gait cycle parameters were measured during all walking trials. Participants wore six APDM Opal inertial sensors (APDM Wearable Technologies, a Clairo Company, Portland, OR, USA) during the overground baseline trials to determine the walking speed for subsequent trials. During the treadmill trials, participants wore 16 retroreflective markers for the collection of three-dimensional motion capture data, sampled at 100 Hz. Participants walked on a custom-built split-belt treadmill instrumented with Bertec force platforms (Model 4060-10, Bertec Corp., Columbus, OH, USA), which collected three-dimensional ground reaction force (GRF), sampled at 1000 Hz. The treadmill consists of two separate belts, each with its own motor, allowing each belt to move at independent speeds. Prior research determined that the fast belt should be under the MA limb to

improve gait symmetry.[20] The MA limb was determined by participant self-reporting and investigator observation during the overground walking trials.

Data Processing

Three-dimensional trajectory and force data were processed using Vicon Nexus software (v. 2.14, Vicon Motion Systems, Oxford, UK). Trajectory positions were filtered using a fifth-order spline-interpolating Woltring filter[21] implemented in Vicon Nexus and utilizing a generalized cross-validation approach, and joint kinematics were calculated using the Vicon Plug-In Gait modeling pipeline. Gait cycle events were identified using a custom MATLAB script (v. 9.13.0, MathWorks Inc., Natick, MA, USA) implemented in Vicon Nexus with a resultant force threshold of 25 N. Kinematic data were divided into stance and swing phases and interpolated to 100 values per phase. Force data were filtered using a fourth-order zero-lag Butterworth filter with a low-pass cutoff frequency of 300 Hz. Force data were also interpolated to 100 values, but only for the stance phase.

Kinematic and GRF data were averaged within each limb for select gait cycles during the treadmill walking trials. A total of five timepoints were defined as follows: Baseline (last 15 gait cycles of 2-minute tied belt trial), Early Adapt (gait cycles 6-15 of split-belt adaptation trial), Late Adapt (last 15 gait cycles of split-belt adaptation trial), Early Post-Adapt (gait cycles 6-15 of post-adaptation trial), and Late Post-Adapt (last 15 gait cycles of post-adaptation Trial). The choice of gait cycles 6-15 was made to ensure the treadmill belts had reached the appropriate speed and steps were consistent.[22] The choice of the last 15 gait cycles was to capture the gait cycles in which participants were most practiced in the respective configuration (i.e. tied- and split-belt), and to normalize them for different walking speeds across participants.

Primary outcome variables included joint range of motion and joint angles for the hip, knee, and ankle, as well as braking, propulsion, and vertical GRF, normalized to a percentage of participant body weight (%BW), of the MA and LA limbs. Using the trapezoidal method of integration, impulse (%BW × %Stance) was calculated for the propulsive portion of the stance phase.[23] From these, asymmetry values were calculated as MA – LA at each timepoint, with a positive value indicating a larger value for the MA limb. For more information on step length asymmetry and other, more global measures of adaptation for this sample I refer the reader to a previous publication (Chapter 2 of this dissertation).[16]

Statistical Methods

Following processing, data were imported into R Statistical Software (v. 4.2.1; R Core Team, Vienna, Austria, 2022) to complete statistical analysis. A 2 × 5 repeated measures analysis of variance (RMANOVA) was calculated for each outcome of interest with limb (MA versus. LA) as a between-factor and timepoint as a within-factor. Residuals versus fitted plots along with quantile–quantile plots were used to confirm normality and equal variance while Mauchly's test was used to confirm sphericity. Following the observation of a significant main effect and an interaction between limb and timepoint, post hoc pairwise comparisons were conducted using false discovery rate (FDR) to correct for multiple comparisons. The reported *p*-values (*p*) are the results from each pairwise comparison with FDR corrections applied. Effect sizes (*d*) were calculated using Cohen's d and correlations were calculated using Pearson's product-moment correlation coefficient (*r*).

Results

Participant Characteristics

In total, 32 PwMS completed this study (69% female). The mean participant age was 50.4 (12.0) years with an average of 12.7 (8.6) years since diagnosis. A total of 84% of participants reported neuropathy. A mean MSWS-12 of 22 (12) and a mean EDSS of 3.7 (0.8) indicated that MS symptoms among this cohort were mild.[24] Further, participants were more active than normative PwMS,[25] exercising 329 minutes per week on average. Table 3.1 provides a comprehensive description of participant characteristics.

Table 3.1: Participant characteristics. Mean and SD or proportion of cohort are reported for select attributes. Collectively, this cohort of PwMS was highly active and reported mild symptoms.

Characteristic	Mean	SD
N	32	
Age (years)	50.4	12.0
Sex	69% Female	
BMI (kg/m²)	24.8	3.6
Activity (min per week)	329	231
Years since diagnosis	12.7	8.6
Falls in prior 6 months	0.3	0.8
Reported neuropathy	84%	
EDSS	3.7	0.8
MFIS	33	15
RAND 36: physical function	78	22
MSWS-12	22	12
BDI	8	7
MOCA	27	2

Peak Propulsion and Dorsiflexion Asymmetry Adaptation

Throughout the time course of the split-belt treadmill adaptation paradigm, participants experienced the largest changes in peak propulsion asymmetry and peak dorsiflexion asymmetry. For peak propulsion, the MA limb immediately produced more propulsion at Early Adapt compared to Baseline ($p < 0.001$, $d = 1.40$). Subsequently, PwMS progressively enhanced propulsion production from Early Adapt to Late Adapt ($p < 0.001$, $d = 0.56$). Following this, the LA limb produced more propulsive force than the MA limb ($p < 0.001$, $d = 0.79$), demonstrating a negative aftereffect which is indicative of successful adaptation (Figure 3.2).

Further results suggest that a reduction in dorsiflexion is a primary contributor to the heightened propulsion observed during adaptation. This inference is supported by a robust negative correlation between propulsion asymmetry adaptation and dorsiflexion asymmetry adaptation ($r = –0.86$, $p < 0.001$). For peak dorsiflexion, the MA limb had an immediate decrease at Early Adapt compared to Baseline ($p < 0.001$, $d = 0.97$). Subsequently, from Early Adapt to Late Adapt, the MA limb exhibited a progressive decline in dorsiflexion ($p = 0.0031$, $d = 0.38$). Following this, the MA limb produced more dorsiflexion than the LA limb during Early Post-Adapt ($p = 0.0051$, $d = 0.55$), demonstrating a negative aftereffect and confirming the occurrence of adaptation (Figure 3.2).

Peak Propulsion Adaptation

To assess force profile changes across the experimental paradigm, peak propulsive force, peak braking force, peak early vertical GRF, and peak late vertical GRF were collected and analyzed for each limb (Figure 3.3, Table B1). For the LA limb, peak propulsive force is reduced during Early Adapt compared to Baseline. Subsequently, peak propulsive forces increase in Late Adapt compared to Early Adapt. However, the shape of the data curve during the adaptation period

is markedly flat. Considering the flat curve in conjunction with the absence of aftereffects indicates that there is insufficient evidence to conclude that propulsive adaptation is occurring in the LA limb. Peak braking, peak early vertical GRF, and peak late vertical GRF in the LA limb all follow a similar pattern where there are no changes during the adaptation period, indicating that changes from baseline are reactive rather than adaptive.

For the MA limb, all four force profiles display a reduction in force during Early Adapt compared to Baseline (Propulsion: $p = 0.0011$, $d = 0.39$ | Braking: $p < 0.001$, $d = 1.16$ | Early Vertical GRF: $p < 0.001$, $d = 0.81$ | Late Vertical GRF: $p < 0.001$, $d = 1.22$) and a subsequent steady increase in force production during the adaptation period, resulting in increased force during Late Adapt compared to Early Adapt (Propulsion: $p < 0.001$, $d = 0.81$ | Braking: $p < 0.001$, $d = 1.51$ | Early Vertical GRF: $p = 0.0027$, $d = 0.45$ | Late Vertical GRF: $p < 0.001$, $d = 0.72$). Notably, during the post-adaptation period, peak propulsion presents a negative aftereffect where propulsion during Early Post-Adapt is reduced compared to propulsion during Baseline ($p < 0.001$, $d = 0.48$), and further, propulsion during Late Post-Adapt is increased relative to Early Post-Adapt and approaching Baseline. Together, the peak propulsion curve during the adaptation period and evidence of negative aftereffects highlight peak propulsion in the MA limb as a key contributor to locomotor adaptation. Peak braking, peak early vertical GRF, and peak late vertical GRF in the MA limb fail to exhibit the same aftereffects and thus, despite having promising adaptation curve profiles, they likely contribute to adaptation but are not the main driving characteristics underlying locomotor adaptation in PwMS.

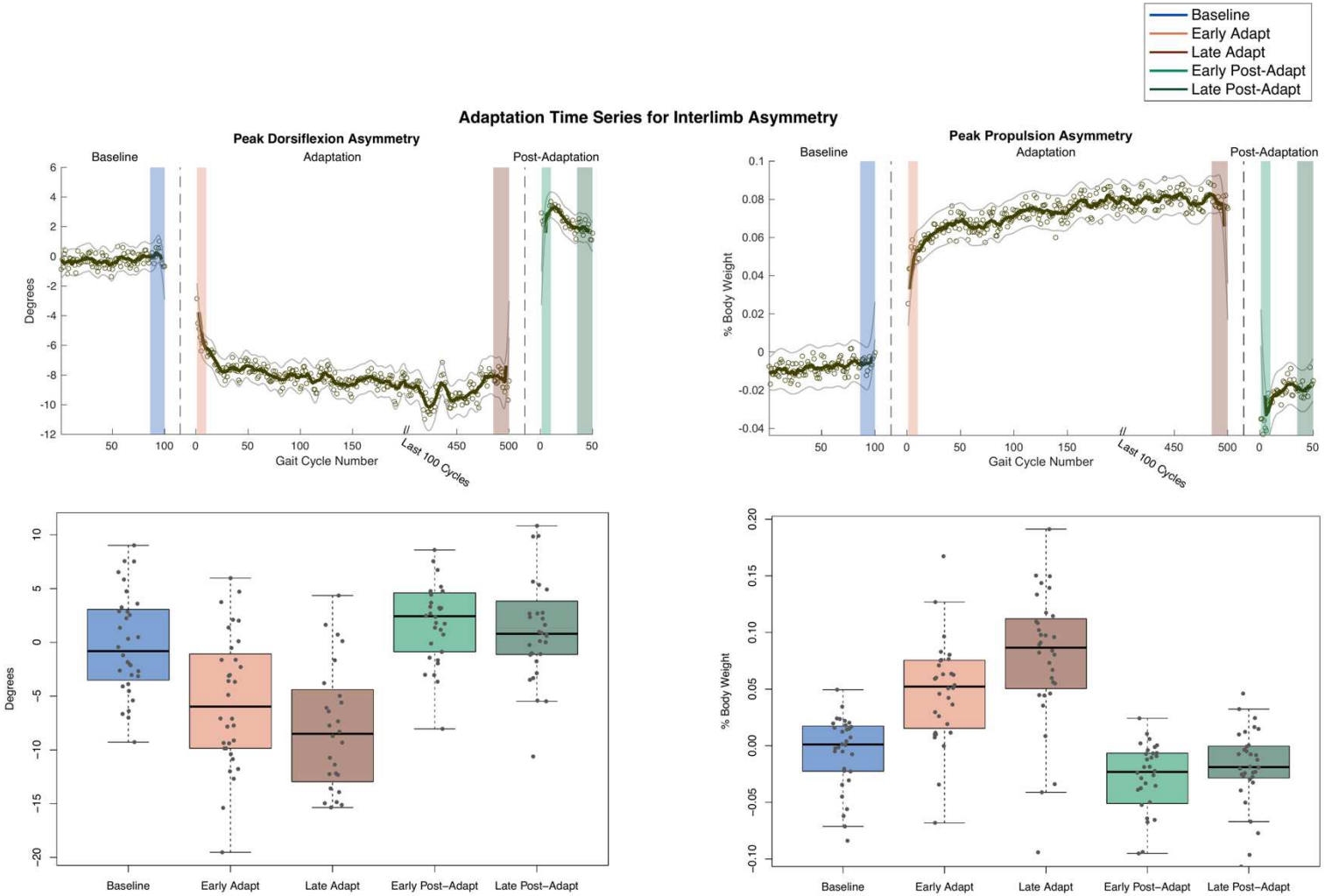

Figure 3.2. Peak propulsion and peak dorsiflexion asymmetry for each gait cycle throughout the split-belt treadmill adaptation time course averaged across all participants. Peak propulsion increased while peak dorsiflexion decreased during adaptation and generated aftereffects. Below the adaptation curves are boxplots containing each participant's data for the specified timepoint.

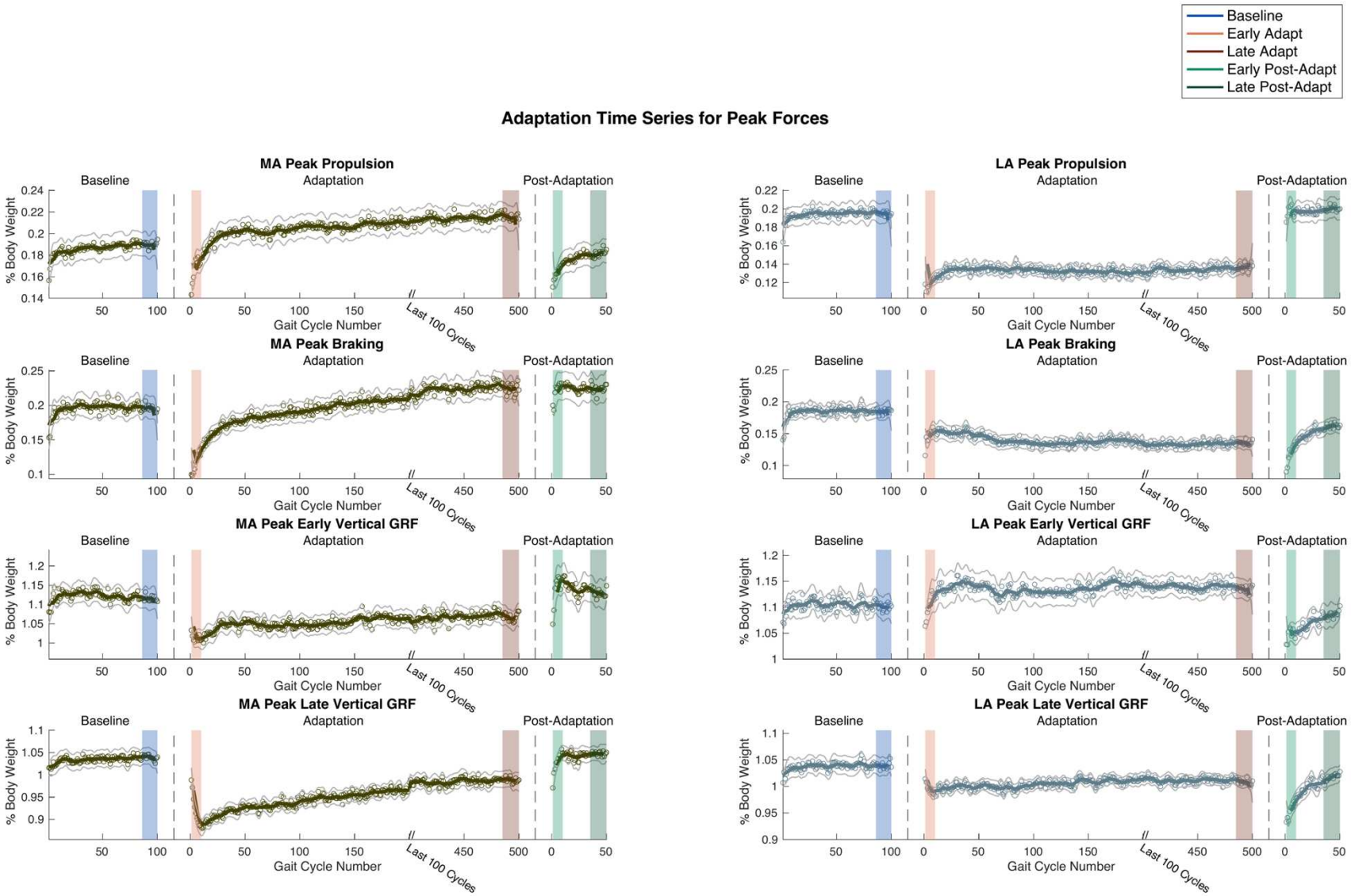

Figure 3.3. Peak propulsive, braking, early vertical, and late vertical ground reaction force (GRF), averaged across all participants, for the more affected (MA) and less affected (LA) limb throughout Baseline, Adaptation, and Post-Adaptation. Light grey lines indicate standard error for each point across all participants.

Peak Joint Angle Adaptation

Examining each limb individually reveals insights into peak joint angles across gait cycles, revealing distinct trends for the MA and LA limbs (Figure 3.4, Table B2). For the LA limb, nearly all joint motions exhibited a reduction in peak angles from Baseline to Early Adapt. Subsequently, during the adaptation period no further changes occurred, leading to the absence of aftereffects during Early Post-Adapt compared to Baseline. This finding indicates that the changes experienced in the LA limb are reactive rather than adaptive. LA peak dorsiflexion was the only LA joint angle that increased, but it followed a similar reactive feedback pattern to all other joints. In contrast, the MA limb exhibited notable adaptive changes, particularly in peak ankle plantarflexion. Peak plantarflexion demonstrated an immediate increase from Baseline to Early Adapt ($p = 0.038$, $d = 0.23$), followed by a slight decrease from Early Adapt to Late Adapt ($p = 0.013$, $d = 0.22$), while still maintaining heightened plantarflexion relative to Baseline. A negative aftereffect in peak plantarflexion further confirmed the occurrence of adaptation ($p = 0.0019$, $d = 0.35$). There was an immediate decrease in MA peak dorsiflexion from Baseline to Early Adapt ($p = 0.0012$, $d = 0.38$), followed by a continued reduction in dorsiflexion from Early Adapt to Late Adapt ($p = 0.078$, $d = 0.24$) that failed to reach significance, likely due to high individual variability at Late Adapt. Further, the negative aftereffect also failed to reach significance during Early Post-Adapt ($p = 0.068$, $d = 0.26$). Additionally, peak hip extension increased during adaptation ($p < 0.001$, $d = 0.44$), but there was no significant aftereffect during Early Post-Adapt. An examination of joint ROM across the split-belt treadmill time series revealed minimal changes in the MA limb and decreases in all LA joint ROM during Early Adapt and Late Adapt, without an aftereffect during Early Post-Adapt (Figure B1). This suggests that the reduction in joint ROM for the LA limb is reactive rather than adaptive, and likely attributable to a slower belt speed under the LA limb.

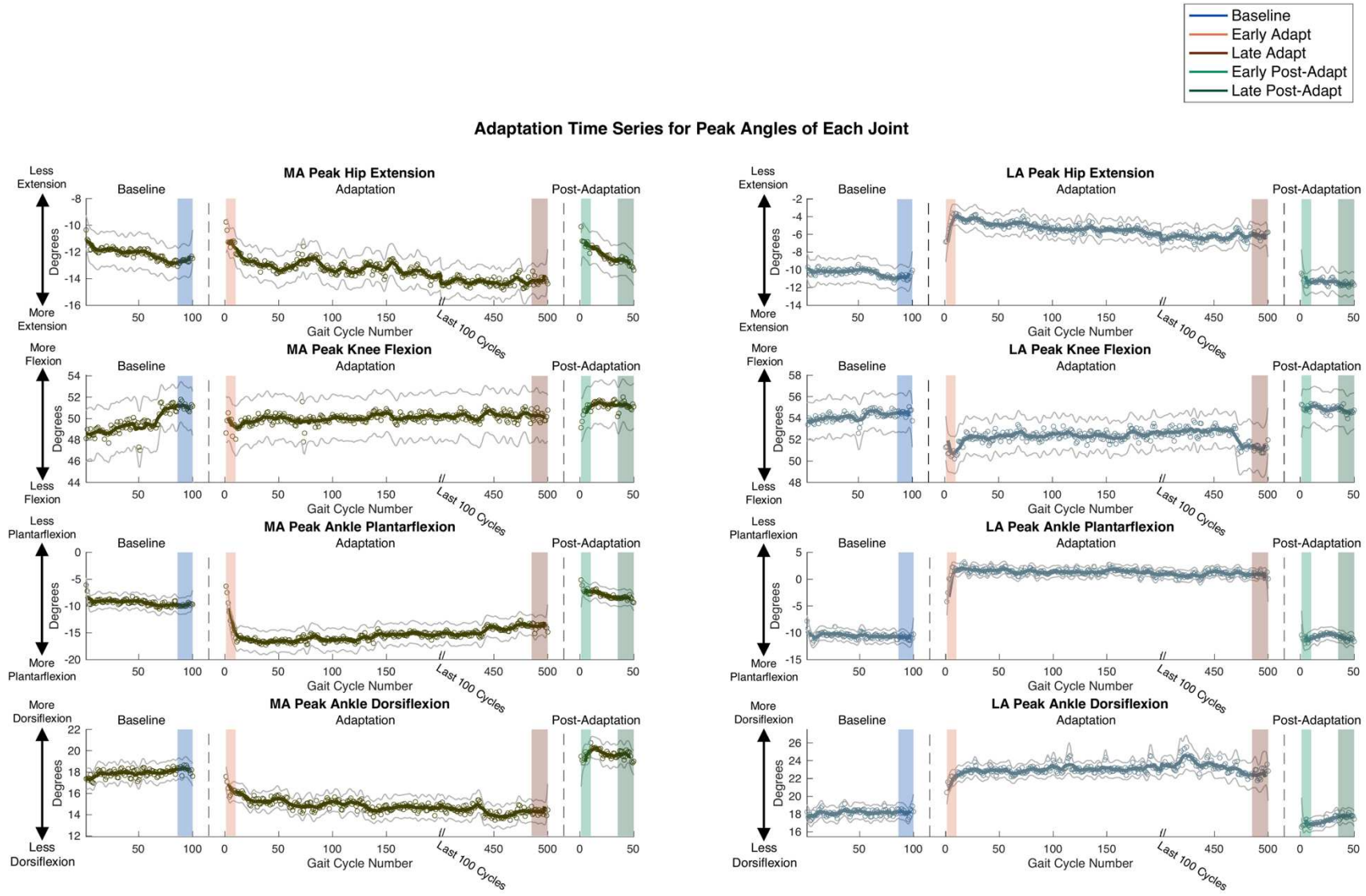

Figure 3.4. Peak joint angles, averaged across all participants, for the more affected (MA) and less affected (LA) hip, knee, and ankle throughout Baseline, Adaptation, and Post-Adaptation. Light grey lines indicate standard error for each point across all participants. MA peak plantarflexion experienced significant adaptative changes while all LA joints had significant reactive changes.

Propulsion Impulse and Ankle Joint Profile Adaptation

With propulsion in the MA limb identified as a key contributor to locomotor adaptation, propulsion impulse in the MA limb was analyzed as force (% BW) over time (% Stance) to obtain a more detailed assessment of propulsion adaptation over the split-belt treadmill paradigm. Despite peak propulsion being less at Early Adapt compared to Baseline in the MA limb, there is no significant change in propulsion impulse from Baseline to Early Adapt ($p = 0.54$, $d = .075$). There is subsequently more propulsion impulse at Late Adapt compared to Early Adapt, showing a steeper rate of force development at Late Adapt ($p < 0.001$, $d = 0.47$). Early Post-Adapt has reduced propulsion impulse compared to Baseline ($p < 0.001$, $d = 0.49$) and furthermore displays similar negative aftereffects to peak propulsion.

An examination of joint profiles throughout the split-belt adaptation paradigm revealed significant changes in peak dorsiflexion timing within the gait cycle. Specifically, peak dorsiflexion in the MA limb occurred 22.3% earlier in the gait cycle during Early Adapt ($p < 0.001$, $d = 1.64$) when compared to baseline and 17.1% earlier during Late Adapt ($p < 0.001$, $d = 1.36$) when compared to baseline, signifying an accelerated onset of plantarflexion (Figure 3.5). This temporal shift, along with related internal musculoskeletal changes, allowed for an extended period of plantarflexion during the gait cycle, facilitating enhanced propulsive force production. Additional joint profiles demonstrated that the LA limb had a decreased ROM during adaptation (Figure B2).

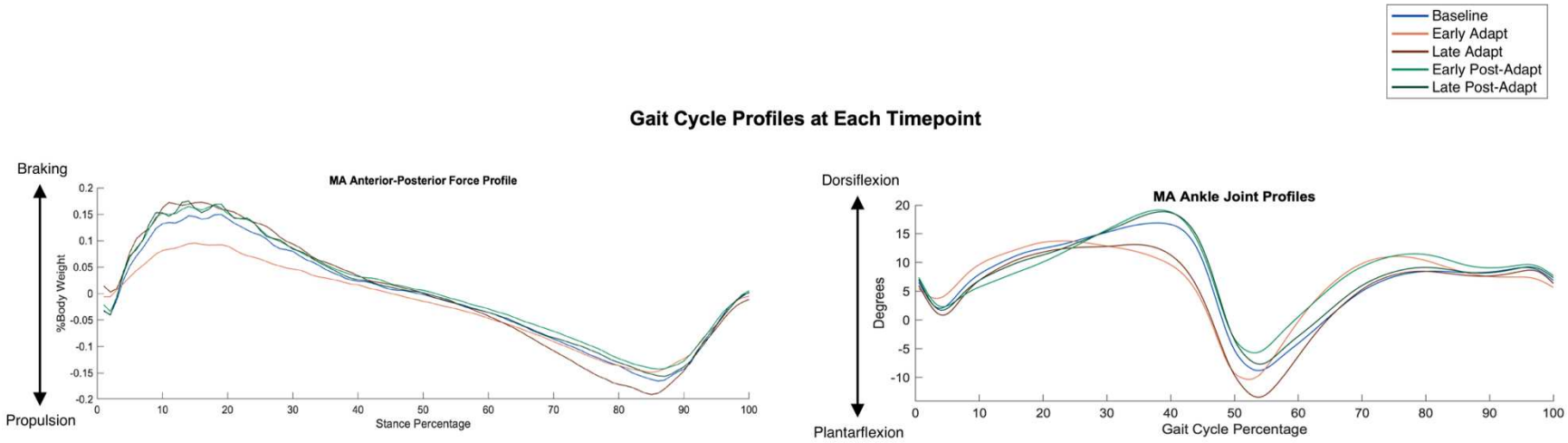

Figure 3.5. More affected **(**MA) anteroposterior (AP) and MA ankle joint profiles, averaged across all participants for each timepoint during split-belt treadmill adaptation. At Early Adapt the ankle joint reaches its peak dorsiflexion angle sooner and thus initiates plantarflexion earlier in the gait cycle. At Late Adapt, while the time of plantarflexion onset is adapting back toward baseline, the increase in plantarflexion is maintained. Stance percentage begins at heel strike and reaches 100% at ipsilateral toe off while gait cycle percentage begins at heel strike and reaches 100% at the subsequent ipsilateral heel strike.

Discussion

In this study, PwMS completed a split-belt treadmill adaptation paradigm and the biomechanical changes that occurred were investigated. The primary finding was that peak propulsion asymmetry between the limbs was increased from Early Adapt to Late Adapt and presented a substantial aftereffect from Baseline to Early Post-Adapt. Additionally, the largest kinematic contributors to these increases in peak propulsion asymmetry were peak dorsiflexion asymmetry, increased MA propulsion impulse, and earlier onset of MA plantarflexion in the gait cycle at the same timepoints. Further, both the kinematic and GRF changes in the MA limb (under the fast belt) experienced predictive feedforward adaptation while the changes in the LA limb (under the slow belt) only experienced reactive feedback adjustments.

Increased Propulsive Forces Drive Split-Belt Treadmill Adaptation in PwMS

Previous literature has established that PwMS exhibit decreased propulsion and decreased ankle power during gait, even in mildly impaired individuals.[18,19,26] Both negative and positive ankle plantarflexion power have been shown to be lower in PwMS compared to healthy controls.[26] Further, positive ankle plantarflexion power has been identified as a robust predictor of step length and walking speed and likely contributes to a reduction in propulsion in PwMS.[18,27] In response to these findings, research efforts have focused on ankle rehabilitation in PwMS to facilitate toe push-off leading to increased propulsive force during gait and a faster walking speed.[28] Similar work has aimed to augment propulsion by increasing propulsive demand by walking at an incline and walking with backward directed resistance in clinical populations and healthy controls.[29–31] With the establishment that neuromuscular function surrounding the ankle is key for propulsion and reduced propulsion impairs gait in PwMS, this study looked to characterize propulsion adaptation during split-belt treadmill training.

Specifically, peak propulsion asymmetry values initially indicate that the LA limb produced more anterior force at Baseline. At the onset of the adaptation period as the belt speeds changed, they increased the propulsive demand for the MA limb and decreased the propulsive demand for the LA limb. As a result, peak propulsion asymmetry values crossed zero indicating that the MA limb produced more propulsive force, which was expected. The MA limb continued to increase propulsive force for the duration of the adaptation period while the LA limb remained relatively unchanged, creating a robust adaptation curve. Finally, during post-adaptation, asymmetry values displayed aftereffects indicating successful adaptation. Thus, increasing the speed of the belt under the MA limb increased the propulsive demand for the MA limb leading to the adaptation of propulsive force generated by the MA limb. These results add to a growing body of literature identifying propulsion as a key factor to gait rehabilitation in clinical populations.

Decreased Dorsiflexion and Early Onset Plantarflexion Modulate Propulsion

It is likely that the observed enhancement in propulsion during adaptation is attributed to a unique strategy of joint motion changes tailored to each participant. Most notably, the musculoskeletal changes that resulted in altered peak dorsiflexion asymmetry and the timing of plantarflexion onset had the largest impact overall. Peak dorsiflexion asymmetry between the limbs had a robust correlation across the split-belt treadmill adaptation time course with peak propulsion asymmetry ($r = –0.86$, $p < 0.001$), implying the importance of reducing peak dorsiflexion as a key driver for heightened propulsion. Specifically, previous literature has identified that excessive dorsiflexion during stance is common in PwMS and hinders propulsion, thus reducing peak dorsiflexion may be a direct mechanism to facilitate propulsion.[18] Consistent with this observation, plantarflexion onset occurred earlier during the stance phase in the adaptation period, allowing for a longer period of plantarflexion during the gait cycle, and, as a

result generating heightened peak propulsion at toe off. This is further corroborated by observing the propulsion impulse profile, which exhibits a steeper and quicker development of propulsive force during the adaptation period (Figure 3.5).

This finding of an earlier plantarflexion onset is contrary to other split-belt treadmill research in young, healthy individuals, suggesting that PwMS have an altered adaptive strategy.[32] Interestingly one study indicated that peak dorsiflexion time (and therefore plantarflexion onset) occurred sooner only at a 4:1 speed ratio and not at lower speed ratios.[22] Given that this study employed a speed ratio of 2:1, it implies that PwMS may adopt this strategy even at a milder belt speed ratio. This adaptation strategy also may be driven by compromised propulsive forces during normal walking in PwMS,[18] necessitating an increased propulsion demand for successful adaptation. Additionally, this observation raises the possibility that the demands faced by PwMS using a 2:1 belt speed ratio are more akin to those encountered by healthy individuals using a 4:1 belt speed ratio.

Following an immediate increase at Early Adapt, peak plantarflexion also adapted and was reduced from Early Adapt to Late Adapt. While this may seem contradictory, due to plantarflexion occurring earlier in the gait cycle continual peak plantarflexion increases are not necessary to continue to produce more propulsion. Moreover, this may suggest that soft tissue loading, by primarily the Achilles tendon, may be an additional strategy to generate more propulsive force.[33] Further, PwMS experience heightened coactivation of the gastrocnemius and tibialis anterior as a result of spasticity and the prioritization of ankle joint stability.[34] This may serve as a plausible explanation for the concurrent reduction in both plantarflexion and dorsiflexion observed during adaptation.

Secondarily, hip extension may assist with propulsion increases during adaptation, but not adapt by itself. In accordance with these data, it can be inferred that split-belt treadmill adaptation likely occurs distal to proximal due to the perturbation being applied to the feet, thereby creating the largest effect at the ankle joint and only residual effects at the hip joint (Figure B2).

The MA Limb Engages in Predictive Feedforward Mechanisms while the LA Limb Relies on Reactive Feedback Mechanisms

Split-belt treadmill adaptation is contingent on a combination of reactive feedback control and predictive feedforward modulation. Findings from lesion studies[35] indicate that immediate gait parameter adjustments utilize feedback mechanisms independent of supraspinal control and are likely mediated through the brainstem and central pattern generators. However, to sustain the effects of split-belt treadmill adaptation and generate feedforward aftereffects, supraspinal influence from the cerebellum[36] and the cerebrum[37] is imperative. Furthermore, it has been recently suggested the microstructural properties of the inferior cerebellar peduncle are associated with locomotor adaptability.[38] In PwMS, the inferior cerebellar peduncle has shown compromised microstructural integrity,[39] which may explain the increased demands observed in PwMS at a 2:1 belt ratio that are absent in neurotypicals until a 4:1 belt speed ratio.

The most prominent adaptive changes observed during the split-belt adaptation paradigm were in peak propulsion asymmetry and peak dorsiflexion asymmetry. The existing literature strongly supports the notion that interlimb parameters are the primary components that undergo adaptation, while intralimb parameters primarily undergo adjustments through reactive feedback but do not exhibit adaptation or aftereffects.[10] The present study aligns with this, as the largest effect sizes of adaptation and aftereffects were identified in propulsion asymmetry and peak

dorsiflexion asymmetry between the limbs. This is consistent with the prevailing understanding of interlimb adaptability.

Notably, when examining individual joint-level changes in each limb, the data suggest that parameters in the LA limb (slow belt) predominantly rely on reactive feedback while parameters in the MA limb (fast belt) undergo adaptation. This suggests that the MA limb plays a primary role in driving the significant adaptive changes observed in peak propulsion asymmetry and peak dorsiflexion asymmetry. It has been historically understood that intralimb parameters, including MA peak propulsion and MA peak plantarflexion, do not adapt during split-belt treadmill adaptation. However, other research in healthy controls has proposed that unlike global intralimb parameters, such as stride length, that are dependent on multiple coordinated joint motions, individual intralimb joints are able to adapt[22] and this finding is corroborated by the presented data.

Limitations

A primary limitation of this study, and many studies involving PwMS, is the considerable distribution of symptom severity. Evidence has indicated that cutaneous perception in the feet plays a role in adaptation, particularly regarding force perception.[32] Many PwMS report neuropathy in their distal limbs,[40] which could impact the biomechanical strategy used to adapt on a split-belt treadmill. Additionally, age is known to affect locomotor adaptability during split-belt treadmill adaptation.[41] Given the wide age range of PwMS in this study, the effects of age alone could potentially impact adaptability, independent of MS-related factors. Further, this sample of PwMS was quite active and relatively healthy compared to normative PwMS. Most participants had a lower disability level than average compared to other PwMS with a similar number of years since diagnosis. However, a fundamental constraint of split-belt treadmill adaptation is the requisite capability to complete the demanding walking task, which unfortunately excludes many

individuals with a higher disability level and limits the generalizability of the findings. With this noted, it is possible that as technology and methodologies improve over time, allowing for the study of locomotor adaptation in individuals with more severe disability, larger effect sizes may be observed from a study with a similar methodology. An additional improvement to this study could involve incorporating a longer tied-belt walking session following split-belt treadmill adaptation. This would enable the recording of the rate of deadaptation after the observed aftereffects and offer valuable insights into the biomechanical strategies and savings during deadaptation.

Future Work

This study presents evidence indicating that propulsive force amplification, specifically generated by the MA limb, has the capacity to augment locomotor adaptation during split-belt treadmill training in PwMS. Therefore, future work should expand on these findings by implementing interventions directly aimed at training and bolstering propulsive force in the MA limb in PwMS combined with or prior to split-belt treadmill training. Previous literature has identified several ways to manipulate propulsion during gait training in clinical and neurotypical populations, including inclined walking,[29,30] walking with backward-directed resistance,[31] and functional electrical stimulation.[42,43] It is anticipated that findings from future studies, paired with the present results, will further highlight the importance of propulsive force in locomotor adaptation in PwMS and promote the need for propulsive intervention in rehabilitation strategies.

To further build on this work, future research should investigate the neural underpinnings that generate split-belt treadmill adaptation, specifically within PwMS. Previous investigations have highlighted the cerebellum,[36,44,45] along with the cortico-cerebellar and cortico-striatal loops,[46] as principal modulators of sensorimotor adaptation. Notably, research has indicated that

PwMS exhibit impaired cerebellar communication,[39,47] adding complexity to their adaptive processes. Given the observed similarity in demands for PwMS at a 2:1 belt ratio compared to neurotypical individuals at a 4:1 belt speed ratio,[22] the identification of the neural substrate responsible for these altered demands becomes a pertinent avenue for exploration. Moreover, determining the neural underpinnings of these demand changes offers valuable insights into optimizing the clinical efficacy of sensorimotor adaptation in PwMS, thereby informing targeted interventions and improving rehabilitation outcomes.

Conclusion

In conclusion, this study investigated the biomechanical adaptations that occurred during split-belt treadmill walking in PwMS. The primary finding was a significant increase in peak propulsion asymmetry between the limbs, with the main contributors being peak dorsiflexion asymmetry and an earlier onset of plantarflexion in the gait cycle. Additionally, this study highlighted that the MA limb played a predominant role in driving adaptive changes, relying on predictive feedforward mechanisms, while the LA limb primarily underwent reactive feedback adjustments. Moreover, this study underscores the importance of gaining further insights into the biomechanics of split-belt treadmill adaptation to fully comprehend its rehabilitative potential. A more comprehensive understanding of the intricacies of locomotor adaptation in PwMS and other populations may contribute to the development of effective and targeted rehabilitation interventions, thereby improving mobility and quality of life for the millions of individuals living with gait dysfunction.

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CHAPTER 4 – TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION ENHANCES LOCOMOTOR ADAPTATION SAVINGS IN PEOPLE WITH MULTIPLE SCLEROSIS[3]

Introduction

Many of the earliest symptoms of multiple sclerosis are sensory in nature, including numbness, tingling, restless limb, and pain,[1,2] affecting around 85% of people with multiple sclerosis (PwMS) in their first year of diagnosis.[3] Importantly, these sensory symptoms are likely the main driver of poor motor performance seen in PwMS.[4–6] Split-belt treadmill walking, where the speed of each limb is controlled independently, is a form of sensorimotor adaptation that can improve spatial, temporal, and kinetic gait symmetry in many populations,[7,8] including PwMS.[9,10] This locomotor adaptation is highly dependent on sensory input and cerebellar function.[11,12] Given the sensory impairments and cerebellar damage in PwMS,[13–15] even slight sensory disruptions could significantly impact adaptation, as the cerebellum relies on the fastest afferent fibers to support timely and accurate prediction.[16] Communication between the cortex and cerebellum is also imperative for locomotor adaptation, with research demonstrating that cerebellar-brain inhibition, or the cerebellum's inhibitory influence on the motor cortex, is proportional to adaptation.[17] Notably, other evidence indicates that, unlike controls, postural adaptation in PwMS is not associated with cortico-cerebellar connectivity strength, implying PwMS may use compensatory strategies, such and increased cortical activation to support motor learning.[18,19] Accordingly, there is a critical need to develop therapeutic strategies that enhance sensory function and support precise motor control, addressing significant gaps in both research and clinical practice.

[3] This chapter is accepted as: Hagen AC, Whittier TT, Stephens JA, Fling BW. Transcutaneous electrical nerve stimulation enhances locomotor adaptation savings in people with multiple sclerosis. *Brain Communications*.

Transcutaneous electrical nerve stimulation (TENS) has been demonstrated to improve sensorimotor integration in healthy and clinical populations, in skilled motor tasks including postural balance,[20] tactile perception,[21,22] manual dexterity,[23,24] and walking.[25–27] However, very few studies have investigated the effect of TENS on motor learning.[28,29] TENS is hypothesized to preferentially recruit Aα and Aβ afferent fibers,[20,30,31] thereby increasing afferent excitability. Although direct measurements of axonal excitability are limited, TENS has been shown to increase H-reflex amplitude and cortical excitability,[20,32–34] suggesting enhanced afferent signaling. These effects have been suggested to reduce sensorimotor uncertainty by improving the accuracy of state estimation during movement.[28] Separately, TENS alters activation patterns in sensorimotor cortical regions,[33,35] indicating broader effects on motor control.

While most research has focused on the cerebellum,[12,36,37] the influence of cortical regions during locomotor adaptation remains less clear.[38–40] Neuroimaging studies have shown that PwMS exhibit greater sensorimotor cortex activation and altered functional connectivity compared to controls during upper and lower extremity motor tasks, likely as a compensatory mechanism for impaired sensory and cerebellar function.[41,42] Building on these observations, the present study aimed to investigate the effect of TENS on locomotor adaptation in PwMS. We hypothesized that TENS would enhance locomotor adaptation, specifically by increasing the rate of stepping symmetry improvements. Additionally, we hypothesized reduced activation in sensorimotor cortical regions, as measured with functional near-infrared spectroscopy (fNIRS), when TENS was applied, indicating less cortical involvement during locomotor adaptation.

Materials and Methods

Participants

In this randomized controlled trial (NCT05878873), eligible participants were aged 18-75 years, and either had a diagnosis of relapsing-remitting multiple sclerosis or were a neurotypical healthy control (HC). PwMS were required to have an Expanded Disability Status Scale (EDSS) score below five and not be in an active relapse. Exclusion criteria included inability to walk 500 meters unassisted, a lower limb injury or surgery within six months, use of medications that impair balance, history of another neural or balance impairment (e.g. traumatic brain injury, stroke, vestibular disease) or current pregnancy. This study adhered to the Declaration of Helsinki and was approved by the Colorado State University Biomedical Institutional Review Board (protocol code: 4795). A preliminary analysis of five PwMS revealed a large effect (d = 1.01) for change in fNIRS-measured activation within the premotor cortex from Baseline to Early Adapt. Based on this effect, a power analysis indicated at least 10 participants per group were needed to maintain an alpha of 0.05 and a power of 0.8. However, to account for smaller effects in other regions, sample size was increased to 15 per group for PwMS. For HCs, 10 participants per group was deemed sufficient based on previous locomotor adaptation studies.[43]

Demographics and disease characteristics were collected after screening and informed consent. Measures included the EDSS, 12-Item Multiple Sclerosis Walking Scale, Modified Fatigue Impact Scale, Beck Depression Inventory, oral Symbol Digit Modalities Test, hallux vibration perception threshold with a Rydel-Seiffer tuning fork, rate of perceived exertion, and Modified Clinical Test of Sensory Integration in Balance (mCTSIB). For the mCTSIB, primary measures included sway during the proprioceptive condition (eyes closed on firm surface) and the composite sway score across all conditions.

Study Design

In this crossover design, PwMS and HC participants were pseudo-randomized to begin in either the TENS First or TENS Second condition. Randomization was performed using a random number generator and was counterbalanced across age, sex, and fast limb to ensure group balance. A predefined list of participant numbers with corresponding randomized assignments was generated prior to enrollment. Participants were enrolled sequentially and assigned to a group based on this list. The TENS first group received active TENS (TENS ON) during the first locomotor adaptation visit and inactive TENS (TENS OFF) during the second visit while the TENS second group received these conditions in the reverse order, with a four-week interval between visits (Figure 4.1A). Importantly, TENS was active only during the adaptation phase of the paradigm, when the treadmill belts moved different at speeds.

Overground walking assessments were conducted in a 30-meter hallway during Visit 1 to determine treadmill speeds. Participants performed two separate two-minute walk trials: one at their preferred speed, and one at their fastest comfortable speed they could maintain for 15 minutes, while turning at each end of the hallway as needed. The 'fast' belt on the split-belt treadmill during locomotor adaptation was set to this fast walk speed while the 'slow' belt was set to half of this fast walk speed (2:1 ratio). The 'fast' limb was the more affected limb in PwMS, confirmed as the shorter overground step length. In HCs, fast limb assignments were counterbalanced to match the distribution observed in PwMS and were aligned by age and sex. During treadmill walking, participants completed a two-minute walk with the belts tied at their preferred speed, followed by a 10-minute adaptation phase with the belts split, and a 10-minute deadaptation phase with the belts tied again at their preferred speed (Figure 4.1B). Participants were secured in a harness without bodyweight support, instructed to hold the handrails, and fixed their gaze on a crosshair target positioned ahead on a large backdrop.

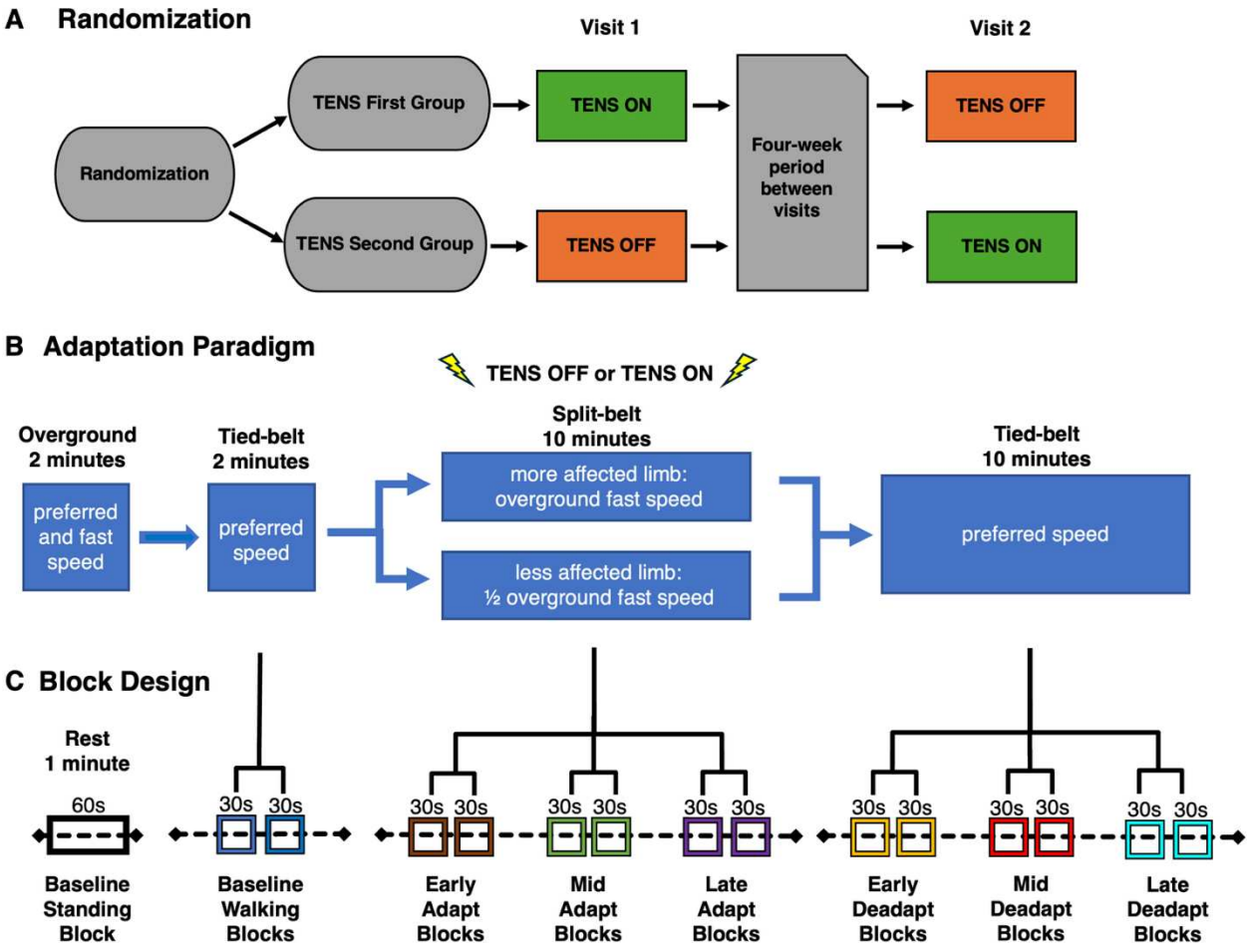

Figure 4.1. Study design. **(A)** Crossover design and randomization of transcutaneous electrical nerve stimulation (TENS) for each visit. The first group received TENS ON during Visit 1 and TENS OFF during Visit 2, while the second group had the reverse order. A four-week washout period separated the visits. **(B)** Locomotor adaptation paradigm. Participants first completed baseline overground walking at preferred and fast speeds, followed by tied-belt treadmill walking at their preferred speed. During the split-belt adaptation phase (10 minutes), the more affected limb was set to the overground fast speed, while the less affected limb was set to half the overground fast speed. TENS was active during adaptation phases only. The protocol concluded with tied-belt treadmill walking (10 minutes) at the preferred speed. **(C)** Functional near-infrared spectroscopy (fNIRS) imaging block design. Following a 60-second standing rest period, pairs of 30-second blocks were averaged to quantify cortical activation for each treadmill walking timepoint in the baseline, adaptation, and deadaptation phases.

TENS Parameters

During all visits, participants had TENS electrodes placed over the muscle bellies of the bilateral tibialis anterior and rectus femoris, while the TENS units (LG–TECELITE,

LGMedSupply) were mounted to the NIRSport2 device. These locations were selected to target muscles involved in dorsiflexion, hip flexion and knee extension, common areas of impairment that affect walking in PwMS.[44,45] Further, previous studies have successfully improved gait performance in PwMS using these electrode locations.[28,46,47] Stimulation consisted of biphasic bursts (seven 0.15 ms pulses) at 5 Hz through 2×4 inch electrode pairs, based on recent evidence suggesting that bursting TENS is most effective during walking.[47] To reduce electrical impedance, the skin over the muscles was shaved to prior to pad placement. Electrode placement and stimulation parameters (e.g., pulse width, frequency) were identical for both PwMS and HC groups. TENS amplitude was individualized for each participant based on their motor threshold. Stimulation intensity was increased in 1 mA increments for each muscle until non-voluntary contractions were observed by the participant or investigator. Contractions were defined as visible muscle twitching or movement at the joint or a palpable muscle response during light manual contact by the investigator. Once threshold was identified, amplitude was set to 2 mA below this threshold for each muscle.[28]

Gait Data Analysis

During overground walking, gait parameters were measured using six APDM opal inertial sensors and processed using APDM Mobility Lab (v. 2.0, APDM Wearable Technologies). During treadmill walking, participants were outfitted with 16 retroreflective markers placed according to the Vicon Plug in Gait Lower Body model, while 10 Vicon T010 infrared cameras surrounding the treadmill captured motion at 100 Hz. This instrumented treadmill (Model 4060-10, Bertec Corp) recorded ground reaction forces at 1000 Hz, and had two motors that allowed independent control of each belt. Force data were filtered using a fourth-order zero-lag Butterworth filter (300 Hz low-pass cutoff) and marker positions were filtered using a fifth-order spline-interpolating

Woltring filter in Vicon Nexus (v. 2.15, Vicon Motion Systems). For this study, the primary measure during treadmill walking was step length asymmetry (SLA), which has been shown to adapt robustly during split-belt treadmill walking.[48] Step length was calculated as the anterior-posterior distance in mm between heel markers at heel strike for each limb. A body-centered model of heel location was used to account for participant translation within strides,[49] and SLA was normalized to participant stride length using the following equation:

$$SLA\ (i)\ = \frac{step\ length_{fast\ limb}(i)\ (mm) - step\ length_{slow\ limb}(i)\ (mm)}{step\ length_{fast\ limb}(i)\ (mm) + step\ length_{slow\ limb}(i)\ (mm)} \quad (1)$$

To improve comparability across participants, the SLA curves for adaptation and deadaptation were baseline-adjusted by subtracting each participant's visit-specific average SLA at Baseline (last 30 strides) from their SLA at each stride. Baseline adjustment removed individual and visit-specific offsets, ensuring that differences in SLA curves reflect changes in adaptation rather than baseline variability.[50] Following, baseline-adjusted adaptation and deadaptation SLA curves were fitted to a single exponential model ($y = a \times e^{bn} + c$) using the algorithm and parameters established by Rashid et al.[51] to more accurately quantify the underlying learning process. Curve fitting was performed on each participant's stride-by-stride data using a particle swarm optimization algorithm to minimize squared error. Although locomotor adaptation is often represented by a two-rate model, the single exponential provided a more consistent fit across our PwMS sample without requiring individualized parameter adjustments.[52] From these exponential models, average SLA was calculated at six timepoints per visit: Initial Adapt (strides 1-5), Early Adapt (strides 6-30), Late Adapt (last 30 strides), Initial Deadapt (strides 1-5), Early Deadapt (strides 6-30), and Late Deadapt (last 30 strides). Subsequently, these averages were used to calculate the following outcomes to characterize locomotor adaptation[53]: (1) early change, defined as the average SLA during the Early Adapt and Early Deadapt phases; (2) adaptation magnitude,

calculated as the difference in SLA between the Early and Late phases of Adapt and Deadapt; (3) aftereffect, defined as the difference between Early Deadapt and Baseline SLA; and (4) savings, quantified as the difference in SLA from Visit 1 to Visit 2 at Initial and Early Adapt, reflecting the rate of relearning.

fNIRS Acquisition

During treadmill walking, participants had a NIRSport2 (NIRx Medical Technologies) mobile fNIRS device attached to their back, which acquired and wirelessly transmitted data to Aurora software (v. 2021.9, NIRx Medical Technologies) at a 6.1 Hz sampling rate. A block design with two 30-second blocks per timepoint was used to quantify cortical activation, and data were averaged across each block pair (Figure 4.1C). The cap montage was designed using the fNIRS Optodes Location Decider toolbox[54] in MATLAB (v. R2022b) and its Brodmann atlas to create source-detector pairs (i.e. channels) over premotor, sensorimotor, and posterior parietal regions of interest (ROIs), as these areas are implicated in gait modulation.[55] This montage contained 16 LED source optodes (760 and 850 nanometers) and 15 detector optodes creating 48 channels that captured changes in oxyhemoglobin (HbO), deoxyhemoglobin (HbR), and total hemoglobin. Eight short-distance detectors measured scalp perfusion, or blood flow to the scalp, as opposed to blood flow in the cortex, and were used during preprocessing to regress out confounding signals including motion, heart rate, and blood pressure.[56] Before collection, signal optimization (i.e. source brightness calibration) and cap preparation steps (e.g. moving hair, adjusting optode tension) were repeated until all channels reached acceptable levels of signal quality (> 0.5 mV).

fNIRS Preprocessing and Analysis

Raw fNIRS data was processed using Satori (v. 1.8, NIRx Medical Technologies). Initial preprocessing steps of conversion and spatial registration are performed automatically in this

software. Raw light intensity data were converted to optical density values and then to concentrations of HbO, HbR, and total hemoglobin using the Modified Beer-Lambert law. The outcomes of this study are based on HbO values, as HbO is the most reported chromophore in fNIRS research and provides the most direct measure of cortical activation.[57] HbO reflects oxygen delivery to active brain regions and is most sensitive to the hemodynamic response. HbR reflects oxygen extracted by the tissue typically showing inverse changes to HbO. HbR results are available in Figure C1. Since the primary outcomes were relative beta weights and the cap montage used a consistent source-detector separation (35-40 mm) across all channels, a differential pathlength factor was not applied, as this minimized pathlength variability and made it unnecessary for comparing relative activation between conditions.[58] Following, individual channel data were spatially registered to the montage and displayed for visual inspection and confirmation of signal quality. Events were manually created for each participant using stimulus trigger markers for each block in the block design.

Subsequently, motion artifact correction and spike removal were performed using the default parameters in Satori. Spikes, or brief, high-amplitude fluctuations typically caused by motion were identified using 10 iterations, 5 second lag, 3.5 threshold, and 0.5 influence. Temporal Derivative Distribution Repair was then applied to restore high frequency bands with monotonic interpolation. Next, short-channel regression was performed for each channel using the highest correlated short-distance detector. Temporal filtering consisted of the default Satori parameters including a high-pass Butterworth filter (0.01 Hz) and low-pass Gaussian smoothing (0.4 Hz). Additionally, Z-transform normalization was performed to make data more comparable between participants. The only default preprocessing step omitted was automatic channel rejection based

on scalp coupling index. However, scalp coupling indexes were calculated for each channel to identify potential outliers post-processing.

Following standardized preprocessing, HbO beta weights were calculated using a general linear model to quantify hemodynamic response strength (double-gamma function model) at each timepoint. These betas isolate task-related activation magnitude between conditions, making them robust to baseline fluctuations and noise. To quantify channel-wide activation, HbO betas were averaged across all channels in the array. Additionally, HbO betas from each channel were organized into different ROI clusters.[59,60] Channels included in each ROI were determined *a priori* based on the highest Brodmann area (BA) specificity, while avoiding channels that overlap multiple ROIs and ensuring symmetrical bilateral correspondence. These bilateral clusters included the dorsal and ventral premotor cortex (PMd, PMv, BA6), primary motor cortex (M1, BA4), primary somatosensory cortex (S1, BA3,1,2), superior parietal lobule (SPL, BA5,7), and inferior parietal lobule (IPL, BA39,40) (Table C1). Contrasts were then created between the averaged Baseline and Early Adapt blocks to isolate activation specific to adaptation alone, and independent of treadmill walking effects.

Statistical Analysis

Linear mixed-effects models were used to identify differences in adaptation and fNIRS outcomes, with group (PwMS vs. HC), 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 were confirmed for each model. Following model creation, a repeated measures ANOVA was performed for each adaptation outcome (adaptation magnitude, early change, aftereffect and savings) and for HbO beta, both at the channel-wide and ROI levels. Type III ANOVAs with Satterthwaite's method for

degrees of freedom estimation were used for all analyses. Post–hoc comparisons were conducted using estimated marginal means (EMM) to further investigate significant main effects and interactions. *P*-values were corrected using the Benjamini–Hochberg false discovery rate within each outcome and group (e.g., ROI *p*-values within the PwMS group were corrected for across all other ROIs) when applicable. Cohen's *d* was calculated to quantify effect sizes. Backward stepwise regression models were conducted to assess the influence of demographic covariates on primary outcome models. Pearson correlations between significant changes in HbO beta and savings were computed to explore their relationship. Finally, linear regression models and backward stepwise regression models were conducted to assess the predictability of HbO beta for savings. All statistical analyses were conducted using the lme4 and emmeans packages within R (v. 4.4.0) and were two-tailed with an alpha threshold set at 0.05.

Results

Participants

A total of 55 participants were screened, and 51 participants (31 PwMS and 20 HCs) were enrolled. Of these, 29 PwMS and 20 HCs completed the study, with one PwMS excluded from analysis due to poor data quality (Figure 4.2). PwMS and HCs did not differ in age, activity, body mass index, RPE during Late Adapt, or baseline SLA. However, PwMS exhibited significantly higher fatigue, greater mCTSIB proprioceptive sway, reduced vibration perception threshold, reduced fast walk speeds, and shorter baseline step lengths (Table 4.1). This sample of PwMS had relatively low impairment (EDSS = 3.2) and was highly active compared to average PwMS.[61]

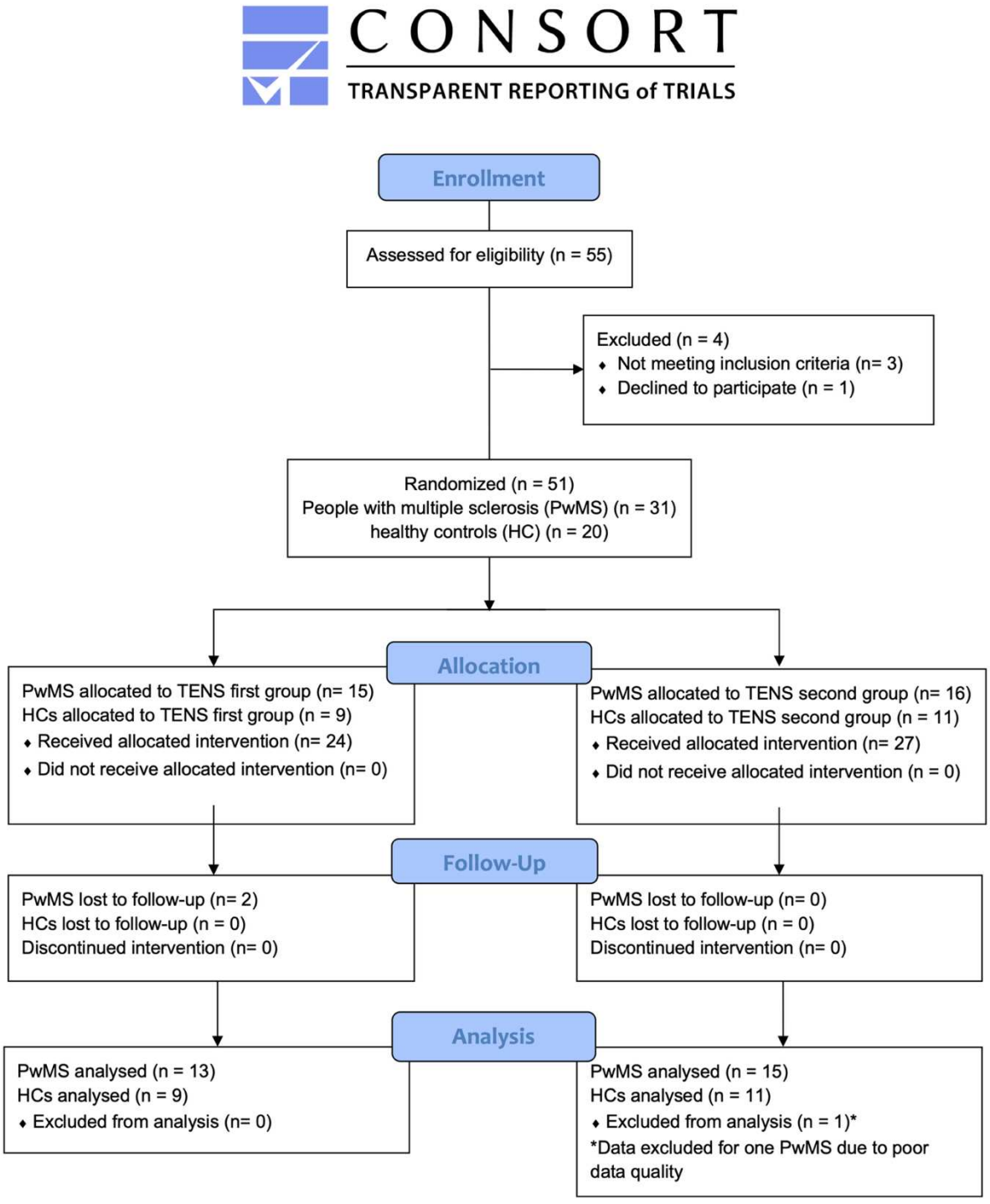

Figure 4.2. Consort diagram. Flowchart illustrating trial recruitment, randomization, and retention of participants. A total of 55 participants were assessed for eligibility, with four excluded (three did not meet inclusion criteria, one declined to participate). The remaining 51 participants were randomized: 31 people with multiple sclerosis (PwMS) and 20 healthy controls (HC). During the trial, two PwMS were lost to follow-up and one had poor data quality, resulting in 28 PwMS and 20 HC participants in this analysis.

Table 4.1. Participant Characteristics. Participant characteristics and baseline clinical measures for people with multiple sclerosis (PwMS) and healthy control (HC) groups, presented as mean ± (standard deviation). *P*-values indicate group differences when applicable.

Characteristic	PwMS mean (SD)	HC mean (SD)	*p*
N	28	20	
Age	53.5 (10.7)	53.8 (14.0)	0.953
Sex (female/male)	19/9	12/8	0.760
Body mass index	24.9 (5.3)	25.4 (4.5)	0.746
Activity (hours per week)	5.1 (3.1)	5.5 (4.4)	0.735
Fallen in past 6 months (yes/no)	6/22	0/20	0.034*
History of sensory impairments (e.g. numbness, tingling) (yes/no)	24/4	0/20	< 0.001***
Years since multiple sclerosis diagnosis[1]	12.8 (17.0)		
Expanded Disability Status Scale[1]	3.5 (1.5)		
12-Item Multiple Sclerosis Walking Scale[1]	16.5 (12.3)		
Modified Fatigue Impact Scale[1]	27.5 (16.8)	4.5 (9.3)	< 0.001***
Beck Depression Inventory[1]	4 (7)	0 (3)	0.008**
Oral Symbol Digit Modalities Test	73.4 (13.6)	77.4 (15.7)	0.368
Vibration perception threshold[1]	5.5 (2.4)	6.4 (1.1)	0.016*
mCTSIB total sway (cm)[1]	205.0 (179.0)	161.5 (57.0)	0.063
mCTSIB proprioception sway (cm)[1]	34.5 (41.5)	25.5 (10.5)	0.005**
Rate of perceived exertion at Late Adapt[1]	3.6 (0.7)	3.4 (1.0)	0.325
Preferred walk speed (m/s)	1.16 (0.20)	1.19 (0.12)	0.454
Fast walk speed (m/s)	1.40 (0.24)	1.55 (0.16)	0.018*
Baseline step length (mm)	563.0 (68.5)	602.4 (55.4)	0.003**
Baseline step length asymmetry	0.021 (0.017)	0.016 (0.012)	0.089
Rectus femoris TENS amplitude (mA)[2]	13.7 (7.9)	11.4 (5.7)	0.103
Tibialis anterior TENS amplitude (mA)[2]	16.8 (8.9)	14.2 (5.7)	0.086

mCTSIB = Modified Clinical Test of Sensory Interaction in Balance. TENS = Transcutaneous Electrical Nerve Stimulation. 1. Data were not normally distributed; values are reported as median (IQR), and group differences were tested using the Mann-Whitney U test. 2. Amplitudes were averaged across the left and right sides for each participant.

SLA Adaptation Outcomes

Adaptation, or changes in SLA during split-belt walking was quantified using four outcomes: adaptation magnitude, early change, aftereffect, and savings. Full results are summarized in Table 4.2.

Table 4.2. Step length asymmetry (SLA) adaptation. Linear mixed-effects model results and subsequent pairwise comparisons using estimated marginal means (EMM; adjusted group means) and standard error (SE) for adaptation magnitude (A), early change (B), aftereffect (C), and savings (D). All models considered group (people with multiple sclerosis (PwMS) vs healthy controls (HC)), visit (Visit 1 vs Visit 2), and transcutaneous electrical nerve stimulation (TENS) condition (TENS ON vs TENS OFF) as fixed effects and participant as a random effect.

A. Adaptation Magnitude

Significant Predictor	***df***	***F***	***p***	
Group	1, 44	5.7	0.021*	
Visit	1, 44	22.9	< 0.001***	
TENS Condition	1, 44	5.0	0.031*	
Pairwise	**EMM (SE)**	***p***	**adj-*p***	***d***
PwMS – HC	–0.022 (0.009)	0.021	0.042*	0.81
PwMS: Visit 2 – Visit 1	–0.021 (0.007)	0.004	0.008**	0.80
HC: Visit 2 – Visit 1	–0.031 (0.008)	< 0.001	0.001**	1.18
PwMS: TENS ON – TENS OFF	–0.019 (0.007)	0.008	0.017*	0.73
HC: TENS ON – TENS OFF	–0.005 (0.008)	0.548	0.5483	0.19

B. Early Change

Significant Predictor	***df***	***F***	***p***	
Visit	1, 44	20.2	< 0.001***	
Pairwise	**EMM (SE)**	***p***	**adj-*p***	***d***
PwMS: Visit 2 – Visit 1	0.024 (0.007)	0.001	0.003**	0.92
HC: Visit 2 – Visit 1	0.025 (0.008)	0.005	0.009**	0.95

C. Aftereffect

Significant Predictor	***df***	***F***	***p***
Visit	1, 44	20.4	< 0.001***

Pairwise	EMM (SE)	*p*	adj-*p*	*d*
PwMS: Visit 2 – Visit 1	0.012 (0.004)	0.002	N/A[1]	0.85
HC: Visit 2 – Visit 1	0.014 (0.004)	0.002	N/A[1]	1.02

D. Savings

Significant Predictor	*df*	*F*	*p*
Initial Adapt: TENS Condition	1, 44	12.7	< 0.001***
Early Adapt: TENS Condition	1, 44	6.3	0.016*

Pairwise	EMM (SE)	*p*	adj-*p*	*d*
Initial Adapt, PwMS: TENS ON – TENS OFF	0.050 (0.014)	< 0.001	0.005**	1.35
Initial Adapt, HC: TENS ON – TENS OFF	0.022 (0.017)	0.199	0.442	0.59
Early Adapt, PwMS: TENS ON – TENS OFF	0.042 (0.014)	0.005	0.014*	1.13
Early Adapt, HC: TENS ON – TENS OFF	0.006 (0.017)	0.732	0.878	0.16

Only statistically significant main effects or interactions are reported. 1. Multiple comparisons corrections were not applied for aftereffect, as it represents a single value across the entire paradigm, with no comparisons across different phases. 2. Random effects were not included in the model, as only one value was calculated per participant.

Adaptation Magnitude

Adaptation magnitude showed significant main effects of group, visit, and TENS condition with no significant interactions (Table 4.2A). Across all visits and TENS conditions, PwMS exhibited reduced adaptation magnitude compared to HCs (EMM = 0.022, adj-p = 0.042, d = 0.81) (Figure 4.3A). As expected, PwMS and HCs had reduced adaptation magnitude at Visit 2. Importantly, with TENS ON, adaptation magnitude was reduced in PwMS (EMM = –0.019, adj-p = 0.017, d = 0.73), but not in HCs (EMM = –0.005, adj-p = 0.548, d = 0.19). In contrast, no significant main effects or group differences were observed for deadaptation magnitude.

Early Change

For early change, visit was the only significant main effect with greater early change at Visit 2 compared to Visit 1, indicating a faster adaptation rate, for both PwMS (EMM = 0.024 adj-p = 0.003, d = 0.92) and HCs (EMM = 0.025, adj-p = 0.009, d = 0.95) (Table 4.2B; Figure

4.3B,3C). When comparing early change at Visit 1 only, no significant difference was observed between TENS ON and TENS OFF conditions in PwMS (EMM = –0.002, $p = 0.898$, $d = 0.09$), despite its visual appearance. Additionally, during deadaptation PwMS and HCs showed reduced early change at Visit 2 compared to Visit 1 (PwMS: EMM = –0.011, adj-$p = 0.023$, $d = 0.62$; HC: EMM = –0.015, adj-$p = 0.009$, $d = 0.86$).

Aftereffect

Aftereffect also showed a significant main effect of visit and was not influenced by group or TENS condition (Table 4.2C). For PwMS and HCs, aftereffect decreased on Visit 2 compared to Visit 1 (PwMS: EMM = –0.012, $p = 0.002$, $d = 0.85$; HC: EMM = –0.014, $p = 0.002$, $d = 1.02$), which is consistent with previous reports.[53]

Savings

For savings, TENS condition was the only significant main effect at both Initial Adapt and Early Adapt (Table 4.2D). Among PwMS, receiving TENS ON at Visit 2 resulted in greater savings compared to receiving TENS OFF (Initial Adapt: EMM = 0.050, adj-$p = 0.005$, $d = 1.35$; Early Adapt: EMM = 0.042, adj-$p = 0.014$, $d = 1.13$) (Figure 4.3B). Conversely, for HCs with TENS ON at visit 2, this effect was not significant (Figure 4.3C). For deadaptation, TENS condition had no effect on savings at either Initial Deadapt or Early Deadapt for either group.

Given significantly greater savings with TENS ON at Visit 2, stepwise regression models predicting savings were performed across all participants, incorporating all demographic, clinical, and functional variables included in Table 4.1. While TENS condition remained significant in both models, savings at Initial Adapt was predicted by vibration perception threshold ($t(44) = 2.6$, $p = 0.013$) and savings at Early Adapt was predicted by mCTSIB proprioceptive sway ($t(42) = 2.3$, $p = 0.024$) and vibration perception threshold marginally ($t(42) = 2.0$, $p = 0.056$).

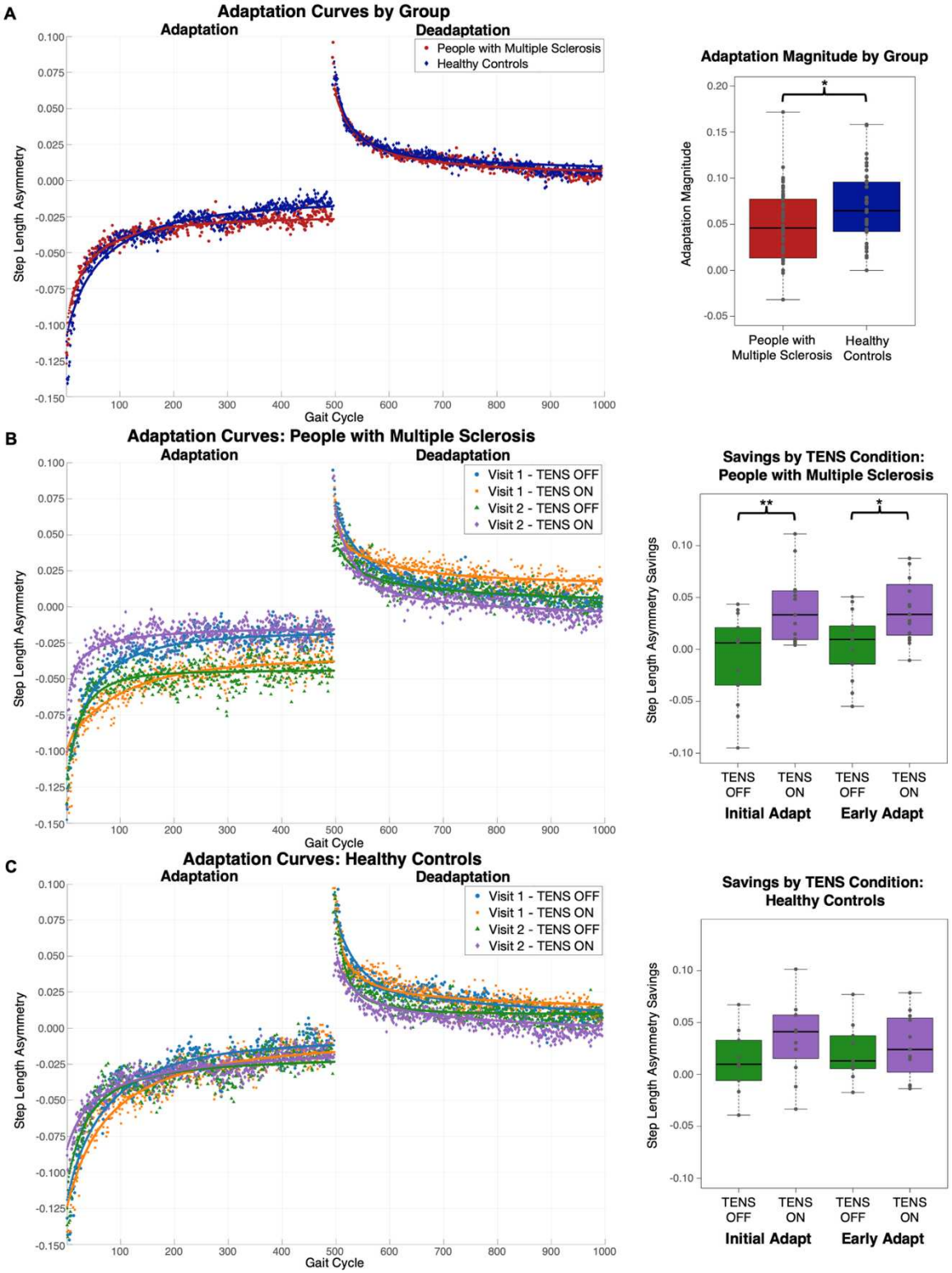

Figure 4.3. Step length asymmetry (SLA) adaptation. (A) SLA adaptation curves and boxplots for people with multiple sclerosis (PwMS) and healthy controls (HC) averaged across all visits and conditions. While early change remained similar, PwMS exhibited reduced adaptation magnitude compared to HCs ($t(44)=-2.39$, adj-$P=0.042$, $d=0.81$). **(B)** SLA adaptation curves and boxplots for PwMS for each visit and condition. Transcutaneous electrical nerve

stimulation (TENS) did not significantly affect early change during adaptation; however, at Visit 2, TENS ON (purple) significantly enhanced adaptation savings at Initial Adapt ($t(44)=3.56$, adj-$P=0.005$, $d=1.35$) and Early Adapt ($t(44)=2.98$, adj-$P=0.014$, $d=1.13$) compared to TENS OFF (green). During deadaptation, PwMS showed reduced early change at Visit 2, while savings did not differ between TENS conditions. **(C)** SLA adaptation curves and boxplots for HC for each visit and condition. TENS had no significant impact on any adaptation variable. However, early change was greater at Visit 2 compared to Visit 1 during adaptation. Similar to PwMS, reduced early change was observed during deadaptation at Visit 2. Each data point in all adaptation curves (A-C) represents the group-average SLA for a single gait cycle (x-axis) with different marker symbols and colors indicating experimental conditions, totaling 1000 data points per condition. For visual purposes SLA curves were interpolated, but all analyses were based on the uninterpolated data. Linear mixed effects model results of all outcomes are reported in Table 4.2.

Channel-Wide Activation

Across all channels, TENS condition had a significant main effect. Surprisingly, there were no effects of visit or group, nor any interactions (Table 4.3A). When comparing timepoints, HbO beta at Early Adapt was significantly greater than Baseline with TENS OFF for PwMS (EMM = 0.054, $p = 0.037$, $d = 0.56$) and HCs (EMM = 0.073, $p = 0.017$, $d = 0.76$). Additionally, when comparing TENS conditions for the Early Adapt – Baseline contrast, TENS ON resulted in significantly reduced HbO beta compared to TENS OFF in both groups (PwMS: EMM = –0.078 $p = 0.007$, $d = 0.76$; HC: EMM = –0.089 $p = 0.008$, $d = 0.88$) (Figure 4.4).

ROI-Level Activation

Linear mixed-effects model results of individual ROIs are shown in Table C2. A similar trend of increased HbO beta during Early Adapt compared to Baseline with TENS OFF was apparent at the ROI level. For PwMS, a large increase in HbO beta at Early Adapt occurred exclusively in PMd (EMM = 0.097, adj-$p = 0.026$, $d = 0.77$), while M1 and S1 showed moderate effects that did not survive corrections. For HCs, HbO beta in PMd (EMM = 0.099, adj-$p = 0.042$, $d = 0.78$) and M1 (EMM = 0.088, adj-$p = 0.042$, $d = 0.81$) was increased at Early Adapt (Table 4.3B).

When comparing TENS OFF to TENS ON conditions across both visits, PwMS exhibited a significant large-effect decrease in HbO beta with TENS ON in PMd (EMM = –0.123, adj-p = 0.019, d = 0.84), and significant moderate-effect decreases in M1, S1 and SPL. For HCs, no comparisons survived corrections for decreased HbO beta with TENS ON, but moderate effects were observed in PMd, M1, S1, and SPL (Table 4.3C).

To evaluate whether demographic, clinical, and functional variables listed in Table 4.1 accounted for variance in cortical activation, stepwise regression models were performed for each ROI. TENS condition remained significant in all ROIs. PMd HbO beta was additionally predicted by vibration perception threshold (t(87) = –2.0, p = 0.048), and body mass index (t(87) = –2.6, p = 0.010).

Table 4.3. Functional near-infrared spectroscopy (fNIRS) oxyhemoglobin (HbO) beta changes. Linear mixed effects model results and estimated marginal means (EMM) of channel-wide and region of interest (ROI) oxyhemoglobin (HbO) beta changes for people with multiple sclerosis (PwMS) and healthy controls (HC). Contrasts include the difference between Early Adaptation and Baseline, as well as TENS ON – TENS OFF for the Early Adaptation – Baseline contrast. ROIs included dorsal and ventral premotor areas (PMd, PMv), primary motor and somatosensory cortices (M1, S1), and superior and inferior parietal lobules (SPL, IPL). Individual ROI ANOVA results available in Table C2.

A. Channel-Wide Activation

Predictor	***df***	***F***	***p***
Group	1, 44	0.3	0.579
Visit	1, 44	0.2	0.692
TENS Condition	1, 44	15.6	< 0.001***
Contrast	**EMM (SE)**	***p***	***d***
PwMS: Early Adapt – Baseline	0.054 (0.026)	0.037*	0.56
HC: Early Adapt – Baseline	0.073 (0.031)	0.017*	0.76
PwMS: TENS ON – TENS OFF	–0.078 (0.027)	0.007**	0.76
HC: TENS ON – TENS OFF	–0.089 (0.032)	0.008**	0.88

B. ROIs: Early Adapt − Baseline Contrast

Group	ROI	EMM (SE)	*p*	adj-*p*	*d*
PwMS	PMd	0.097 (0.034)	0.004	0.026*	0.77
HC	PMd	0.099 (0.040)	0.014	0.042*	0.78
PwMS	PMv	0.021 (0.043)	0.626	0.626	0.13
HC	PMv	0.085 (0.051)	0.093	0.186	0.54
PwMS	M1	0.064 (0.029)	0.030	0.090	0.58
HC	M1	0.088 (0.035)	0.012	0.042*	0.81
PwMS	S1	0.065 (0.034)	0.056	0.113	0.51
HC	S1	0.039 (0.040)	0.329	0.329	0.31
PwMS	SPL	0.055 (0.036)	0.124	0.187	0.41
HC	SPL	0.044 (0.042)	0.303	0.329	0.33
PwMS	IPL	0.037 (0.037)	0.316	0.380	0.27
HC	IPL	0.061 (0.043)	0.160	0.239	0.45

C. ROIs: TENS ON − TENS OFF Contrast

Group	ROI	EMM (SE)	*p*	adj-*p*	*d*
PwMS	PMd	–0.123 (0.040)	0.003	0.019*	0.84
HC	PMd	–0.090 (0.047)	0.060	0.090	0.61
PwMS	PMv	–0.007 (0.049)	0.888	0.888	0.04
HC	PMv	–0.061 (0.59)	0.301	0.301	0.33
PwMS	M1	–0.085 (0.038)	0.029	0.043*	0.61
HC	M1	–0.105 (0.045)	0.023	0.069	0.75
PwMS	S1	–0.106 (0.038)	0.008	0.024*	0.75
HC	S1	–0.113 (0.045)	0.017	0.069	0.79
PwMS	SPL	–0.104 (0.040)	0.013	0.026*	0.70
HC	SPL	–0.094 (0.047)	0.054	0.090	0.63
PwMS	IPL	–0.059 (0.045)	0.197	0.237	0.35
HC	IPL	–0.088 (0.054)	0.110	0.131	0.52

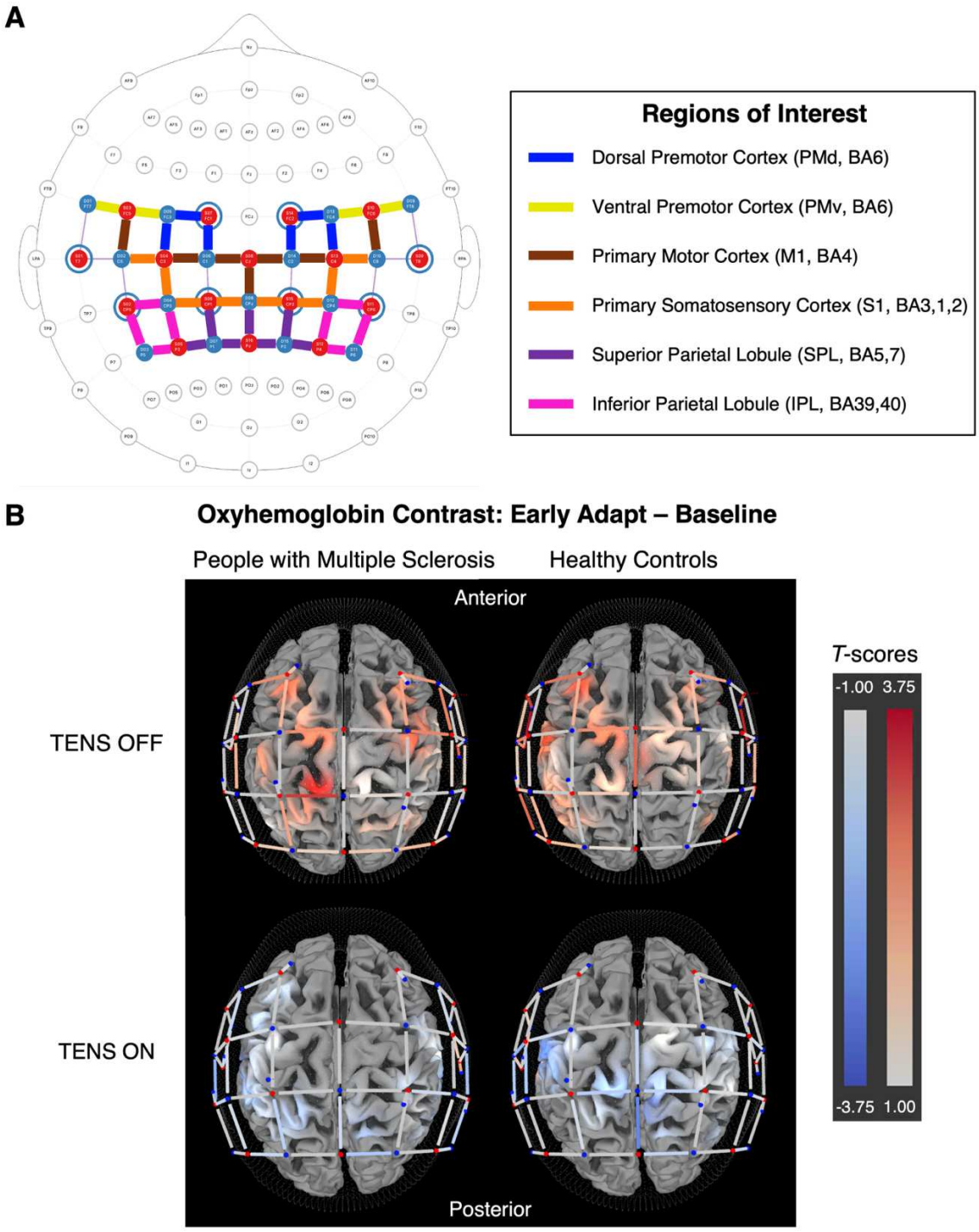

Figure 4.4. Functional near-infrared spectroscopy (fNIRS) results. **(A)** fNIRS channel locations are shown relative to the 10-10 system. Sources are represented by red circles, detectors by blue circles, and short-distance detectors by blue rings. Each source-detector line indicates an individual channel, with the thick colored lines highlighting channels assigned to a specific region of interest (ROI) based on their Brodmann areas (BA). ROIs included dorsal and ventral premotor areas (PMd, PMv), primary motor and somatosensory areas (M1, S1), and superior and inferior parietal lobules (SPL, IPL). **(B)** Cortical activation with TENS OFF and TENS ON**.** Group level *t*-score maps oxyhemoglobin (HbO) beta change for the Early Adapt – Baseline

contrast across premotor, sensorimotor, and posterior parietal regions for people with multiple sclerosis (PwMS) (N=28) and healthy controls (HC) (N=20). With TENS OFF, both groups exhibited channel-wide increases in activation, including significant activation of the dorsal premotor cortex (PMd) during Early Adapt compared to Baseline. In contrast, with TENS ON, channel-wide activation was significantly reduced compared to TENS OFF for both groups, with no increase from Baseline to Early Adapt. Among only PwMS, this reduced activation with TENS ON was observed specifically in the PMd, primary motor (M1), primary sensory (S1), and superior parietal lobule (SPL) regions of interest (ROIs). Linear mixed effects model results of all ROIs are reported in Table 4.3.

Relationship Between Savings and Cortical Activation

In line with our hypothesis that TENS modulates activation in sensorimotor regions and the finding of significantly greater savings with TENS, correlations between HbO beta and savings at Early Adapt were examined. While no significant correlations were observed at the ROI level, a trending relationship in PwMS at PMd (r = –0.63, adj-p = 0.119) was insignificant after correction, prompting an exploratory analysis of channel-specific correlations within the hypothesized regions of PMd, PMv, SPL, and IPL. In PwMS with TENS OFF, a strong correlation was observed in a PMd channel (S14-D14: r = –0.72, adj-p = 0.034) and a potential correlation in a SPL channel (S16-D17: r = 0.56, adj-p = 0.326) that lacked significance after correction. Interestingly, these correlations were absent for PwMS with TENS ON and for HCs in both TENS conditions suggesting that individuals who required greater recruitment of PMd during adaptation tended to show poorer savings across visits (Figure 4.5).

To examine whether broader cortical activation patterns predicted savings, we computed a stepwise linear regression model including HbO beta from all ROIs. In PwMS with TENS OFF, activation in PMd ($t(8)$ = –4.5, p = 0.001) and SPL ($t(8)$ = 2.9, p = 0.016) contributed significantly to the model, which explained a substantial proportion of variance in savings (R^2 = 0.68, adj-R^2 = 0.61, $F(2,10)$ = 10.4, p = 0.004) further supporting the relationship between activation in these regions and savings in PwMS. No meaningful model contributions were observed in PwMS with TENS ON (R^2 = 0.32, adj-R^2 = 0.14, $F(3,11)$ = 1.7, p = 0.219).

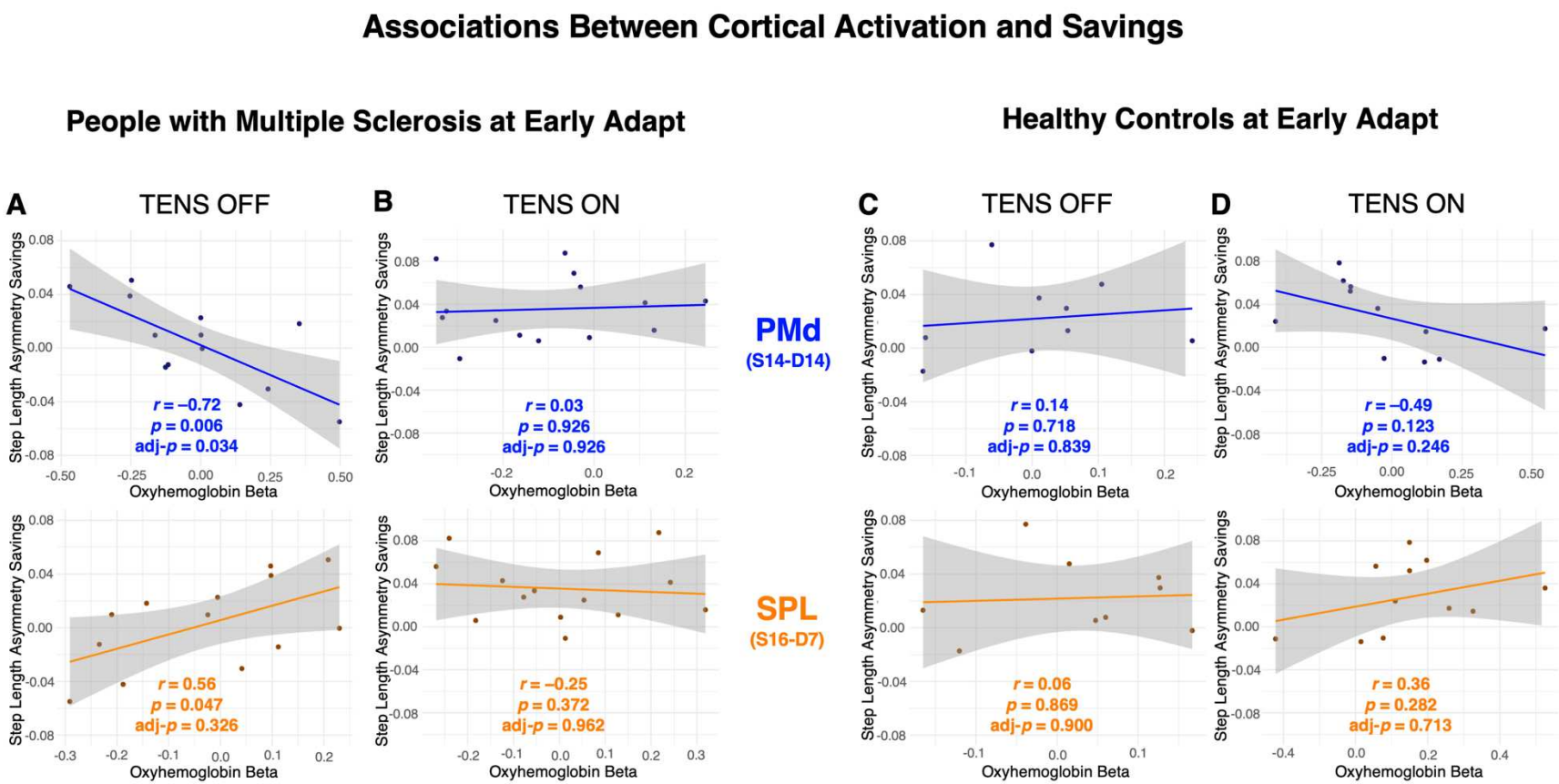

Figure 4.5. Associations between cortical activation and savings**.** Correlation plots showing relationships between step length asymmetry (SLA) savings and cortical activation, quantified as oxyhemoglobin (HbO) beta, at Early Adapt. **(A)** In people with multiple sclerosis (PwMS), a strong negative correlation was observed between savings and HbO beta in a dorsal premotor cortex (PMd) channel, with a potential correlation in a superior parietal lobule (SPL) channel (N=13). **(B)** With transcutaneous electrical nerve stimulation (TENS) ON, these correlations were no longer present in PwMS (N=15). In healthy controls (HCs), no correlations were observed at these channels under either **(C)** TENS OFF (N=9), or **(D)** TENS ON (N=11) conditions. Pearson correlations were used for all associations shown. Each data point represents an individual participant's average step length asymmetry savings and corresponding HbO beta in the specified channel.

Discussion

This randomized controlled trial investigated the impact of TENS on locomotor adaptation in PwMS and HCs. Contrary to our hypothesis, TENS did not improve adaptation rate. Rather, TENS significantly enhanced adaptation savings in PwMS, as reflected by faster relearning from Visit 1 to Visit 2, suggesting that TENS may not influence the immediate adaptation process but instead enhances retention or retrieval of previous learning. Additionally, TENS reduced cortical activation in both groups, particularly in PMd, which may indicate a shift towards more automatic control and reduced cortical involvement during adaptation.

TENS Enhances Locomotor Adaptation Savings in PwMS

Contrary to our hypothesis, TENS did not impact early change, which reflects adaptation rate. Instead, savings were significantly greater at Initial Adapt and Early Adapt for PwMS who received TENS ON during Visit 2, compared to those who received TENS OFF. Relatedly, adaptation magnitude was decreased with TENS ON as increased savings caused the SLA adaptation curve to begin at a higher point, inherently limiting the extent of further adaptation. This suggests that TENS may not improve the initial learning rate but facilitates the recall of prior learning and accelerates relearning. One possible explanation for the lack of effect on early change is that PwMS had similar early change compared to HCs adaptation, leaving limited room for further improvement. Alternatively, TENS may primarily act on the retrieval processes rather than modulating online learning. Previous work from our group corroborates this finding. Whittier et al.[28] found the greatest improvement across visits in HCs who received TENS ON during Visit 2 during a visuomotor stepping task. For HCs in our study, a similar pattern of improved savings with TENS ON during Visit 2 was observed with a moderate effect size (d = 0.59), though not significant. This suggests that while TENS could impact savings in HCs, its effects are more

pronounced in PwMS. This disparity may reflect a ceiling effect in HCs, limiting the observable benefits of TENS for those without clinical sensory impairment.

Despite a clear effect of visit on aftereffect,[52] TENS condition did not influence aftereffect. Sensorimotor adaptation relies on a two-rate process: an initial, rapidly flexible mechanism followed by a gradual, implicit recalibration of the forward model, or the neural process that predicts sensory consequences from motor commands.[62–64] Forward model recalibration, driven by sensory prediction errors (i.e. mismatches between expected and actual sensory feedback), is considered the primary mechanism underlying aftereffects upon exposure to a different environment.[65–67] Since the present data show enhanced savings at Initial Adapt and Early Adapt with TENS ON, yet no changes in aftereffect, TENS is likely modulating the rapidly flexible mechanism, rather than recalibration. This may indicate TENS is more effective when sensory prediction errors are large and when recalling a previously established forward model.[68] TENS is suggested to reduce sensorimotor uncertainty for state estimates,[28] which may be particularly beneficial when large sensory prediction errors occur[37,68] and in turn promote more precise error adjustments which are used to refine the established forward model. Mechanistically, prior work suggests applying TENS primes ascending Aα and Aβ sensory fibers potentially by altering membrane properties that enhance excitability and thus firing rate.[20,69–71] This effect would increase the quantity of sensory information transmitted from the periphery to the CNS.[32,34,72] Separately, TENS, can enhance the detection of weak sensory signals through stochastic resonance, in which the presence of low level electrical noise enhances signal processing in the nervous system.[73–75] This improved signal detection likely supports better sensorimotor integration at cortical and cerebellar levels, facilitating more accurate state estimates and sensory predication errors. In addition to TENS condition, greater vibration perception threshold and mCTSIB

proprioceptive sway were predictive of greater savings, emphasizing the importance of sensory and proprioceptive input in generating adaptation savings, which is line with other motor learning findings.[11,76–79] Prior work has also shown that TENS can reduce sway and improve proprioception, further highlighting the relevance of TENS for improving these inputs.[20,25,71,80]

Notably, the Visit 1–TENS OFF and Visit 2–TENS ON curves reflect performance from the same individuals, as do the Visit 1–TENS OFF and Visit 2–TENS ON curves. This within-participant consistency likely contributes to the visual similarity between the adaptation profiles across visits in PwMS, particularly at Late Adapt (Figure 4.3B). However, there were no differences between TENS OFF and TENS ON conditions at Late Adapt for Visit 1 (EMM = –0.019, adj-p = 0.282, d = 1.00) or Visit 2 (EMM = 0.021, adj-p = 0.225, d = 1.12), but large effects at both visits may suggest some highly varying, non-significant differences that reflect individual variability rather than an effect of TENS. Importantly, these curves were baseline adjusted and demographic factors were consistent across groups, minimizing the likelihood that these observed differences stem from overt sample disparities. By design, our analysis compares savings within participants, ensuring the improvements with TENS ON during Visit 2 are not influenced by sample heterogeneity.

TENS Reduces Cortical Activation

PwMS and HCs exhibited greater cortical activation, represented by increased HbO beta, during Early Adapt compared to Baseline. This increase was significant at the channel-wide level and at the ROI level for PMd in both groups and M1 for HCs only. Comparing activation between TENS conditions, TENS ON resulted in less cortical activation during the Early Adapt – Baseline contrast, which quantified the adaptation-related activation independent of treadmill walking. Unlike the savings findings, this effect was consistent across visits and groups, with both PwMS

and HCs exhibiting decreased activation with TENS ON for both visits. Somewhat surprisingly, PwMS did not have more activation than HCs; however, this study did not measure activation in the prefrontal cortex, a primary area where increased activation is commonly observed during motor tasks in PwMS.[19,42] This effect of TENS was strongest at the channel-wide level but also demonstrated significant differences with moderate to large effect sizes at the ROI level. Among the ROIs, PMd, M1, S1, and SPL exhibited significant decreases in activation with TENS ON for PwMS but not HCs. PMd is particularly relevant for sensorimotor adaptation as it is highly influential in motor planning and a primary node in the cortico-cerebellar loop.[81–83] During feedforward control, sensory prediction errors processed by the cerebellum relay through the ventrolateral thalamus to PMd, M1, S1, and SPL where they contribute to refining motor plans, integrating sensory information, and updating forward models of limb position and movement.[82–84] The extent of premotor activation during this process depends on the magnitude of sensory prediction errors and the complexity of motor planning required to update subsequent motor output.[83] In this study, greater PMd activation was additionally predicted by worse vibration perception threshold, emphasizing the impact of sensory function on cortical activation.

Similar to its effect on improved savings, TENS likely reduces cortical activation by enhancing firing rate of Aα and Aβ sensory fibers. These fibers are preferentially activated due to the longer pulse widths used in TENS, which selectively target large-diameter afferents without inducing muscle contractions.[20,31,70,85] By reducing sensorimotor uncertainty and enhancing input, TENS may facilitate more efficient integration of sensory prediction errors within the cortico-cerebellar loop, thereby reducing the reliance on cortical regions, such as PMd, for complex motor planning. This observed reduction in cortical activation suggests a shift away from higher-order motor control, promoting greater automaticity during adaptation. With less cortical involvement,

the reliance on subcortical structures, particularly the cerebellum, likely increases. Notably, increased cerebellar engagement, as evidenced by reduced cerebellar brain inhibition, has been associated with greater locomotor adaptation,[17] suggesting that strategies relying more on cerebellar-driven control are likely less demanding and more effective for sensorimotor adaptation.[12]

While no previous studies have investigated the effect of TENS on functional cortical activation, other work has demonstrated cortical changes with the application of similar sensory stimulation using other imaging modalities.[34] Using transcranial magnetic stimulation, Celnik et al.[33] identified that sensory stimulation improved dexterity and reduced GABAergic intracortical inhibition, which is also linked with increased cortical representation.[86] Interestingly, one group found that cortical motor representations, were increased after three weeks of daily TENS in HCs[35] but in a separate study found decreased representation in PwMS.[87] Additionally, EEG data have reported that high-frequency vibration shortened task completion time and decreased beta power over the sensorimotor cortex in people with Parkinson's disease, indicating enhanced automaticity of the task.[88] Other work has also suggested that TENS reduces sensorimotor excitability, reflected by increased alpha-2 power, during a single-leg balance task.[89] Conversely, while one study identified improved motor skill acquisition as a result of sensory stimulation, this was associated with increased N30 amplitudes in the sensorimotor areas.[29] However, this task involved sequence learning, which is less cerebellar-driven and more reliant on cortical processes compared to sensorimotor adaptation.[67] Overall, while our findings and others' suggest TENS may improve automaticity, further research is needed to uncover the distinct neural changes that occur due to TENS.

Cortical Activation Predicts Savings in PwMS with TENS OFF Only

Greater HbO beta in a PMd channel was associated with reduced savings, highlighting that increased PMd activation, reflecting higher-order motor involvement, may impede the efficiency of relearning during sensorimotor adaptation. Interestingly, this strong association was present only in PwMS with TENS OFF. With TENS ON this correlation was absent in PwMS, suggesting that adaptation is less influenced by PMd activation with TENS, potentially reflecting a shift toward more automatic or cerebellar-mediated control. In contrast, HCs exhibited no associations between activation and savings, indicating motor control strategies in PwMS more closely resemble those of HCs when TENS is applied. These correlations were further supported through regression models. In a model including all ROIs, PMd and SPL, explained a substantial portion of the variance in savings across participants only in PwMS with TENS OFF. These models were not significant in PwMS with TENS ON, reinforcing the idea that TENS may alter the neural contributions underlying savings in PwMS. While this pattern suggests that PwMS may rely on compensatory PMd engagement with TENS OFF, these analyses were exploratory, not corrected for all possible comparisons across subgroups, and the study was not powered for subgroup analyses. Increased PMd activation could reflect heightened cognitive or attentional demands in those with poorer adaptation, or maladaptive recruitment of motor planning circuits that interfere with recalibration. Future studies using imaging methods such as fMRI are needed to determine whether TENS reduces reliance on compensatory cortical mechanisms and enhances cerebellar engagement or connectivity during adaptation.

Limitations

One primary limitation of this study was absence of a group that received TENS OFF or TENS ON across both visits. These groups would have been particularly relevant, as savings from Visit 1 to Visit 2 was the primary behavioral outcome influenced by TENS. Without these

reference groups, it is possible that TENS ON during Visit 1 may have impaired savings at Visit 2, rather than TENS ON during Visit 2 improving savings as suggested throughout this article. However, Visit 1–TENS OFF was not significantly different than Visit 1–TENS ON for early change, late adaptation, adaptation magnitude, or aftereffect in PwMS or HCs, limiting the likelihood of this alternative hypothesis. Another design limitation is the absence of a sham TENS condition. This decision was intentional, as most sham protocols involve an initial stimulation period before ramping down, which would likely affect the initial and early adaptation phases that were central to our outcome measures. Importantly, because locomotor adaptation is an implicit process with minimal volitional control, the influence of placebo effects is likely limited.

Additionally, while the acquired sample size aligns with the *a priori* power analysis and with similar studies,[43] the counterbalancing between groups was imperfect due to participant dropout during the study, which affected randomization. Further, achieving equal group sizes between PwMS and HCs could have enhanced statistical power. Another sampling limitation is that compared to other cohorts of PwMS with a similar number of years since diagnosis, this sample had lower disability scores and was quite active. Accordingly, split-belt treadmill walking is a relatively complex motor task that excludes many PwMS with higher disability, limiting the generalizability of these results. Additionally, while fNIRS measures neural activation during ecologically valid tasks such as walking, it is limited by its lower spatial resolution and depth compared to other neuroimaging methods like fMRI, which is especially important for ROI analyses. Further, fNIRS can only measure cortical surface activation and does not capture subcortical or cerebellar contributions, which are essential for adaptation. Importantly, each ROI included multiple channels that were determined by their highest Brodmann area specificity. Averaging across multiple anatomically constrained channels helps reduce misclassification and

improves the reliability of ROI-level interpretations. However, our conclusions regarding specific cortical regions should be interpreted as reflecting surface-level activity within these general anatomical boundaries, rather than precise localization.

Clinical Implications and Future Directions

While previous studies have demonstrated improvements in motor control with TENS, this is the first to emphasize the importance of treatment timing during motor learning. Specifically, TENS appears to enhance retention of motor learning in populations with sensory dysfunction and may be most effective when applied after initial skill acquisition, rather than during early learning. Consequently, clinicians may consider strategically introducing TENS in later rehabilitation sessions to reinforce previously learning skills and support motor automaticity, though the optimal timing of stimulation may depend on the specific task.[90] This approach may help transition patients from conscious, effortful control to more automatic strategies. This shift is particularly important for functional movements, like walking or grasping, allowing patients to regain independence and reduce dual-task impairments. While this study applied TENS continuously during motor learning, future work should examine the optimal stimulation duration, intensity, and potential impact of nerve accommodation during extended clinical use. Moreover, TENS may offer benefits for other rehabilitation strategies in neurological conditions by reducing reliance on compensatory neural recruitment and facilitating more natural motor control strategies.

Further research is needed to better understand the mechanisms of TENS-induced improvements in motor control, especially during motor learning. Investigating how TENS affects perceptual components of adaptation or proprioceptive testing would further elucidate its effects on the sensory system.[52,91] To assess its generalizability, future studies should investigate the effect of TENS on other sensorimotor adaptations, including reaching and visuomotor perturbations,

along with different motor learning modalities, like reinforcement learning.[67] As a next step, the impact of TENS on overground transfer of locomotor adaptation should be explored. Recent evidence suggests that gradual adaptation improves overground transfer,[92] likely due to modifying credit assignment of stepping errors.[43] By decreasing sensorimotor uncertainty, TENS may augment credit assignment during adaptation, or potentially only be beneficial during the transfer stage of the paradigm, providing further insights into which stages of learning are the most responsive to TENS.

Conclusion

This study is the first to demonstrate that TENS enhances sensorimotor adaptation in PwMS, highlighting the importance of treatment timing on savings. Specifically in PwMS with mild to moderate disability (median EDSS = 3.5), TENS did not affect adaptation rate during initial exposure to the split-belt treadmill but did enhance adaptation savings. This indicates that TENS may be particularly effective in enhancing the recall of prior learning or motor memories, resulting in faster relearning. The reduction in cortical activation in both groups, suggests a shift toward reduced cortical reliance and greater automaticity during learning, even without behavioral change in HCs. These findings indicate that TENS may have broader utility in populations with sensory impairments to amplify the retention of motor learning and promote greater automaticity, factors that are key for regaining functional independence during motor rehabilitation.

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CHAPTER 5 – DISCUSSION OF OVERALL FINDINGS, IMPLICATIONS, AND FUTURE DIRECTIONS

Summary of Doctoral Work

This dissertation represents one of the first investigations to assess locomotor adaptation in people with multiple sclerosis (PwMS), a population with significant gait and sensory dysfunction.[1,2] Much existing clinical locomotor adaptation research has focused on populations such as stroke and Parkinson's disease,[3,4] and PwMS remain an understudied population that stand to benefit greatly from this intervention, due to their high prevalence of gait asymmetry.[5] Through this series of studies, it has been demonstrated that PwMS maintain the ability to adapt, even with significant sensory impairments and central nervous system damage.[6] Additionally, it was identified that propulsion and ankle joint dynamics displayed the greatest biomechanical contribution to adaptation. Most notably, this dissertation established that transcutaneous electrical nerve stimulation (TENS), a neuromodulation technique that augments sensory neurons in the periphery, enhanced adaptation savings four weeks after initial learning in PwMS. These findings elucidate both the mechanisms of locomotor adaptation in PwMS and the potential for neuromodulatory interventions to enhance motor learning in this population.

In Chapter 1, this dissertation discussed the current understanding of locomotor adaptation, with a specific focus on how different modulators impact adaptation dynamics. It also highlighted the major existing clinical gap regarding adaptation savings and overground transfer of laboratory-induced locomotor adjustments. It is well-established that locomotor adaptation on a split-belt treadmill involves both reactive and adaptive gait parameters, with interlimb measures, particularly step length asymmetry, displaying a stereotypical adaptation curve and aftereffects once the belts

returned to equal speeds.[7] This chapter also compiled the extensive research on the neural mechanisms of adaptation and storage of this new walking pattern. Overall, sensory prediction errors originating in the cerebellum[8,9] are heavily implicated in the adaptive process as demonstrated by studies using cerebellar lesions,[10,11] neuromodulation,[12,13] and neuroimaging.[14,15] Additionally, researchers have explored strategies to modulate adaptation, such as error size,[16,17] sensory environments,[18] and feedback,[19] each revealing unique effects on adaptation dynamics. Despite these advances, there remains a dearth of studies examining retention and overground transfer of this adaptation, which are the dynamics most relevant for clinical utility.

In the first empirical study, Chapter 2 examined the efficacy of locomotor adaptation in PwMS. Namely, do PwMS maintain the ability to adapt their stepping in space and in time in response to the split-belt perturbation? This study suggested that while PwMS do maintain the ability for locomotor adaptation, this does not result in improved gait symmetry for all participants. Instead, greater baseline asymmetry in both spatial and temporal parameters was predictive of larger symmetry improvements following adaptation. Additionally, spatial and temporal adaptation occurred independently, consistent with findings in other populations.[20]

Chapter 3 assessed the biomechanical contributions toward locomotor adaptation in PwMS. Propulsive force modulation throughout the adaptation paradigm had the largest kinetic contribution, while ankle joint dynamics had the largest kinematic contribution. Specifically, peak propulsion asymmetry increased significantly during the split-belt perturbation, demonstrated aftereffects, and was closely associated with a decrease in peak dorsiflexion asymmetry. During adaptation, PwMS exhibited an earlier onset of plantarflexion during the stance phase, a response not typically occurring in healthy controls unless belt speed ratios are more drastic.[21] Additionally,

both the kinetic and kinematic changes in the fast limb experienced predictive feedforward adaptation, whereas the changes in slow limb only relied on reactive feedback adjustments.

In a randomized controlled trial, Chapter 4 investigated the impact of transcutaneous electrical nerve stimulation (TENS) on locomotor adaptability in PwMS and healthy controls. Contrary to the hypothesis, TENS did not alter adaptation rate. However, perhaps more meaningfully, TENS enhanced adaptation savings, demonstrating an improved retention and retrieval of learning when re-exposed to the split-belt perturbation four weeks later. Interestingly, this effect was only exhibited in PwMS, suggesting that the augmented sensory input from TENS is particularly beneficial for individuals with sensory impairments. Further, TENS reduced cortical activation in both PwMS and healthy controls, indicating decreased cortical reliance and greater automaticity during learning.

Clinical Implications

In the United States, 12.2% of adults have a mobility disability with serious difficulty walking,[22] making the preservation and improvement of walking ability a major public health priority. This is especially relevant for individuals with neurological conditions such as multiple sclerosis, stroke, Parkinson's disease, and spinal cord injury, as well as older adults who have age-related mobility decline. In PwMS specifically, over 93% experience some degree of walking impairment over the disease course,[6] with gait dysfunction consistently reported as one of the most debilitating symptoms affecting quality of life.[23,24] Walking impairments in PwMS lead to increased fall risk, reduced participation in daily activities, and social withdrawal, emphasizing the need for effective interventions to enhance walking function and independence in this population.[23,25,26]

This dissertation shows that PwMS maintain the ability to adapt locomotion despite significant central nervous system damage. Many PwMS in these studies reported sensory dysfunction, primarily in the lower limbs, yet were still able to adapt their gait when exposed to the split-belt treadmill. This finding is clinically meaningful because it suggests that the nervous system prioritizes adaptability and retains plasticity, even in the presence of white matter degeneration. However, the extent of adaptation in PwMS was significantly lower than in healthy controls, indicating some disruption in learning. Additionally, vibration perception and proprioceptive balance were predictive of adaptation savings across PwMS and healthy controls, reinforcing the role of sensory input in motor learning. These results suggest that interventions targeting sensory function may make locomotor adaptation more effective in PwMS, potentially leading to improved rehabilitation outcomes.

A key finding from this dissertation is that TENS can enhance the retention of locomotor adaptation in PwMS. Specifically, TENS improved adaptation savings when participants were re-exposed to the split-belt perturbation four weeks later. Importantly, this effect was observed only when TENS was applied during the retention test, not during initial learning. This result suggests that the effectiveness of TENS is dependent on treatment timing and could be used strategically after initial motor learning to reinforce consolidation and improve long-term retention. Healthy controls did not exhibit enhanced savings with TENS, suggesting that the intervention is most beneficial for individuals with sensory impairments. Importantly, TENS also reduced cortical activation in both PwMS and healthy controls, indicating that TENS may facilitate motor learning by reducing compensatory neural activation and promoting more automatic movement patterns. These findings indicate that TENS has potential as a clinical tool to improve long-term motor learning in PwMS, making it a promising addition to existing rehabilitation strategies.

Future Directions

Despite the growing body of research on locomotor adaptation, its long-term outcomes, particularly savings and overground transfer, remain understudied. Yet these are the most pertinent aspects for developing clinically relevant, long-term gait rehabilitation strategies. While locomotor adaptation on a split-belt treadmill has been shown to acutely decrease gait asymmetry, few studies have successfully translated this paradigm into lasting improvements in gait coordination.[27] From this dissertation, the clear next direction would be to assess overground transfer of locomotor adaptation in PwMS, as many clinical populations actually have increased overground transfer compared to healthy controls.[28] Understanding the extent of overground transfer in PwMS would help determine both the clinical feasibility of locomotor adaptation as a rehabilitation tool, and the underlying neural mechanisms of overground transfer, by assessing this ability in a population with sensory dysfunction.

A principal question moving forward is whether TENS can enhance overground transfer both in acute testing and in the long term. The timing and frequency of TENS during acute overground transfer or during repeated interventions (e.g. three sessions per week for eight weeks) should be carefully considered. This dissertation suggests that TENS improves adaptation retention when applied during re-exposure rather than initial learning, but whether this pattern holds in a longitudinal intervention remains unknown. If TENS primarily strengthens retrieval rather than acquisition, it may be hypothesized that TENS will be most effective when administered during overground transfer, rather than during learning. Further, although healthy controls did not show improved retention with TENS in this study, they did exhibit reduced cortical activation, raising the possibility that TENS during overground transfer or longitudinal interventions could reveal benefits even in neurologically intact individuals.

Beyond overground transfer, future research should explore the broader applications of sensory augmentation in motor learning. Studies should investigate whether TENS has similar effects on locomotor adaptation in other neurologically impaired populations, such as stroke or Parkinson's disease, and whether pairing TENS with other motor learning strategies, including use-dependent plasticity or reinforcement, could further enhance learning retention and functional outcomes.

Ultimately, translating locomotor adaptation research into clinical practice will require studies that move beyond short-term outcomes and assess functional benefits in real-world settings. Examining how well PwMS retain and generalize adapted walking patterns across different environments and the impact of TENS on generalizability will be critical for determining the rehabilitative potential of locomotor adaptation and TENS.

Conclusion

This dissertation examined the locomotor adaptability of PwMS, demonstrating that despite central nervous system damage, PwMS maintained the ability to adapt feedforward control to successfully alter spatial and temporal walking patterns similarly to other populations. A biomechanical analysis of this adaptation in PwMS suggested that increases in propulsive force and decreases in dorsiflexion during split-belt walking are the main contributors generating this new walking pattern. Importantly, applying TENS during re-exposure to the split-belt perturbation improved adaptation retention and reduced compensatory neural activation. Together, these findings show the potential for improving gait symmetry in PwMS using locomotor adaptation that can be enhanced by neuromodulation, warranting future investigation into the generalizability of this learning to overground walking and rehabilitations settings.

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APPENDIX A – CHAPTER 2 ADDITIONAL DATA AND COMMENTARY

In Chapter 2 of this dissertation, the primary analysis assessed if split-belt treadmill walking improved gait symmetry relative to baseline in people with multiple sclerosis (PwMS). This chapter did not directly examine the level of adaptability in this sample, rather it only focused on whether adaptation lead to improved gait symmetry (i.e. step length asymmetry (SLA) approaching zero). A large portion of this sample had low baseline asymmetry, which may have caused them to overshoot an SLA of zero during adaptation and become more asymmetrical in the opposite direction. Here, additional data investigates if PwMS exhibit adaptability on the split-belt treadmill, regardless of if this led to improved symmetry. All 35 participants adapted successfully and demonstrated a robust response to the split-belt treadmill walking paradigm. Specifically, participants exhibited a significant group-level change in SLA ($p < 0.001$, $d = 1.46$) from baseline to post-adaptation (i.e., SLA aftereffect), indicating storage of a new walking pattern (Table A1). This suggests that while not all participants improve symmetry relative to baseline, likely due to the overshoot effect, all PwMS maintained the ability to adapt on the split-belt treadmill and generate aftereffects.

Table A1. Step length asymmetry (SLA) aftereffect (change from baseline to post-adaptation) in people with multiple sclerosis following the split-belt treadmill walking paradigm. Reported as mean (standard deviation).

SLA Baseline (mm)	SLA post-adaptation (mm)	SLA Aftereffect (mm)	*p*	*d*
–18.81 (69.13)	73.00 (55.87)	91.81 (48.81)	$< 0.001^{***}$	1.46

APPENDIX B – CHAPTER 3 SUPPLEMENTAL MATERIAL

Table B1. 2 x 5 RMANOVA pairwise results for each force variable. Following identification of a significant interaction effect pairwise comparisons between each timepoint were made using false discovery rate to correct for multiple comparisons. MA = more affected, LA = less affected

Variable	Contrast	Interaction Effect (*F*)	Estimate (% BW)	Effect Size (*d*)
Propulsion Asymmetry[1]	Early Adapt – Baseline	38.78***	0.054***	1.40
	Late Adapt – Early Adapt		0.030***	0.56
	Early Post-Adapt – Baseline		-0.028***	0.79
MA Peak Propulsion Impulse[2]	Early Adapt – Baseline	11.51***	-0.12	0.075
	Late Adapt – Early Adapt		0.80***	0.47
	Early Post-Adapt – Baseline		-0.71***	0.49
LA Peak Propulsion Impulse[2]	Early Adapt – Baseline		-1.61***	1.19
	Late Adapt – Early Adapt		0.15	0.13
	Early Post-Adapt – Baseline		0.11	0.07
MA Peak Propulsion	Early Adapt – Baseline	12.09***	-0.022**	0.39
	Late Adapt – Early Adapt		0.048***	0.81
	Early Post-Adapt – Baseline		-0.026***	0.48
LA Peak Propulsion	Early Adapt – Baseline		-0.076***	1.61
	Late Adapt – Early Adapt		0.018**	0.48
	Early Post-Adapt – Baseline		0.0015	0.03
MA Peak Braking	Early Adapt – Baseline	8.12***	-0.078***	1.16
	Late Adapt – Early Adapt		0.11***	1.51
			0.025*	0.25

	Early Post-Adapt – Baseline			
LA Peak Braking	Early Adapt – Baseline		-0.039***	0.62
	Late Adapt – Early Adapt		-0.010	0.18
	Early Post-Adapt – Baseline		-0.067***	1.15
MA Peak Early Vertical GRF	Early Adapt – Baseline	4.92***	-0.10***	0.81
	Late Adapt – Early Adapt		0.054**	0.45
	Early Post-Adapt – Baseline		0.025	0.15
LA Peak Early Vertical GRF	Early Adapt – Baseline		0.0016	0.012
	Late Adapt – Early Adapt		0.032	0.26
	Early Post-Adapt – Baseline		-0.055***	0.51
MA Peak Late Vertical GRF	Early Adapt – Baseline	6.51***	-0.12***	1.22
	Late Adapt – Early Adapt		0.068**	0.72
	Early Post-Adapt – Baseline		-0.013	0.14
LA Peak Late Vertical GRF	Early Adapt – Baseline		-0.044**	0.60
	Late Adapt – Early Adapt		0.013	0.24
	Early Post-Adapt – Baseline		-0.083***	0.97

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, 1. In the case of interlimb parameters, this is a main effect of timepoint rather than an interaction effect of limb x timepoint. 2. Units for impulse are %BW x %Stance.

Table B2. 2 x 5 RMANOVA pairwise results for each kinematic variable. Following identification of a significant interaction effect pairwise comparisons between each timepoint were made using false discovery rate to correct for multiple comparisons. MA = more affected, LA = less affected

Variable	Contrast	Interaction Effect (*F*)	Estimate (degrees)	Effect Size (*d*)
Peak Dorsiflexion Asymmetry[1]	Early Adapt – Baseline	17.72***	-5.27***	0.97
	Late Adapt – Early Adapt		-2.98**	0.38
	Early Post-Adapt – Baseline		2.57**	0.55
MA Peak Dorsiflexion Time[2]	Early Adapt – Baseline	41.17***	-22.34***	1.64
	Late Adapt – Early Adapt		5.21	0.32
	Early Post-Adapt – Baseline		2.25	0.35
LA Peak Dorsiflexion Time[2]	Early Adapt – Baseline		8.86**	0.79
	Late Adapt – Early Adapt		1.48	0.19
	Early Post-Adapt – Baseline		-16.66***	0.94
MA Peak Hip Extension	Early Adapt – Baseline	3.97**	1.51**	0.24
	Late Adapt – Early Adapt		-2.97***	0.44
	Early Post-Adapt – Baseline		1.32	0.20
LA Peak Hip Extension	Early Adapt – Baseline		5.80***	0.86
	Late Adapt – Early Adapt		-1.31	0.20
	Early Post-Adapt – Baseline		-0.50	0.72
MA Knee Flexion[3]	Early Adapt – Baseline	0.30	-0.93	0.079
	Late Adapt – Early Adapt		0.35	0.042
	Early Post-Adapt – Baseline		-0.57	0.054
LA Knee Flexion[3]	Early Adapt – Baseline		-3.69	0.38

	Late Adapt – Early Adapt		2.05*	0.22
	Early Post-Adapt – Baseline		0.47	0.049
MA Peak Plantarflexion	Early Adapt – Baseline	17.10***	-2.23*	0.23
	Late Adapt – Early Adapt		-2.67*	0.52
	Early Post-Adapt – Baseline		2.82**	0.35
LA Peak Plantarflexion	Early Adapt – Baseline		9.92***	1.40
	Late Adapt – Early Adapt		1.54	0.26
	Early Post-Adapt – Baseline		0.29	0.04
MA Peak Dorsiflexion	Early Adapt – Baseline	13.90***	-1.87**	0.38
	Late Adapt – Early Adapt		-1.18	0.24
	Early Post-Adapt – Baseline		1.35	0.26
LA Peak Dorsiflexion	Early Adapt – Baseline		3.40***	0.71
	Late Adapt – Early Adapt		2.40*	0.38
	Early Post-Adapt – Baseline		-1.23	0.29

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, 1. In the case of interlimb parameters, this is a main effect of timepoint rather than an interaction effect of limb x timepoint. 2. Units for peak dorsiflexion time are % gait cycle. 3. In cases of an insignificant interaction effect, pairwise comparisons were performed for demonstrative purposes but did not test for significance.

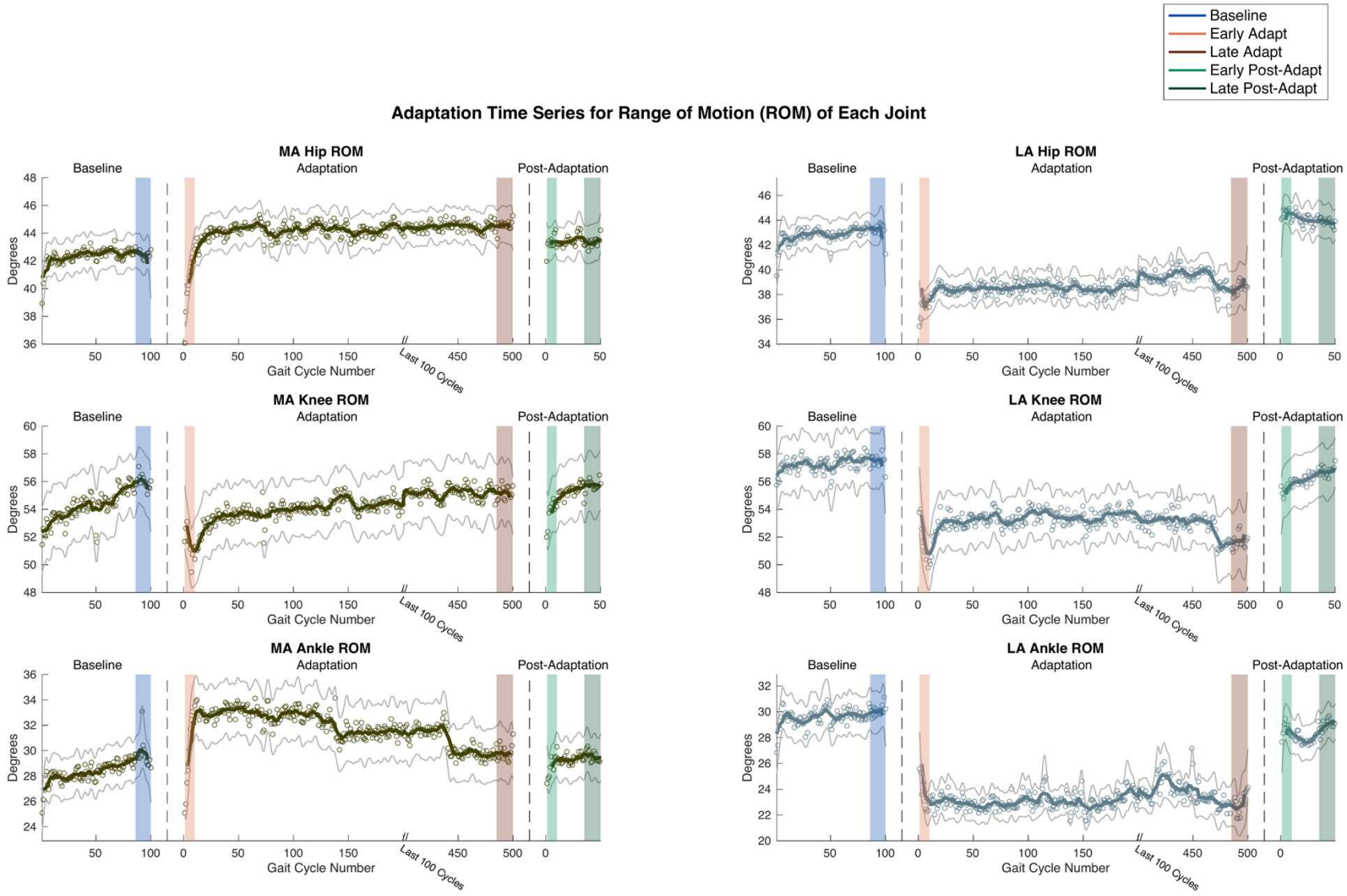

Figure B1. Range of motion (ROM) adaptation for the more affected (MA) and less affected (LA) hip, knee, and ankle for Baseline, Adaptation, and Post-Adaptation. Light grey lines indicate standard error for each point across all participants. The LA limb had immediate decreases in ROM in all joints from Baseline to Adaptation but demonstrated no adaptation.

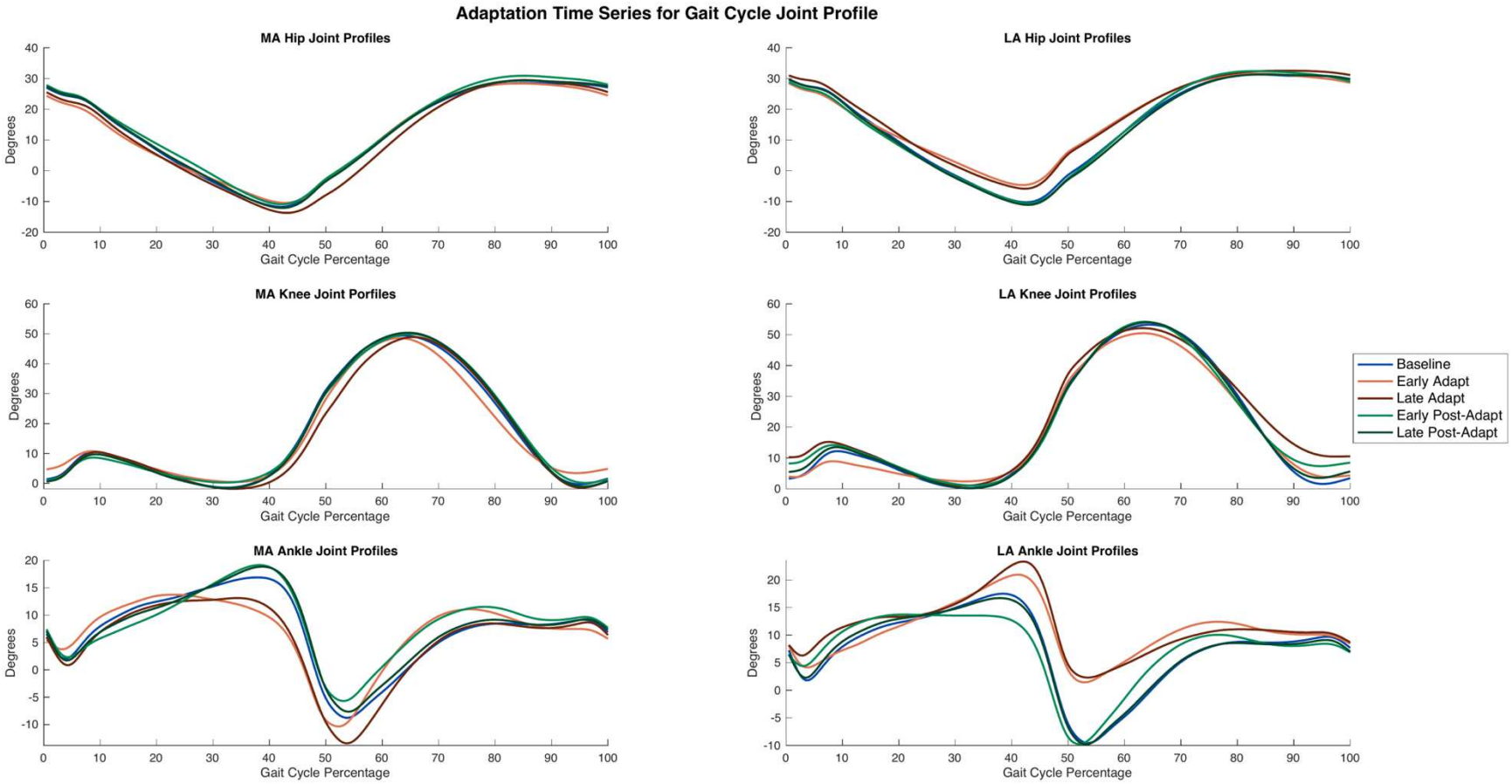

Figure B2. Joint profiles for the more affected (MA) and less affected (LA) hip, knee, and ankle for each timepoint of the split-belt adaptation paradigm. Joint profiles are averaged across all participants. Majority of change between timepoints occurred at the ankle joint, with less change occurring in the hip and knee joints.

APPENDIX C – CHAPTER 4 SUPPLEMENTAL MATERIAL

Table C1. Channel locations and region of interest (ROI) designations. Channels formed by source-detector pairs were localized using Montreal Neurological Institute (MNI) coordinates and anatomical landmarks, identified through the Brodmann atlas. ROIs were determined based on the Brodmann area with the highest specificity for each channel. ROIs included dorsal and ventral premotor areas (PMd, PMv), primary motor and somatosensory cortices (M1, S1), and superior and inferior parietal lobules (SPL, IPL).

Channel	Source	Detector	Channel coordinates (MNI)	Brodmann areas	ROI Designation
1	1	1	(-63,-9,-12)	21	
2	1	2	(-65,-18,4)	22,21	
3	2	2	(-63,-32,23)	22	
4	2	3	(-57,-57,21)	39	IPL
5	2	4	(-57,-48,38)	40,39	IPL
6	3	1	(-59,11,9)	6,44	PMv
7	3	2	(-62,-3,23)	4,43	M1
8	3	5	(-55,12,34)	6,44	PMv
9	4	2	(-60,-18,37)	3,2	S1
10	4	4	(-52,-34,52)	2,3,1	S1
11	4	5	(-50,-3,50)	6	PMd
12	4	6	(-42,-20,62)	4	M1
13	5	3	(-46,-72,30)	39	IPL
14	5	4	(-46,-61,46)	39,40	IPL
15	5	7	(-32,-73,47)	7	SPL
16	6	4	(-39,-48,60)	2,3,40	S1
17	6	6	(-27,-36,71)	4,3	
18	6	7	(-24,-62,62)	7	SPL
19	6	8	(-16,-50,72)	1,5	S1
20	7	5	(-38,12,55)	6,9	PMd

21	7	6	(-26,-5,68)	6	PMd
22	8	6	(-17,-20,74)	4,6	M1
23	8	8	(1,-35,75)	4	M1
24	8	14	(17,-21,75)	4,6	M1
25	9	9	(66,-8,-12)	21	
26	9	10	(67,-19,4)	22,21	
27	10	9	(61,11,8)	6,44	PMv
28	10	10	(64,-5,22)	4,43	S1
29	10	13	(56,12,33)	6,44	PMv
30	11	10	(65,-33,23)	22	
31	11	11	(58,-58,22)	39	IPL
32	11	12	(58,-48,38)	40,39	IPL
33	12	11	(47,-72,30)	39	IPL
34	12	12	(46,-62,47)	39,40	IPL
35	12	15	(33,-74,48)	7	SPL
36	13	10	(62,-20,37)	2,3,1	S1
37	13	12	(53,-35,52)	2,3,1	S1
38	13	13	(52,-4,48)	6	PMd
39	13	14	(42,-21,62)	4	M1
40	14	13	(39,12,54)	6,9	PMd
41	14	14	(27,-4,68)	6	PMd
42	15	8	(17,-50,73)	3,1,5	S1
43	15	12	(39,-49,60)	2,3,40	S1
44	15	14	(28,-36,71)	4,3	
45	15	15	(25,-62,63)	7	SPL
46	16	7	(-13,-73,56)	7	SPL
47	16	8	(2,-61,66)	5,7	SPL
48	16	15	(15,-73,57)	7	SPL

Table C2. Region of interest (ROI) model results. Fixed-effects ANOVA results from linear mixed-effects models of oxyhemoglobin (HbO) change across individual regions of interest (ROIs). Surprisingly, only one significant interaction was present. ROIs included dorsal and ventral premotor areas (PMd, PMv), primary motor and somatosensory cortices (M1, S1), and superior and inferior parietal lobules (SPL, IPL). FDR = false discovery rate.

PMd Activation

Predictor	*df*	*F*	*p*	FDR-adj. *p*
Group	1, 44	0.3	0.595	0.838
Visit	1, 44	0.3	0.614	0.737
TENS Condition	1, 44	12.1	0.001	0.003**

PMv Activation

Predictor	*df*	*F*	*p*	FDR-adj. *p*
Group	1, 44	0.5	0.484	0.838
Visit	1, 44	0.3	0.577	0.737
TENS Condition	1, 44	0.8	0.378	0.378
Group:Visit Interaction	1,44	7.7	0.008	0.049*

M1 Activation

Predictor	*df*	*F*	*p*	FDR-adj. *p*
Group	1, 44	0.2	0.627	0.838
Visit	1, 44	0.5	0.491	0.737
TENS Condition	1, 44	10.6	0.002	0.003**

S1 Activation

Predictor	*df*	*F*	*p*	FDR-adj. *p*
Group	1, 44	0.8	0.366	0.838
Visit	1, 44	0.8	0.383	0.737
TENS Condition	1, 44	13.7	< 0.001	0.003**

SPL Activation

Predictor	*df*	*F*	*p*	FDR-adj. *p*
Group	1, 44	0.0	0.838	0.838
Visit	1, 44	0.0	0.968	0.968

TENS Condition	1, 44	10.2	0.002	0.003**

IPL Activation

Predictor	***df***	***F***	***p***	**FDR-adj. *p***
Group	1, 44	0.0	0.772	0.838
Visit	1, 44	1.1	0.307	0.737
TENS Condition	1, 44	4.4	0.039	0.047*

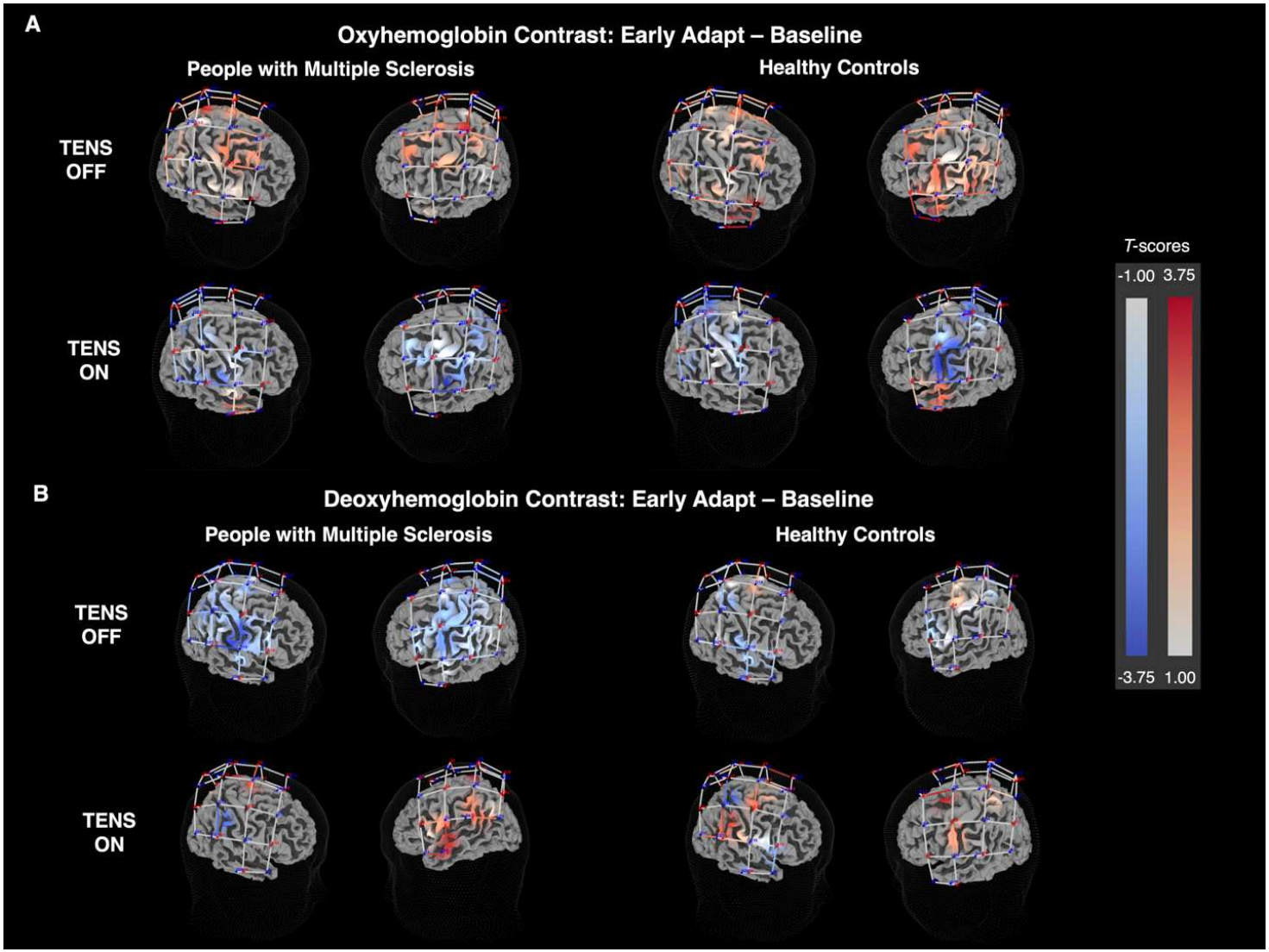

Figure C1. Oxyhemoglobin (HbO) and deoxyhemoglobin (HbR) changes from Baseline to Early Adapt. **(A)** Bilateral views and *t*-score map showing HbO beta changes for the Early Adapt – Baseline contrast across groups and TENS conditions. **(B)** Bilateral views and *t*-score map of HbR beta changes for the Early Adapt – Baseline contrast across groups and TENS conditions. T-scores were calculated using a random effects model including 28 people with multiple sclerosis and 20 healthy controls.