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Characterizing Interactions Between Descending Cortical and Spinal Sensorimotor Circuits Across the Adult Lifespan and their Implications for Balance Control

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Abstract

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Successful balance performance remains a crucial prerequisite for human beings to safely navigate activities of daily living. However, deficits in balance ability across the human lifespan remain important intrinsic factors for increased risk of falls. The aging process can substantially contribute to poor balance function due to changes in underlying neural circuitry. Human sensorimotor circuits in the cortex and spinal cord have been evaluated using non-invasive stimulation techniques to characterize their physiological function and connectivity. Yet, the complex interactions between cortical and spinal sensorimotor circuits across the adult lifespan and their relation to successful balance control remain poorly understood. To advance our understanding of balance control in humans, the use of non-invasive paired stimulation paradigms has enabled novel insights into interactions between descending cortical and spinal sensorimotor circuits. This dissertation investigates the neurophysiological correlates of balance control by characterizing interactions between descending cortical and spinal sensorimotor circuits across the adult lifespan. In Study 1, we replicated previous research that has demonstrated the ability of descending cortical circuits to modulate the excitability of spinal reflexes; however, our results revealed that modulation occurred throughout the physiological recruitment order of spinal reflexes and descending cortical influences contributed to changing the reflex gain in healthy adults. These findings can inform future studies investigating neurophysiologic processes that modulate recruitment order and reflex gain in conditions of abnormal balance control. Additionally, in Study 2, we examined the relative timing of converging descending cortical inputs with respect to ascending afferent inputs within the spinal cord. While standardized timing between converging inputs resulted in significant descending modulation of spinal reflexes, individual-specific timing between inputs resulted in significantly greater descending modulation of spinal reflexes. These findings suggest that using individual-specific stimulation parameters when measuring descending modulation of spinal reflexes can inform future studies on the complex array of connections between descending cortical and spinal sensorimotor circuits. Finally, in Study 3, we investigated the effects of aging-related changes and task-related activation on descending modulation of spinal reflexes. We demonstrated that task-related activation resulted in differential modulation of spinal reflexes via direct and indirect descending circuits, and aging resulted in greater descending inhibitory control of spinal reflexes during task-related activation. Taken together, our results suggest that descending cortical and spinal sensorimotor circuits have differentially important roles throughout the adult lifespan in the regulation of spinal circuit activity, and these interactions remain highly implicated in the neural control of balance ability.

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Chapter 1: General Introduction

1.1 Introduction

Across the lifespan, balance ability remains an important health- and activity-related component of everyday life (e.g., walking, climbing stairs). Balance control involves static and dynamic conditions, which can be subdivided into four types: static steady-state balance (e.g., standing), dynamic steady-state balance (e.g., walking), proactive balance (e.g., anticipated slip on a wet floor), and reactive balance (e.g., tripping) (Shumway-Cook & Woollacott, 2016). While some previous studies have implied that these different types of balance were hardly associated and showed only small correlations among each other (Bohannon, 2006; Springer et al., 2007), others have suggested that the various types of balance were highly interlinked (Meinel & Schnabel, 2018; Schnabel et al., 2014). While the above-mentioned four types of balance may or may not be representatives of one general balance ability, successful balance performance remains a crucial prerequisite for human beings to safely navigate activities of daily living.

Deficits in balance performance have been identified as important intrinsic factors increasing the risk of falling and sustaining injuries for all age groups (Fousekis et al., 2011; Razmus et al., 2006; Rubenstein, 2006; H.-K. Wang et al., 2006). In particular, the process of aging appears to be a substantial contributor to deficits in balance performance, which can lead to increased fall risk. Falls remain a major concern for older adults and a leading cause of accidental death in older adults (Deandrea et al., 2010), where 1 in 4 older adults fall every year and 1 out of 5 falls result in serious injury. The medical costs associated for both fatal and non-fatal falls are approximately $50 billion per year (Florence et al., 2018), and the number of falls and fall-related injuries will continue to increase as the population of older adults increases (Moreland et al., 2020). With a sharp rise in the older adult population in the United States and other developed countries, the importance of characterizing how aging-related changes impact the underlying neural circuitry responsible for successful balance control becomes imperative.

Aging results in structural and functional changes across the neural axis, which influences motor task performance (Papegaaij et al., 2014). Structural degeneration of gray and white matter (Ge et al., 2002; McGinnis et al., 2011; Raz et al., 2004) and peripheral nerves (Rivner et al., 2001) cause functional deterioration, which can trigger the need for functional compensation (Bernard & Seidler, 2012; Mattay et al., 2002) to successfully perform motor tasks. These aging-related changes occur on the cortical level, as well as the peripheral and spinal level. For example, aging results in greater cortical engagement during performance of balance tasks, which appears to be a compensatory mechanism for older adults with deficits in balance (Palmer et al., 2021; Payne et al., 2021). Additionally, aging causes a decrease in the strength of signal transmission along the neural axis, which stems from a decrease in the number of myelinated fibers and degeneration of remaining myelin sheaths (Scaglioni et al., 2003; Verdú et al., 2000). Thus, the age-related reorganization of cortical and spinal circuits responsible for balance control may be a substantial contributor to increased falls risk in older adults. Yet, there are still significant knowledge gaps in our understanding of aging-related effects on neural mechanisms contributing to cortical and spinal circuit function, specifically the interactions and relationship between these circuits during motor tasks. Understanding how aging impacts neural circuitry responsible for balance control is a crucial prerequisite to developing neurobiology-informed treatments to improve balance and reduce falls risk in older individuals, with future work parsing out the effects of aging versus the effects of neuropathologies such as stroke or Parkinson's disease.

1.2 Neural Circuits Implicated in Balance

An orchestrated integration of sensorimotor processes throughout the neural axis is needed for successful balance maintenance. It is well known that visual, proprioceptive, auditory, and vestibular systems contribute to one's ability to maintain balance, as well as the integration of those sensory inputs and the resulting motor output to musculature responsible for balance. Here, I will explore and discuss the neural circuits implicated in balance control.

1.2.1 The Spinal Reflex Pathway

The spinal reflex pathway, which mediates stretch reflexes, consists of group Ia afferent fibers that make excitatory connections with alpha (α)-motoneurons at the spinal segmental level (H. H. Chen et al., 2003; Meunier & Pierrot-Deseilligny, 1989; Pierrot-Deseilligny & Mazevet, 2000; Z. Wang et al., 2012) (**Figure 1-1**). The Hoffmann (H-) reflex is the electrical analogue of the monosynaptic stretch reflex that bypasses the muscle spindle and fusimotor activity, is used to evaluate the excitability of the monosynaptic spinal reflex pathway (Gassel, 1969; Knikou, 2008; Schieppati, 1987), and is commonly measured in upper and lower extremity muscles using surface electromyography (EMG) (Burke, 2016; Gassel & Diamantopoulos, 1966; Granit & Job, 1952). H-reflexes are evoked by low-intensity surface electrical stimulation of a mixed peripheral nerve (peripheral nerve stimulation, PNS), which trans-synaptically excites the lower motor neuron (LMN) pool and are influenced by pre- and post-synaptic mechanisms (Earles et al., 2002; Komiyama et al., 1999; Milanov, 2000). Average range of onset latencies of soleus H-reflexes in healthy adults aged 18 to 35 years are typically 34 ± 3 msec (Taube et al., 2006) and have been shown to be positively correlated with leg length (Falco et al., 1994). H-reflex responses recorded over a range of PNS intensities generates the H-reflex recruitment curve, which provides useful information regarding reflex gain and metrics of spinal reflex excitability such as the peak H-reflex amplitude (Funase et al., 1994; Mazzocchio et al., 2001). Understanding the shape and behavior of the H-reflex recruitment curve may provide novel insights into the physiologic processes that modulate the recruitment order of spinal LMNs.

The H-reflex can be used as a probe to study spinal inhibitory circuits and neuronal pathways and mechanisms at rest and during movement in humans. Presynaptic inhibition of Ia afferents (Hayes et al., 2009; Meunier & Pierrot-Deseilligny, 1998; Milanov, 2000), reciprocal Ia inhibition (Hanna-Boutros et al., 2015; Hultborn, 1976; Rossi et al., 1988), non-reciprocal, or Ib, inhibition (Faist et al., 2006; Stephens & Yang, 1996), and recurrent inhibition via Renshaw cells (Bussel & Pierrot-Deseilligny, 1977; Ross et al., 1975, 1976) have all been shown to modulate the H-reflex response. When H-reflex recordings are conducted with participants at rest, influences from supraspinal centers are anticipated to be minimal and spinal inhibitory interneurons (i.e., Renshaw cells, Ia / Ib inhibitory interneurons) that are affected by muscle contraction might be less active. If the H-reflex is recorded during voluntary sustained contraction of the homonymous muscle, the amplitude of the H-reflex may be affected by changes of α-motor neuron excitability due to descending excitation, contraction-associated sensory feedback, decrease of Ib inhibition (Marchand-Pauvert et al., 2002), and changes in recurrent inhibition or presynaptic inhibition (Hultborn et al., 1987). However, methodologies that investigate spinal inhibitory circuits during rest and active motor tasks do not typically evaluate the influence of descending corticomotor pathways on spinal reflexes. Hence, alternative techniques are needed to determine the influence of descending corticomotor inputs on spinal reflex excitability.

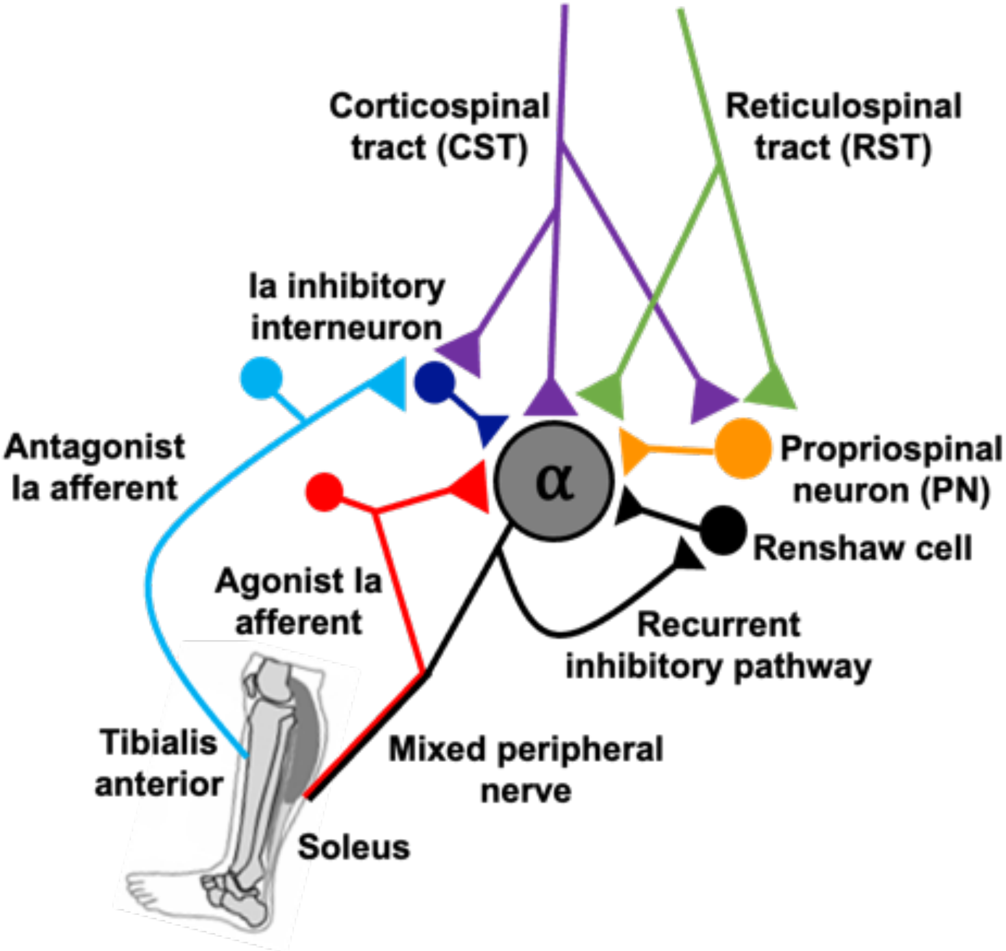

Figure 1-1. **Simplified illustration of human spinal circuitry involved in regulating the monosynaptic reflex arc.** The circuitry of the soleus reflex pathway is depicted. The main and principal circuit is the Ia afferent - alpha (α)-motor neuron pathway within a mixed peripheral nerve from the soleus muscle (ascending red line and descending black line). α-motor neurons receive inputs from multiple sources: descending tracts (purple and green lines), peripheral afferents (light blue and red lines), spinal interneurons (dark blue line), propriospinal neurons (orange line), and the recurrent inhibition pathway (black lines). To activate peripheral musculature for movement, whether volitional or reflexive, the activation of α-motor neurons and resulting efferent signal to the muscle become the final common path in the motor system.

1.2.2 Descending Cortical and Subcortical Pathways

Unlike the spinal reflex pathway, volitional movement is generated when pyramidal tract neurons (PTNs) in the primary motor cortex (M1) activate and conduct descending excitatory signals to spinal lower motor neurons (LMNs) that innervate target muscle groups. Subcortical and brainstem-mediated descending pathways can also project onto spinal LMNs, thus influencing the excitability of the spinal LMN pool (Bagust et al., 1985; Büschges, 2017; Nielsen & Petersen, 1995b). Descending corticomotor pathways can be described in two distinct categories: direct or fast-conducting and indirect or slower-conducting pathways. Direct pathways mainly originate in the cerebral cortex and typically have faster-conducting descending tracts that connect directly with spinal LMNs. The corticospinal tract (CST) originates mainly from pyramidal cells in layer V of the cerebral cortex, and projections descend into the spinal cord to synapse directly and/or indirectly onto spinal LMNs. Importantly, CST neurons also have many collateral fibers that make connections in a number of subcortical and brainstem areas including the basal ganglia, thalamus, and multiple sensory nuclei. Previous studies in non-human primates and human subjects report the average conduction velocity of these tracts to be approximately 70 ± 5 m/s (Kraskov et al., 2019; Ugawa et al., 1995).

Alternatively, indirect pathways, which cortical motor areas activate indirectly via neurons in the brainstem, typically have slower-conducting descending tracts that directly or indirectly terminate on LMNs in the spinal cord. Two examples of indirect pathways are the reticulospinal tracts, which arise in the pontine and medullary areas of the reticular formation, and the vestibulospinal tracts, which arise from lateral and medial vestibular nuclei. Reticulospinal fibers can influence alpha (α)-motor neurons through monosynaptic or polysynaptic connections, and there is significant overlap in the termination of corticospinal and reticulospinal projections to spinal segmental interneurons (Baker, 2011; Lemon, 2008). Vestibulospinal fibers act with reticulospinal fibers in the control of muscle tone, potentiating the stretch reflex of antigravity muscles through monosynaptic connections with alpha and gamma motor neurons as well as via interneurons. Together, the reticulospinal and vestibulospinal tracts provide balanced excitatory and inhibitory descending regulation of the spinal sensorimotor circuitry (Li & Francisco, 2015). Regardless of the types of descending inputs, spinal LMNs remain the final common pathway for all motor output (**Figure 1-1**).

One non-invasive technique to probe the influence of descending inputs that is commonly used in humans is transcranial magnetic stimulation, or TMS (Iriarte & George, 2018; Klomjai et al., 2015; Ziemann et al., 2015). TMS delivered over the primary motor cortex (M1) is used to non-invasively characterize excitability of descending cortical pathways (Barker et al., 1985; Groppa et al., 2012; Hallett, 2007). A single supra-threshold TMS pulse delivered over M1 elicits a motor evoked potential (MEP) in the contralateral targeted muscle (Barker et al., 1985; Rossini et al., 2015). Although the amplitude of the MEP provides an overall index of corticomotor excitability, standalone TMS is unable to specifically characterize contributions of different descending cortical pathways that influence spinal circuit activity. Thus, each non-invasive stimulation technique (i.e., PNS-induced H-reflexes, TMS-induced MEPs) provides useful but limited information on how converging synaptic inputs within the spinal cord integrate and shape motor output.

1.3 Influence of Descending Pathways on Spinal Reflexes

1.3.1 Paired Non-Invasive Stimulation to Probe Sensorimotor Integration within the Spinal Cord

Combining PNS with subthreshold TMS provides a unique tool to index descending corticomotor influences on spinal reflex excitability (Geertsen et al., 2011; Nielsen et al., 1993; Nielsen & Petersen, 1995b). When a sub-threshold TMS conditioning pulse is delivered before or after PNS, the H-reflex response is typically facilitated, inhibited, or unchanged depending on the temporal interval, or timing between the paired pulses. Direct descending pathway modulation, also often referred to as short interval facilitation of the H-reflex, occurs when a subthreshold TMS pulse is delivered 1-5 milliseconds (ms) *after* a PNS pulse, allowing the direct, fastest descending volley to arrive at the spinal LMN pool prior to the afferent signal, and enhance the H-reflex amplitude by modulating the excitability of the LMN pool (Gray et al., 2017; Nielsen & Petersen, 1995b). Indirect descending pathway modulation, also referred to as long interval facilitation of the H-reflex, occurs when a TMS pulse is delivered *before* PNS, allowing indirect, slower descending volleys to arrive prior to the afferent signal (Gray et al., 2017; Nielsen & Petersen, 1995b). Thus, direct and indirect pathway modulation of the soleus H-reflex provide neurophysiologic measures to non-invasively probe the specific sites and mechanisms of sensorimotor circuit connections between cortical and spinal circuitry (**Figure 1-2**).

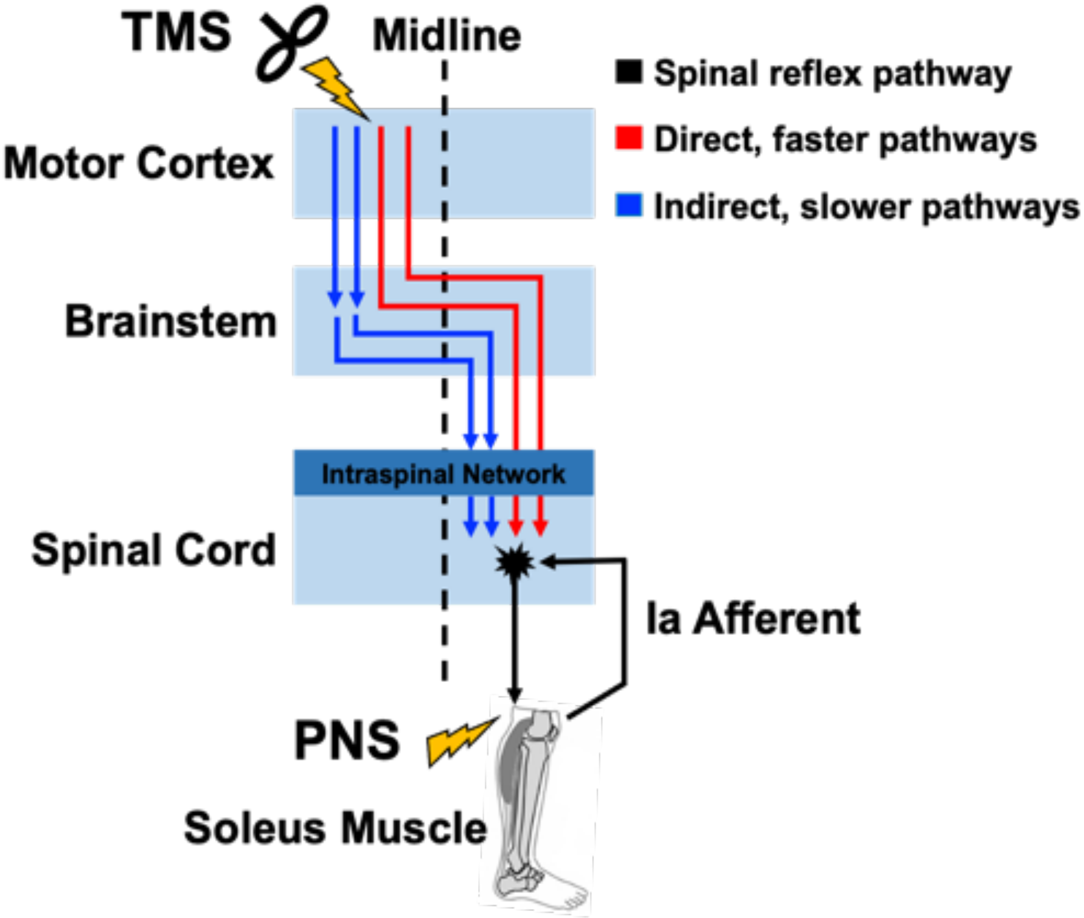

Figure 1-2. Schematic representation of the ability of paired non-invasive stimulation [i.e., transcranial magnetic stimulation (TMS), peripheral nerve stimulation (PNS)] to probe the influence of descending pathways on the spinal reflex pathway. The black lines represent the monosynaptic Ia afferent-alpha motor neuron pathway, the red lines represent the direct, monosynaptic, fast-conducting descending pathways, and the blue lines represent the indirect, polysynaptic, slower-conducting descending pathways.

1.4 Gap in Knowledge

There are significant knowledge gaps in our understanding of aging-related effects on neural mechanisms contributing to spinal sensorimotor circuit function and balance control. Spinal circuitry is complex, where alpha motor neurons receive inputs from many varying sources: descending tracts, peripheral afferents, and intraspinal circuits (i.e., Ia / Ib inhibitory interneurons, Renshaw cells, propriospinal neurons). While the activation of alpha motor neurons becomes the final common path in the motor system, the influence of synaptic inputs onto alpha motor neurons are typically studied individually, not concurrently. It is important to investigate the interactions between descending cortical and spinal reflex pathways to better understand their relation to the control and maintenance of balance. Understanding how aging impacts neural circuitry responsible for balance control is a crucial prerequisite to (1) developing neurobiology-informed treatments to improve balance and reduce falls risk in older individuals; and (2) future work parsing out the effects of aging versus the effects of neuropathology such as stroke or Parkinson's disease. However, we still do not know how these neuromotor circuits change with age, and during task-related activation. Therefore, determining how task-related changes and aging-related effects impact interactions between cortical and spinal neural circuits remains an important objective.

1.5 Dissertation Overview

The current chapter (**Chapter 1)** serves as an introduction to the dissertation by providing an overview of balance control and the underlying neural circuitry implicated in the control and maintenance of balance. Interactions between these neural circuits that underlie successful balance control remain poorly understood (**Figure 1-1**), yet a methodological approach in humans provides a unique ability to non-invasively investigate the relationship between descending cortical and spinal reflex pathways (**Figure 1-2**).

While this paired non-invasive neurostimulation approach has been utilized in previous studies, **Chapter 2** provides insight into the ability of descending cortical pathways to modulate the recruitment gain of spinal reflexes in healthy young adults, with PNS intensity as a methodological consideration. **Chapter 3** highlights an important methodological consideration related to the temporal profile, or timing, of descending cortical modulation of spinal reflexes in healthy young adults. However, aging causes structural and functional changes in cortical and spinal neural networks, which can impact performance of motor tasks. **Chapter 4** examines the effects of task-related activation and aging-related changes on descending cortical modulation of spinal reflexes. Finally, **Chapter 5** includes a summary discussion of findings from these studies as well as outlines alternative methodologies and clinical implications for future studies.

Chapter 2: Integration of Convergent Sensorimotor Inputs Within Spinal Reflex Circuits in Healthy Adults

This chapter is reproduced with minor edits from:

Lopez AJ*; Xu J*; Hoque MM; McMullen C; Kesar TM; Borich MR; "Integration of convergent sensorimotor inputs within spinal reflex circuits in healthy adults" *Frontiers in Human Neuroscience*, 2020. doi: 10.3389/fnhum.2020.592013

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2.1 Abstract

The output from motor neuron pools is influenced by the integration of synaptic inputs originating from descending corticomotor and spinal reflex pathways. In this chapter, using paired non-invasive brain and peripheral nerve stimulation, we investigated how descending corticomotor pathways influence the physiologic recruitment order of the soleus Hoffmann (H-) reflex. Eleven neurologically unimpaired adults (9 females; mean age 25 ± 3 years) completed an assessment of transcranial magnetic stimulation (TMS)-conditioning of the soleus H-reflex over a range of peripheral nerve stimulation (PNS) intensities. Unconditioned H-reflex recruitment curves were obtained by delivering PNS pulses to the posterior tibial nerve. Subsequently, TMS-conditioned H-reflex recruitment curves were obtained by pairing PNS with subthreshold TMS at short (−1.5 ms) and long (+10 ms) intervals. We evaluated unconditioned and TMS-conditioned H-reflex amplitudes along the ascending limb, peak, and descending limb of the H-reflex recruitment curve. Our results revealed that, for long-interval facilitation, TMS-conditioned H-reflex amplitudes were significantly larger than unconditioned H-reflex amplitudes along the ascending limb and peak of the H-reflex recruitment curve. Additionally, significantly lower PNS intensities were needed to elicit peak H-reflex amplitude (Hmax) for long-interval facilitation compared to unconditioned. These findings suggest that the influence of descending corticomotor pathways, particularly those mediating long-interval facilitation, contribute to changing the recruitment gain of the motor neuron pool, and can inform future methodological protocols for TMS-conditioning of H-reflexes. By characterizing and inducing short-term plasticity in circuitry mediating short- and long-interval TMS-conditioning of H-reflex amplitudes, future studies can

investigate supraspinal and spinal circuit contributions to abnormal motor control, as well as develop novel therapeutic targets for neuromodulation.

2.2 Introduction

Coordination between descending corticomotor and peripheral sensory inputs that converge at the spinal segmental level is needed for typical motor control. The monosynaptic spinal reflex pathway, which mediates stretch reflexes, consists of group Ia afferent fibers that make excitatory connections with alpha (α)-motoneurons at the spinal segmental level (Chen et al., 2003; Meunier & Pierrot-Deseilligny, 1989; Pierrot-Deseilligny & Mazevet, 2000; Wang et al., 2012). The Hoffman (H-) reflex is the electrical analogue of the monosynaptic stretch reflex that bypasses the muscle spindle and fusimotor activity and is used to evaluate the excitability of the monosynaptic spinal reflex pathway (Gassel, 1969; Knikou, 2008; Schieppati, 1987). Unlike the spinal reflex pathway, volitional movement is generated when pyramidal neurons in the primary motor cortex (M1) activate and conduct descending excitatory signals to spinal lower motor neurons (LMNs) that innervate target muscle groups. Subcortical and brainstem-mediated descending pathways can also project onto spinal LMNs, thus influencing the excitability of the spinal LMN pool (Bagust et al., 1985; Büschges, 2017; Nielsen & Petersen, 1995b; Ross et al., 1975, 1976). Therefore, spinal LMNs are the final common pathway for all motor output, whether reflexive or volitional. Neuropathologies such as stroke or multiple sclerosis can compromise motor control circuitry resulting in altered spinal reflex activity and impaired motor control (Bakheit et al., 2005; Bhagchandani & Schindler-Ivens, 2012; Perez & Cohen, 2009; Schindler-Ivens et al., 2008). To better understand neural mechanisms of abnormal motor control, descending corticomotor influences on spinal α-motoneurons in the unimpaired nervous system need to be understood.

Reflexive motor output that occurs when a mixed peripheral nerve is electrically stimulated (Rossini et al., 2015; Walsh et al., 1998) is commonly measured in upper and lower extremity muscles using surface electromyography (EMG) (Burke, 2016; Gassel & Diamantopoulos, 1966; Granit & Job, 1952; Knikou, 2008). H-reflexes are evoked by low-intensity surface electrical stimulation of a mixed peripheral nerve (peripheral nerve stimulation, PNS), which trans-synaptically excites the LMN pool, and are influenced by pre- and post-synaptic mechanisms (Komiyama et al., 1999; Milanov, 2000). H-reflex responses recorded over a range of PNS intensities generates the H-reflex recruitment curve, which provides useful information regarding reflex gain and metrics of spinal reflex excitability such as the peak H-reflex amplitude (Funase et al., 1994; Mazzocchio et al., 2001). Understanding the shape (e.g., slope) and behavior (e.g., leftward shift) of the H-reflex recruitment curve may provide novel insights into the physiologic processes that modulate the recruitment order and gain of LMN pools. However, a current limitation of classic H-reflex methodology is the inability to differentially assess the influence of cortical, subcortical, brainstem, and intraspinal inputs that target the LMN pool. The influence of Renshaw cells (Ross et al., 1972, 1976) and presynaptic inhibition (Meunier & Pierrot-Deseilligny, 1998; Milanov, 2000) on the monosynaptic spinal reflex pathway has been previously studied using paired PNS methods or antagonist muscle activation. However, methodologies to parse out contributions of different *descending* corticomotor inputs (e.g., direct corticospinal tract projections from the motor cortex versus polysynaptic descending projections that travel through brain stem centers) on LMN pools are lacking. Additionally, methodologies that investigate Renshaw cells or presynaptic inhibition do not evaluate the influence of descending corticomotor projections. Hence, alternative techniques are needed to determine the influence of descending corticomotor inputs on the recruitment gain of the LMN pool.

Transcranial magnetic stimulation (TMS) delivered over the primary motor cortex (M1) is used to non-invasively characterize excitability of descending corticomotor pathways (Barker et al., 1985; Groppa et al., 2012; Hallett, 2007). When a brief, high-intensity magnetic field is generated by electric current through a magnetic coil, the resulting magnetic field can excite or inhibit a small area of cortical tissue below the coil. All parts of cortical tissue just beneath the skull can be influenced, but most studies have been of M1 where a focal muscle twitch can be produced in target musculature called the motor evoked potential, or MEP. A single supra-threshold TMS pulse delivered over M1 elicits a MEP in the contralateral targeted muscle (Barker et al., 1985; Rossini et al., 2015). Although the amplitude of the MEP provides an overall index of corticomotor excitability, standalone TMS is unable to specifically characterize contributions of different descending corticomotor pathways that influence spinal circuit activity.

Combining PNS with subthreshold TMS provides a unique tool to index descending corticomotor influences on spinal reflex excitability (Geertsen et al., 2011; Nielsen et al., 1993; Nielsen & Petersen, 1995b). When a sub-threshold TMS conditioning pulse is delivered before or after PNS, the H-reflex response is typically increased. Short-interval facilitation (SIF), also often referred to as short latency facilitation of the H-reflex, occurs when a subthreshold TMS pulse is delivered 1-5 milliseconds (ms) after a PNS pulse, allowing the direct, fastest descending volley to arrive at the spinal LMN pool prior to the afferent signal, and enhance the H-reflex amplitude by modulating the excitability of the LMN pool (Gray et al., 2017; Nielsen & Petersen, 1995b). Long-interval facilitation (LIF), also referred to as long latency facilitation, occurs when a TMS pulse is delivered before PNS, allowing indirect, slower descending volleys to arrive prior to the afferent signal (Gray et al., 2017; Nielsen & Petersen, 1995b). Thus, SIF and LIF provide measures to non-invasively probe the specific sites and mechanisms of neuromotor circuit connections between cortical and spinal circuitry, which are previously poorly understood.

A single PNS intensity is commonly used when evaluating SIF and LIF (Geertsen et al., 2010, 2011; Petersen et al., 2003). In previous work, PNS intensities were set to elicit an H-reflex amplitude equivalent to 10-30% of the maximal muscle response (Mmax) to investigate both SIF and LIF (Geertsen et al., 2010; Nielsen et al., 1993; Nielsen & Petersen, 1995b; Pyndt & Nielsen, 2003), with the H-reflex amplitude (typically) on the ascending limb of the recruitment curve considered sensitive to facilitatory H-reflex conditioning (Di Lazzaro & Rothwell, 2014; Gray et al., 2017; Taube et al., 2017). Changes in the strength of output from the motor neuron pool can be attributed to different excitation thresholds of afferent fibers and motor axons (Cecen et al., 2018; Funase et al., 1994; Pierrot-Deseilligny & Mazevet, 2000). Additionally, different descending corticomotor pathways that are recruited or become activated due to varying TMS intensity, muscle activation state, or postural activation can influence the activation threshold of different motor neuron pools (Bawa & Lemon, 1993; Costa et al., 2011). While the influence of TMS parameters (e.g. intensity, direction of induced current) on H-reflex facilitation have been explored in previous studies (Andrews et al., 2020; Leukel & Kurz, 2021; Niemann et al., 2018), to our knowledge, the effect of PNS intensity on TMS-conditioning of the H-reflex has not been previously reported. We posit that evaluating the effects of TMS-conditioning at multiple PNS intensities across the H-reflex recruitment curves can provide novel insights and a more comprehensive characterization of the mechanisms underlying direct and indirect descending cortical connections that influence spinal segmental reflex circuitry.

Therefore, the overall objective of this study was to investigate the influence of descending corticomotor and peripheral sensory inputs on the physiologic recruitment order of soleus H-reflexes. To characterize different recruitment profiles in response to the integration of sensorimotor inputs onto the motor neuron pool, we evaluated TMS-conditioning of the H-reflex across a range of PNS intensities. We hypothesized that short and long interval facilitation of soleus H-reflexes will occur across multiple PNS intensities, and that the introduction of descending corticomotor inputs will change the characteristics of the H-reflex recruitment curve.

2.3 Methods

2.3.1 Study participants

Eleven young, neurologically unimpaired participants (9 females; mean age 25 ± 3 years) were recruited and completed the experimental protocol. Exclusion criteria included: 1) any known neurologic, neurodegenerative, orthopedic or musculoskeletal disorder, or psychiatric diagnosis, 2) outside the age range of 18–35 years old, or 3) contraindications to TMS (Rossini et al., 2015). Each participant provided written informed consent in accordance with the Declaration of Helsinki. Study procedures were approved by the Emory University Institutional Review Board (IRB#00067708).

2.3.2 Experimental design

Participants completed a single experimental session lasting approximately 3 hours, and study procedures were completed at a similar time of day (± 2 hours) across participants to account for potential circadian influences. Participants were asked to refrain from strenuous physical activity for 12 hours before the session to prevent potential excitability changes induced by strenuous physical activity (Cerqueira et al., 2006; Walton et al., 2003). The right soleus muscle was tested in all participants. Measurements were obtained with the participant seated in a semi-recumbent position with hips and knees in 30° of flexion and the ankles secured in neutral position in a paired rigid boot. Proper participant positioning was ensured using inelastic straps at mid-point of the shank (or lower leg) and thigh bilaterally to prevent hip rotation and abduction.

2.3.3 Electromyography (EMG) procedures

Following standard skin preparation procedures, two surface electrodes (2-cm diameter, EL503 Biopac Systems Inc., Goleta, CA) were affixed to the skin overlying the posterolateral aspect of the right soleus and the tibialis anterior (TA) muscle belly. A ground electrode was placed over the ipsilateral lateral malleolus. To verify proper EMG sensor placement, participants were asked to contract their right soleus and TA while an experimenter confirmed EMG activation (Biopac Systems AcqKnowledge Software Version 4.4). After verifying proper EMG sensor placement, soleus EMG activity was measured during 30 seconds of quiet standing, and the average EMG amplitude was recorded and used as a target for background EMG during the remaining experimental procedures.

2.3.4 Unconditioned soleus H-reflex recruitment curve

Soleus H-reflexes were evoked by stimulating the tibial nerve within the popliteal fossa using a monopolar electrode (round, 2.5 cm), with the anode (square, 5 cm) placed at the midline proximal to the patella (Biopac Systems Inc, Goleta, CA). The stimulating electrodes were self-adhering carbon rubber electrodes. With the participant lying prone, single pulses were delivered at random intervals as the experimenter moved the cathode within the posterolateral popliteal fossa to determine the optimal location to elicit H- and M- waves along with a pure plantar flexion response. Next, with the participant sitting in the semi-reclined test position, threshold stimulating intensities (lowest intensity where an H-reflex response was visible and lowest intensity where the Mmax was observed) were obtained. To control for the effects of varying background EMG activation on spinal and cortical excitability (Guzmán-López et al., 2015; Nielsen et al., 1993), participants were requested to maintain the right soleus background EMG activity at a low-level (matched to the level of each individual's voluntary EMG during quiet standing, ~10% maximum voluntary contraction (MVC)) by plantarflexing into a small block placed in the rigid boot (Gray et al., 2017). Real-time visual feedback of ongoing soleus root mean square EMG was provided on a computer monitor placed in front of the participant to ensure consistent soleus activity levels during data collection.

Soleus H-reflex and M-wave recruitment curve stimulation intensities were defined by the lowest PNS intensity eliciting an observable (0.1mV peak-to-peak amplitude) H-reflex response (H-threshold), and the highest PNS intensity resulting in a plateau of the M-wave (Mmax) (Burke, 2016; Knikou, 2008). To collect the H-reflex recruitment curve, approximately 50 stimulation pulses were delivered at increasing intensities (0.2 – 1 mA) with a variable inter-pulse interval ranging from 4 – 8 sec. A 6th order polynomial curve was fitted to the individual H-reflex and M-wave responses, generating an H-reflex/M-wave recruitment curve. Using the polynomial curve fit, we extracted H-reflex and M-wave amplitudes equivalent to 20% Mmax, Mmax, H-threshold, 50% Hmax, Hmax, 150% Hmax, and H-curve-endpoint, and used the values from these intensity conditions as the primary outcome measures. The H-threshold was defined as the intensity along the ascending limb of the H-reflex recruitment curve at which consecutive PNS pulses elicited visible H-reflexes. 50% Hmax was defined as the midway point along the ascending limb of the H-reflex recruitment between H-threshold and Hmax. 150% Hmax was defined as the midway point along the descending limb of the H-reflex recruitment curve between Hmax and H-curve-endpoint. The H-curve-endpoint was defined as the intensity along the descending limb of the H-reflex recruitment curve at which consecutive PNS pulses did not elicit a visible H-reflex.

2.3.5 Transcranial magnetic stimulation (TMS) procedures

To identify the M1 hotspot for the right soleus muscle, MEPs were elicited with single TMS pulses delivered with a custom 70 mm figure-of-eight batwing coil (Magstim Company Ltd., Dyfed, UK) connected to a monophasic stimulator (Magstim 200^2) (Gray et al., 2017; Kesar et al., 2018). The hotspot was defined as the optimal coil position that elicited the largest MEP response from the right soleus muscle at a fixed suprathreshold stimulation intensity. Consistency and accuracy in coil placement were maintained using stereotaxic neuronavigation using a standard brain template (Brainsight v. 2.2.14, Rogue Research Inc., Canada). Active motor threshold (AMT) was determined as the lowest stimulator intensity needed to evoke a soleus MEP of ≥ 100 μV peak-to-peak amplitude in at least 3 out of 5 trials during a volitional contraction (~10% MVC) (Gray et al., 2017). AMT intensity values ranged from 41% to 69% MSO (mean=53% ± 9% maximum stimulator output or MSO).

2.3.6 TMS-conditioned H-reflex recruitment curves

To investigate TMS-conditioning of the soleus H-reflex, sub-threshold TMS (90% AMT) was delivered at two different inter-stimulus intervals relative to electrical stimulation of the tibial nerve. To elicit SIF, the subthreshold TMS pulse was delivered 1.5 ms after delivery of PNS (-1.5 ms) and a TMS pulse was delivered 10 ms prior to PNS (+10 ms) to index LIF (**Figure 2-1A**). Both inter-stimulus intervals have been shown to reliably elicit significant H-reflex facilitation in participants with similar demographic characteristics (Gray et al., 2017). During TMS-conditioning experiments, each recruitment curve dataset was recorded by sequentially delivering interleaved unconditioned (UC) and conditioned (SIF, LIF) pulses (ex: UC_n, SIF_n, LIF_n, UC_{n+1}, SIF_{n+1}, LIF_{n+1}, …) with increasing PNS intensity. The same range of PNS intensities were used for the initial UC H-reflex recruitment curve as well as the SIF and LIF curves. We did not randomize delivery of PNS intensities for each recruitment curve in order to capture the rise, peak, and descent of each recruitment curves in increasing sequential order that was also individualized for each participant.

2.3.7 Calculation of H-reflex and M-response amplitude, Hmax, and Mmax

Peak-to-peak amplitude for H-reflex and M-wave responses were identified for each trial and used to generate the unconditioned and TMS-conditioned recruitment curves (**Figure 2-1B**). Evoked responses that failed to exceed background EMG threshold (0.05 mV) were excluded from the analysis. We fit a polynomial curve (6th order) to each individuals' unconditioned, SIF, and LIF H-reflex recruitment curve dataset (R^2 values between 0.8 and 0.99). Hmax and Mmax were calculated as the average of the three largest H-reflex or M-wave amplitudes, respectively (Gray et al., 2017). TMS-conditioned H-reflex recruitment curves (SIF and LIF) were analyzed using the same methods as unconditioned H-reflexes. All conditioned and unconditioned H-reflex amplitudes were plotted as a function of PNS intensity.

2.3.8 Determination of magnitude of TMS-induced facilitation

The unconditioned H-reflex recruitment curve was used to find the intensity that elicited an H-reflex amplitude that was equivalent to about 20% of Mmax, as H-reflexes of this size have been shown to be sensitive to inhibitory and facilitatory conditioning (Crone et al., 1990; Geertsen et al., 2011; Nielsen & Petersen, 1995b). TMS-conditioned H-reflex amplitudes for SIF and LIF were also recorded at the same PNS intensity (derived using the UC H-reflex recruitment curve) and used to compute the magnitude of facilitation. Thus, for each participant, the same PNS intensities were delivered for all 3 H-reflex recruitment curves – unconditioned, SIF, and LIF. The magnitude of facilitation for soleus H-reflexes was expressed as the H-reflex amplitude normalized to each individual's Mmax for UC, SIF, and LIF.

Unconditioned and conditioned (SIF, LIF) H-reflex recruitment curves were plotted at 5 points along the H-reflex recruitment curve (H-threshold, 50% Hmax, Hmax, 150% Hmax, H-endpoint) (**Figure 2-2**). To evaluate the magnitude of facilitation along the ascending, peak, and descending portions of the H-reflex recruitment curve, both unconditioned and conditioned (SIF, LIF) H-reflex amplitudes were normalized to each individual's Mmax amplitude and compared at 3 intensity conditions (50% Hmax, Hmax, 150% Hmax) (**Figure 2-3A**).

2.3.9 Statistical analyses

Normality was assessed using the Shapiro-Wilk test and quantile-quantile plots. Sphericity was assessed using Mauchly's test. If sphericity could not be assumed, Greenhouse-Geisser correction was applied to the degrees of freedom. Significant main effects were followed by post-hoc contrasts and corrected for multiple comparisons using the Bonferroni method. To first confirm the presence of SIF and LIF at a single, standard PNS intensity eliciting an H-reflex equivalent to 20% of Mmax (Geertsen et al., 2011; Nielsen & Petersen, 1995b), a one-way repeated-measures analysis of variance (ANOVA) was used to evaluate the effect of TMS-conditioning on H-reflex amplitude (unconditioned, SIF, LIF). A two-way repeated measures ANOVA was then performed to evaluate the effect of PNS intensity (3 standardized points along the recruitment curve) and TMS-conditioning (unconditioned, SIF, LIF) on the H-reflex amplitudes normalized to Mmax. For the two-way repeated measures ANOVA, we compared 3 intensities along the recruitment curve that represented the ascending limb (50% Hmax), peak (Hmax), and descending limb (150% Hmax). Simple effects analyses were performed to compare the H-reflex amplitudes normalized to Mmax at each of the 3 intensity conditions. For each TMS-conditioning level, pairwise comparisons were performed to compare the magnitude of facilitation between the 3 intensity conditions. A repeated measures two-way ANOVA was performed to assess the effect of PNS intensity and facilitation condition on the facilitation ratios (conditioned / unconditioned H-reflex amplitudes). Additionally, two separate one-way repeated-measures ANOVAs were performed to evaluate the effects of TMS-conditioning (unconditioned, SIF, LIF) on the Hmax/Mmax ratio and the intensity at which Hmax occurred. All statistical tests were run in Statistical Package for the Social Sciences (IBM SPSS version 26) and the critical alpha level was set to $p<.05$.

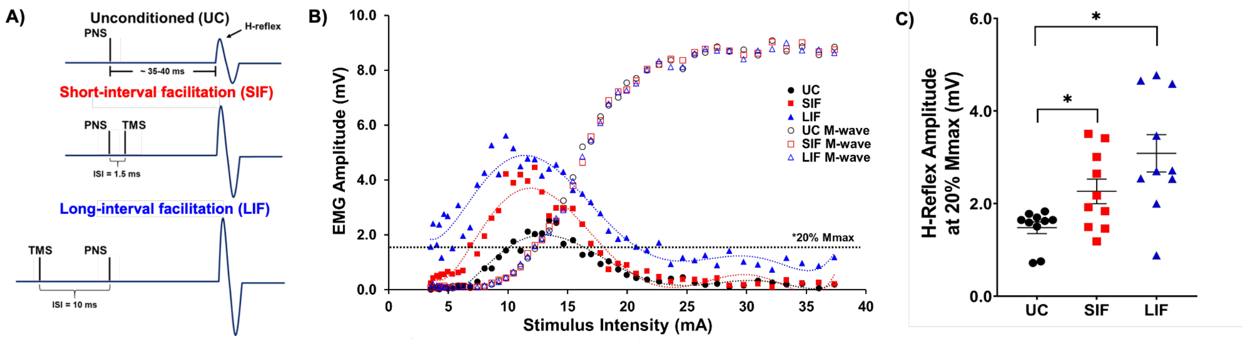

Figure 2-1. Demonstration of short-interval facilitation (SIF) and long-interval facilitation (LIF) of soleus H-reflexes. (A) Schematic representation of stimulation timing for unconditioned (UC) soleus H-reflex response and expected short-interval facilitation (SIF) and long-interval facilitation (LIF) of soleus H-reflexes. Inter-stimulus intervals (ISIs) of –1.5 and +10 ms were used to elicit SIF and LIF of soleus H-reflexes, respectively. **(B)** Unconditioned (UC), short-interval facilitation (SIF), and long-interval facilitation (LIF) recruitment curves were plotted from a representative participant. A 6th order polynomial curve was fitted for each recruitment curve and used to extract H-reflex and M-wave amplitudes at multiple intensities across the curve. Note facilitation of the H-reflex for the SIF and LIF recruitment curves compared to the UC recruitment curve, and an apparent leftward shift of the SIF and LIF recruitment curves. **(C)** The graph above shows the mean and standard error of H-reflex amplitudes (mV) at 20% Mmax (N = 10) for the UC (1.60 ± 0.15), SIF (2.36 ± 0.29), and LIF (3.29 ± 0.38) conditions. H-reflex amplitudes were significantly increased for both SIF ($p < 0.03$) and LIF ($p = 0.001$) compared to UC.

2.4 Results

Complete datasets were collected for 10 of the 11 participants; data from one participant were not included in the analyses due to methodological issues during the experimental session.

2.4.1 Demonstration of short- and long-interval facilitation of H-reflexes at a single PNS intensity (20% Mmax)

To confirm short- and long-interval facilitation (SIF, LIF) of the H-reflex at the 20% of Mmax intensity, we performed a one-way repeated measures ANOVA which revealed a significant main effect of conditioning (unconditioned, SIF, LIF) on H-reflex amplitude ($F_{2,18}=15.51$, $p<.001$; $\eta^2_p=.63$). Post hoc pairwise comparisons showed significantly greater H-reflex amplitudes for LIF compared to unconditioned (p=.002) and SIF compared to unconditioned (p=.018) at the 20% of Mmax intensity (**Figure 2-1C**).

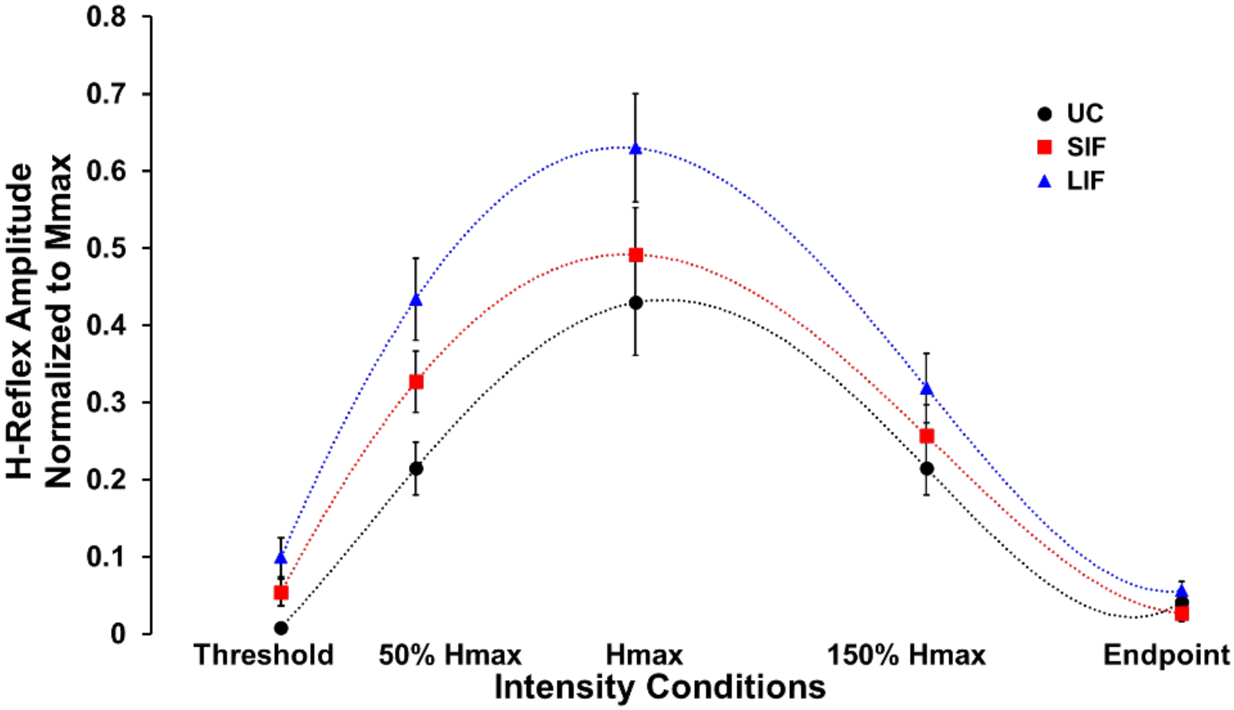

Figure 2-2. Average H-reflex amplitude normalized to Mmax for each condition (N = 10) at five different PNS intensity conditions (Threshold, 50% Hmax, Hmax, 150% Hmax, and Endpoint) along the unconditioned (UC), short-interval facilitation (SIF), and long-interval facilitation (LIF) recruitment curves. A 6th order polynomial curve was fitted for each recruitment curve and used to extract H-reflex and M-wave amplitudes at multiple intensities across the curve.

2.4.2 Evaluation of short- and long-interval facilitation of H-reflexes at a range of PNS intensities

Across the range of PNS intensities evaluated, both the conditioned H-reflex curves (SIF and LIF conditions) demonstrated larger H-reflex amplitudes compared to the unconditioned H-reflex recruitment curve (**Figure 2-2**). The two-way repeated measures ANOVA revealed a significant main effect for facilitation ($F_{2,18}$=13.50, p<.001;

η^2_p=.60), intensity ($F_{2,18}$=51.34, p<.001; η^2_p=.85), and an interaction between facilitation and intensity ($F_{4,36}$=4.39, p=.005; η^2_p=.33). Simple effects analysis revealed a significant effect of TMS-conditioning at the 50% Hmax ($F_{2,81}$=4.59, p=.013) and Hmax ($F_{2,81}$=4.01, p=.022) intensities. Post hoc pairwise comparisons revealed significantly higher H-reflex amplitudes normalized to Mmax for LIF compared to unconditioned at 50% Hmax (p=.010) and Hmax (p=.021) (**Figure 2-3A**). Additionally, post hoc pairwise comparisons revealed significantly greater magnitude of SIF at 50% Hmax compared to 150% Hmax (p=.005), and significantly greater magnitude of LIF at Hmax compared to 50% Hmax (p=.025) and 150% Hmax (p<.001). No other comparisons were significant (p>.076). To assess the effect of PNS intensity and facilitation condition on facilitation ratios, the two-way repeated measures ANOVA revealed a significant main effect for PNS intensity ($F_{1.21,10.91}$=6.80, p=.021; η^2_p=.43). Across PNS intensities, there was greater facilitation for the LIF condition compared to the SIF condition (p=.037). Post-hoc testing revealed greater facilitation was observed for the LIF compared to SIF condition at Hmax compared to 50% Hmax (p=.023).

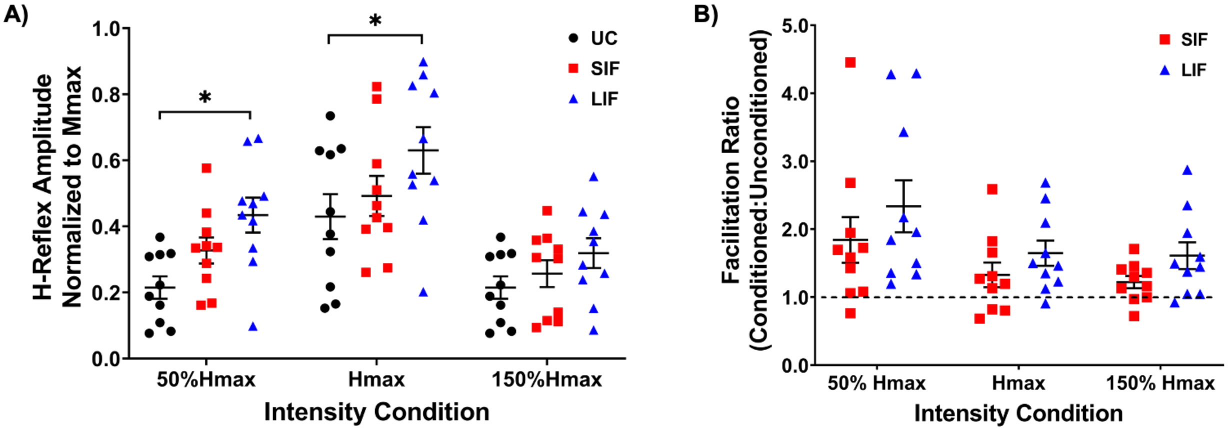

Figure 2-3. Soleus H-reflex amplitudes normalized to Mmax and the facilitation ratio of conditioned soleus H-reflexes over unconditioned (UC) soleus H-reflexes. (A) The graph shows the mean and standard error for H-reflex amplitudes normalized to Mmax for UC, short-interval facilitation (SIF), and long-interval facilitation (LIF) at three PNS

intensity conditions along the ascending (50% Hmax), peak (Hmax), and descending (150% Hmax) portions of the H-reflex recruitment curve. Significant facilitation was observed at 50% Hmax for LIF (0.434 ± 0.053) compared to UC (0.215 ± 0.034), and at Hmax for LIF (0.630 ± 0.070) compared to UC (0.430 ± 0.068). *$p < 0.05$. **(B)** The graph above shows the mean and standard error for the facilitation ratio, calculated as the conditioned (SIF, LIF) H-reflex amplitude over the unconditioned H-reflex amplitude (conditioned/unconditioned), at three PNS intensity conditions (50% Hmax, Hmax, 150% Hmax).

2.4.3 Comparison of unconditioned and conditioned Hmax and intensity required to elicit Hmax

The one-way repeated measures ANOVA revealed a significant main effect of TMS-conditioning (unconditioned, SIF, LIF) on the Hmax/Mmax ratio ($F_{2,18}$=10.13, p=.001; η^2_p=.53). Post hoc pairwise comparisons showed significantly greater Hmax/Mmax ratio for LIF when compared to unconditioned (p=.005) and SIF (p=.041) (**Figure 2-4A**). One-way repeated measures ANOVA revealed a significant main effect of conditioning ($F_{2,18}$=3.554, p=.050; η^2_p=.28) on the intensity used to obtain Hmax, which was normalized to the intensity used to obtain unconditioned Hmax. Post hoc pairwise comparisons showed a significantly lower intensity at which Hmax was obtained for LIF compared to unconditioned (p=.006), suggesting a leftward shift of the H-reflex curve. No significant differences in intensity to elicit Hmax were observed between SIF compared to unconditioned (p=.815) or SIF compared to LIF (p=.818). Although we did not find a statistically significant difference in intensity eliciting Hmax between SIF compared to unconditioned, individual participant data revealed that for all but one participant, Hmax occurred at a lower stimulus intensity during the SIF versus the unconditioned condition (**Figure 2-4B**). Additionally, all participants demonstrated that Hmax occurred at a lower intensity during LIF versus the unconditioned condition (**Figure 2-4B**).

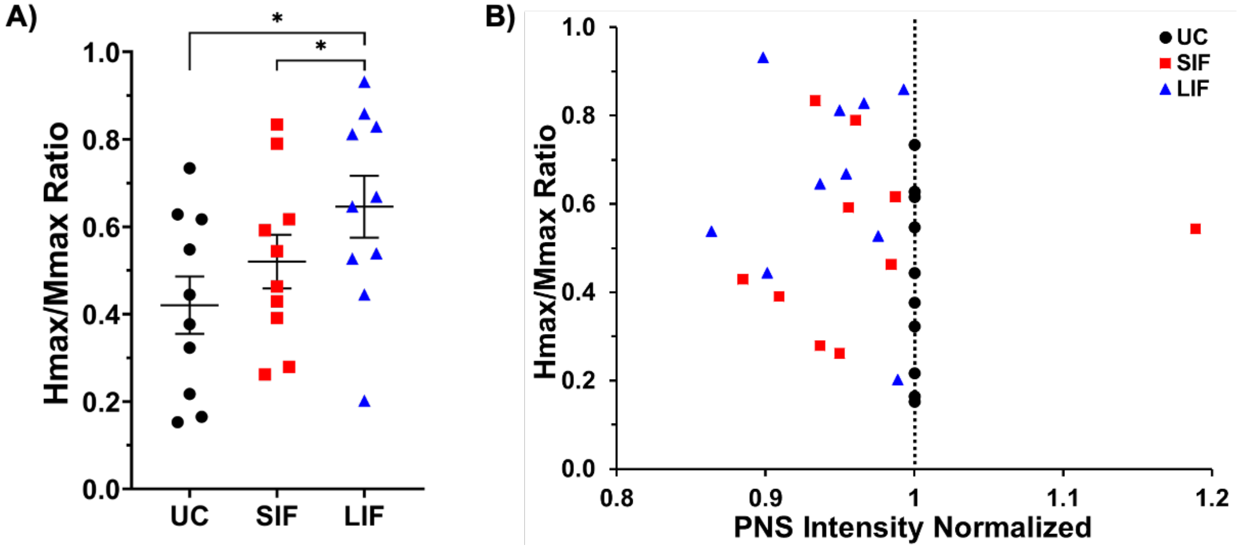

Figure 2-4. Hmax/Mmax ratios depicted during unconditioned (UC), short-interval facilitation (SIF), and long-interval facilitation (LIF). (A) The graph above shows the mean and standard error for Hmax values normalized to Mmax for UC, SIF, and LIF. For LIF, the Hmax/Mmax ratio (0.630 ± 0.070) was significantly greater than the Hmax/Mmax ratios for UC (0.429 ± 0.068; p = 0.005) and SIF (0.492 ± 0.061; p = 0.041). **(B)** Normalized Hmax values as a function of stimulus intensity (normalized to unconditioned). TMS-conditioned Hmax was achieved at a lower stimulus intensity (normalized PNS intensity < 1) compared to unconditioned Hmax for all but one participant.

2.5 Discussion

Our results revealed that across participants, at the same PNS intensity used for each condition (UC, SIF, LIF), TMS-conditioned H-reflexes, specifically for LIF, were significantly greater than unconditioned H-reflexes at multiple PNS intensities across the H-reflex recruitment curve. For LIF, proposed to be mediated by indirect, slower descending projections, TMS-conditioned H-reflex amplitudes were significantly larger than unconditioned H-reflex amplitudes on the ascending limb (50% Hmax) and peak (Hmax) of the H-reflex recruitment curve. Additionally, for LIF, we observed a lower PNS intensity needed to elicit the same soleus H-reflex amplitude as UC (i.e., the leftward shift of the conditioned H-reflex recruitment curve). Taken together, our finding that TMS-induced facilitation of soleus H-reflexes occurs at multiple PNS intensities across the H-reflex recruitment curve provides novel insight into the ability of convergent synaptic inputs (from descending cortical and spinal reflex pathways) to augment the physiologic recruitment order of the soleus H-reflex.

Previous studies evaluating SIF and LIF have typically measured H-reflex facilitation at a single PNS intensity, usually an intensity along the ascending limb of the H-reflex curve (e.g., an intensity eliciting an H-reflex amplitude at about 20% of Mmax) (Geertsen et al., 2011; Nielsen et al., 1993; Petersen et al., 2003). Mechanisms that may explain this facilitatory phenomenon in soleus include a decrease in the amount of presynaptic inhibition acting on Ia afferents (Costa et al., 2011), as well as temporal summation of excitatory postsynaptic potentials (EPSPs) from corticospinal projections and/or indirectly from polysynaptic pathways (Nielsen & Petersen, 1995b). To our knowledge, SIF and LIF have not been previously evaluated at a range of PNS intensities, which is critical to understand the putative mechanisms of H-reflex facilitation across the recruitment curve. Therefore, the current study provides a novel, systematic, and comprehensive approach that probes convergent synaptic inputs on physiologic recruitment of spinal sensorimotor circuitry.

During recording of the unconditioned H-reflex curve, trans-synaptic recruitment of LMNs occurs in accordance with the size principle (Binboga & Turker, 2012; Pierrot-Deseilligny & Mazevet, 2000), with smaller diameter LMNs contributing to the compound H-reflex amplitude at lower PNS intensities (at H-threshold and ascending limb of the H-curve), and larger diameter LMNs being recruited at higher PNS intensities (peak of H-curve and descending limb) (Davies et al., 1993; Gorassini et al., 2002). The Hmax/Mmax ratio represents the largest proportion of LMNs that are trans-synaptically recruited in response to the Ia afferent volleys. However, the direct stimulation of motor axons by PNS also simultaneously elicits an antidromic volley that recruits the larger-diameter motor axons before the smaller-diameter motor axons (Burke et al., 1979; Funase et al., 1994; Gottlieb & Agarwal, 1976). The "collision" of these antidromic volleys in the LMNs with the descending orthodromic LMN volley contributes to the reduced amplitude of the H-reflex (e.g., the descending limb of the curve), and ultimately abolishes the H-reflex with only M-waves visible (H-reflex curve endpoint). Based on our findings, during TMS-conditioning, we posit that the TMS-induced descending volleys result in membrane depolarization of the LMNs trans-synaptically, which also follows physiological recruitment order (Bawa & Lemon, 1993). The TMS pulse, although delivered at a sub-threshold intensity, may cause depolarization of a sub-population of LMN cell membranes (Bawa & Lemon, 1993), potentially inducing a greater change in membrane potential of smaller and medium diameter LMN fibers (Davies et al., 1993; Gorassini et al., 2002). Thus, at PNS intensities matched to the *unconditioned, no-TMS* condition, in the presence of TMS-facilitation, the upward shift of the conditioned H-reflex recruitment curves may represent a greater relative contribution of the smaller and medium-diameter LMNs to the compound H-reflex amplitude. We propose that the modulation of recruitment profiles of LMNs contributing to the compound H-reflex explain the upward shift of the conditioned H-reflex recruitment curves (e.g., lower stimulus intensities eliciting increased H-reflex amplitudes across the TMS-conditioned curves).

Another potential explanation for the observed finding is that TMS-conditioning may alter the membrane excitability of the LMN pool effectively lowering the activation threshold such that lower PNS stimulus intensities would recruit a greater proportion of the LMN pool when "primed" by TMS, potentially explaining the leftward shift of the curve. TMS-conditioning may also enable a greater proportion of medium and smaller diameter LMNs to be recruited in the compound H-reflex response, allowing for the measurement of a greater H-reflex amplitude, before occlusion of the H-reflex in the descending limb of the curve. Medium diameter LMNs that may have been "canceled out" by the collision with the antidromic signal for the *unconditioned* condition then could potentially contribute to the compound H-reflex in the SIF and LIF condition, explaining the increase in Hmax with LIF.

SIF of the H-reflex is thought to index the most direct, fastest descending volleys that arrive at the spinal segment prior to the arrival of the Ia afferent signal (Nielsen et al., 1993; Petersen et al., 1998; Taube et al., 2017). The descending cortical pathways that contribute to these observations of "early facilitation" have been described as the fastest, presumably monosynaptic, corticomotoneuronal connections between M1 and the LMN pool (Butler et al., 2007; Geertsen et al., 2011; Nielsen & Petersen, 1995b). Our finding of significant SIF of soleus H-reflexes at the H-reflex amplitude equivalent to 20% Mmax suggests that in contrast to LIF, measuring SIF across multiple PNS intensities may not be necessary to adequately characterize the influence of these direct descending projections on spinal excitability.

In contrast to SIF, LIF is thought to be mediated by indirect, slower descending volleys arriving prior to the Ia afferent signal (Geertsen et al., 2011; Gray et al., 2017). We observed significant H-reflex facilitation for LIF at multiple points along the curve (i.e., at 20% Mmax, 50% Hmax, and Hmax). Previous studies have reported that LIF pathways induce a larger magnitude of facilitation compared to the SIF pathway (Nielsen et al., 1993; Nielsen & Petersen, 1995b). For LIF pathways, greater magnitude of TMS-induced facilitation could be due to contributions from multiple descending pathways that synapse within subcortical, brain stem, and spinal cord regions (Geertsen et al., 2011; Leukel et al., 2015; Niemann et al., 2018). Potentially, the longer latency between TMS and PNS for the LIF condition allows sufficient time for descending volleys of multiple indirect, polysynaptic descending projections to arrive at the spinal segmental level, enabling a greater modulation of membrane excitability of the spinal motoneuron pool.

Previous studies have also suggested that several different corticofugal pathways, such as cortico-rubrospinal or cortico-reticulospinal, may play a role in the facilitation observed at the LIF latency of 10 ms (Gray et al., 2017; Nielsen & Petersen, 1995b; Niemann et al., 2018). A larger magnitude of LIF may also be explained by the arrival of TMS-induced descending volleys at the spinal segmental level at varying times, providing greater opportunity for varied temporal summation of excitatory post-synaptic potentials (EPSPs) (Geertsen et al., 2011; Gray et al., 2017). This could explain why LIF was greater and occurred at more sites along the H-reflex curve, than SIF. Although it is known that these indirect, polysynaptic pathways are involved in multiple neurophysiological processes (Deliagina et al., 2008; Fregosi et al., 2017; Honeycutt et al., 2013), future studies are required to characterize the unique and combined influences on spinal reflex excitability.

Taken together, our results show that evaluating H-reflex facilitation at multiple PNS intensities reveal additional insights into the mechanisms of descending supraspinal influences on spinal circuitry and reflex gain that may have practical applications for future investigations including individuals with neurologic impairments, such as stroke or spinal cord injury (SCI). Descending corticomotor projections are important components of neural circuitry controlling voluntary movement (Burke et al., 1993; Butler et al., 2007; Sidhu et al., 2012). Current non-invasive neurophysiologic measures, such as standalone single-pulse TMS or H-reflex amplitudes, are unable to specifically evaluate the connections between cortical and spinal circuits in humans, leading to a gap in our understanding of the salient mechanisms underlying recovery of movement associated with rehabilitative interventions. Additionally, evaluating both SIF and LIF could determine whether rehabilitation interventions differentially modulate the excitability of direct and indirect descending projections, an area of training-induced neuroplasticity that is not well-understood. Future studies are needed to examine the influence of other methodological parameters (i.e., muscle activation, posture, task, TMS intensity), and in the context of neurologic conditions (i.e., stroke, SCI), on TMS-induced H-reflex facilitation. Taken together, SIF and LIF may provide valuable neurophysiologic outcome variables for studying rehabilitation-induced neuroplasticity of direct and indirect descending corticomotor projections onto spinal LMNs.

2.5.1 Study limitations

In this study, the same PNS intensities were used for the unconditioned and conditioned (SIF and LIF) H-reflex recruitment curves for each participant. The same subthreshold TMS intensity, determined at the beginning of the experimental session, was used for the conditioned (SIF and LIF) H-reflex recruitment curves. Furthermore, the EMG sensor placement location, PNS stimulation site, TMS coil location, and muscle activation state were the same for each individual participant across all 3 conditions. Thus, our experimental design was such that H-reflex facilitation observed in the SIF and LIF conditions compared to unconditioned cannot be ascribed to differences in PNS intensity, TMS intensity, or methodological concerns.

However, this study had limitations. It is possible that an order effect in the delivery of stimulation pulses during collection of unconditioned and conditioned H-reflex recruitment curves may influence our findings. For example, an effect of delivering LIF pulses prior to UC and SIF pulses may artificially increase the magnitude of facilitation. Thus, future studies should consider randomizing the order of stimulation delivery to avoid any potential order effects. TMS-conditioning of the H-reflex can be used to evaluate the influences of descending corticomotor pathways on spinal reflex excitability, but this technique is limited to muscles in which it is possible to elicit a stable H-reflex. The study is limited by a relatively small sample size although the size is in line with previous studies (Gray et al., 2017; Guzmán-López et al., 2015; Nielsen et al., 1993) and our preliminary findings yielded significant results. Findings from the current study provide an important proof of concept that will aid in the design and power analysis of future studies on the influence of convergent sensorimotor inputs within the spinal cord.

We did not objectively assess pain perception during the experiment. Previous findings have indicated that some participants may perceive pain in response to a standardized protocol to elicit soleus H-reflexes (Motl et al., 2002, 2004) but participants in the current study did not report significant discomfort with study procedures. Additionally, in the present study, data were collected while participants were in an active state (maintaining a sustained low-level volitional muscle activation) and in a specific body position (seated). Throughout the experiment, we monitored and displayed to the participant real-time visual feedback regarding the ongoing background EMG activation with respect to the target EMG. However, small variations in background EMG may have influenced the study results. Previous studies have also shown that muscle activation state, body position and length, and posture can influence both cortical and spinal excitability (Guzmán-López et al., 2015; Nielsen et al., 1993; Nielsen & Petersen, 1995a; van den Bos et al., 2017).

Here, we assessed SIF and LIF at a single standardized ISI respectively for all participants that does not account for inter-individual variability in conduction velocities, limb length, or other individual characteristics. Individualizing the ISI for each participant to optimize the magnitude of facilitation (Nielsen et al., 1993; Taube et al., 2017), and accounting for each individuals' body or limb length parameters, as well as nerve conduction velocities, would be valuable directions for future study. Finally, during collection of unconditioned and TMS-conditioned H-reflex recruitment curves, H-reflex responses were recorded once at each increasing PNS intensity for all conditions (UC, SIF, LIF) due to experimental time limitations. Thus, evaluating SIF and LIF using a greater number of trials throughout the H-reflex recruitment curve, as well as collecting a wider range of PNS intensities across the H-reflex recruitment curve (i.e., prior to H-reflex onset), could establish reliability and reproducibility, and may be an important methodological consideration for future studies.

2.6 Conclusions

Findings from this study demonstrated that TMS-conditioning of soleus H-reflexes resulted in greater magnitude of facilitation (LIF) at multiple PNS intensities on the H-reflex recruitment curve (50%Hmax, Hmax), and that TMS-conditioning resulted in an increased Hmax/Mmax ratio in the LIF condition, inducing a leftward shift of the H-reflex recruitment curve. Our findings suggest that evaluating SIF and LIF over a range of PNS intensities offers a non-invasive approach to characterize descending corticomotor influences on spinal circuit activity. Additionally, our findings further elucidate the ability of convergent sensorimotor inputs to modulate the recruitment profiles of spinal LMNs. Further optimization of approaches used to characterize integration of ascending sensory and descending motor signals at the spinal segmental level offer novel opportunities to improve our understanding of neurophysiologic mechanisms of abnormal spinal circuit activity and motor function.

Chapter 3: Temporal Profile of Descending Cortical Modulation of Spinal Excitability: Group and Individual-Specific Effects

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3.1 Abstract

Sensorimotor control is modulated through complex interactions between descending corticomotor pathways and ascending sensory inputs. Pairing sub-threshold transcranial magnetic stimulation (TMS) with peripheral nerve stimulation (PNS) modulates the Hoffmann's reflex (H-reflex), providing a neurophysiologic probe into the influence of descending cortical drive on spinal segmental circuits. However, individual variability in the timing and magnitude of H-reflex modulation is poorly understood. Here, we varied the inter-stimulus interval (ISI) between TMS and PNS to systematically manipulate the relative timing of convergence of descending TMS-induced volleys with respect to ascending PNS-induced afferent volleys in the spinal cord to: (1) characterize effective connectivity between the primary motor cortex (M1) and spinal circuits, mediated by both direct, fastest-conducting, and indirect, slower-conducting descending pathways; and (2) compare the effect of individual-specific vs. standard ISIs. Unconditioned and TMS-conditioned H-reflexes (24 different ISIs ranging from −6 to 12 ms) were recorded from the soleus muscle in 10 able-bodied individuals. The magnitude of H-reflex modulation at individualized ISIs (earliest facilitation delay or EFD and individual-specific peak facilitation) was compared with standard ISIs. Our results revealed a significant effect of ISI on H-reflex modulation. ISIs eliciting earliest-onset facilitation (EFD 0 ms) ranged from −3 to −5 ms across individuals. No difference in the magnitude of facilitation was observed at EFD 0 ms vs. a standardized short-interval ISI of −1.5 ms. Peak facilitation occurred at longer ISIs, ranging from +3 to +11 ms. The magnitude of H-reflex facilitation derived using an individual-specific peak facilitation was significantly larger than facilitation observed at a standardized longer-interval ISI of +10 ms. Our results suggest that unique insights can be provided with individual-specific measures of top-down effective connectivity mediated by direct

and/or fastest-conducting pathways (indicated by the magnitude of facilitation observed at EFD 0 ms) and other descending pathways that encompass relatively slower and/or indirect connections from M1 to spinal circuits (indicated by peak facilitation and facilitation at longer ISIs). By comprehensively characterizing the temporal profile and inter-individual variability of descending modulation of spinal reflexes, our findings provide methodological guidelines and normative reference values to inform future studies on neurophysiological correlates of the complex array of descending neural connections between M1 and spinal circuits.

3.2 Introduction

Motor evoked potentials (MEPs) generated in response to transcranial magnetic stimulation (TMS) (Abbruzzese & Trompetto, 2002; Barker et al., 1985; Hallett, 2007; Kesar et al., 2018; Kobayashi & Pascual-Leone, 2003) and Hoffman reflexes (H-reflexes) elicited in response to peripheral nerve stimulation (PNS) (Burke, 2016; Perez et al., 2007; Pierrot-Deseilligny & Mazevet, 2000; Schieppati, 1987) have each been individually used to evaluate the excitability of cortical and spinal sensorimotor circuitry, respectively. Studies using TMS-evoked MEPs have shown modulation of corticospinal excitability with immobilization (Clark et al., 2008, 2010; Leukel et al., 2015; Opie et al., 2016), rehabilitation (Kantak et al., 2013; Keller et al., 2018; Roosink & Zijdewind, 2010), somatosensory stimulation (Brown et al., 2018; Meehan et al., 2008; Veldman et al., 2016), and motor learning (Celnik, 2015; Kantak et al., 2018; Palmer et al., 2018; Stefan, 2005). Similarly, H-reflex studies have demonstrated spinal circuit plasticity in response to electrical stimulation (Bae & Kim, 2017; Kuck et al., 2018; Rozand et al., 2015), aerobic exercise (Hodapp et al., 2009; Meunier et al., 2007; Tanuma et al., 2017), balance training (Behrens et al., 2015; Taube et al., 2007), operant conditioning (Thompson & Wolpaw, 2014, 2015), and immobilization (Clark et al., 2010; Leukel et al., 2015; Lundbye-Jensen & Nielsen, 2008). However, TMS-evoked MEPs or H-reflexes measured in isolation are limited in their ability to specifically elucidate whether the specific site of neuroplasticity is within the cortex, descending projections between M1 and the spinal cord, spinal segmental reflex circuits, or the spinal motoneuron pool, which is the final common pathway for both reflexive and voluntary motor commands (McNeil et al., 2013).

The H-reflex, providing an electrical analog of the excitability of spinal segmental reflexes, can be influenced or modulated by descending corticomotor volleys evoked by electrical or magnetic brain stimulation (Cowan et al., 1986; Nielsen et al., 1993). Therefore, pairing of sub-threshold TMS and PNS has been used as a neurophysiologic technique to evaluate the strength of descending physiologic connections (i.e., effective connectivity) between M1 and spinal circuits (Crone, 2003; Keller et al., 2018; Urbin et al., 2017). TMS-conditioning of the H-reflex can index the excitability of fastest-conducting or direct as well as relatively slower or indirect descending corticomotor projections onto spinal motoneurons (Nielsen et al., 1993; Taube et al., 2017). The effect of pairing TMS with PNS manifests as a change in the amplitude of H-reflex response, when the PNS-induced afferent volley transmitted via the Ia afferents and TMS-induced descending volleys transmitted via descending corticomotor pathways converge at the level of the spinal motoneuron pool (Niemann et al., 2018). This paired, non-invasive stimulation technique can provide information about the ability of descending corticofugal pathways to modulate spinal reflex excitability in humans.

Many previous studies of TMS-conditioned H-reflexes have used two standardized inter-stimulus intervals (ISIs) to evaluate early and late interval facilitation (Keller et al., 2018; Nielsen et al., 1993; Nielsen & Petersen, 1995b; Taube et al., 2017). For instance, when sub-threshold TMS is delivered 1-5ms *after* PNS (ISI -1 to -5ms), the resulting early onset facilitation of the H-reflex is thought to be mediated via direct, faster-conducting descending projections onto spinal motoneurons. When TMS is applied 5-10ms *before* PNS (ISI +5 to +10), the resulting longer interval facilitation is hypothesized to modulate the H-reflex response through an array of relatively slower and/or indirect corticomotor descending pathways. By varying the relative timing of TMS-induced descending corticomotor inputs with respect to the PNS, varying magnitudes of H-reflex facilitation can be elicited and quantified, which in turn probe the excitability of multiple, descending pathways that influence the excitability of spinal segmental reflexes.

Previously, using a single or standard ISI, we showed moderate-to-good reliability of TMS-induced H-reflex facilitation over multiple test sessions (Gray et al., 2017). However, several methodological factors can influence the inter-individual variability and magnitudes of TMS-induced H-reflex facilitation. Previous studies have evaluated the effect of TMS intensity (Niemann et al., 2018), coil direction (Niemann et al., 2018), and muscle activation (Keller et al., 2018) on TMS-facilitation of H-reflexes. Recently, we demonstrated the effect of PNS intensity on H-reflex facilitation, albeit only at two standardized ISIs (-1.5ms for early-onset facilitation and +10ms for longer interval facilitation) (Lopez et al., 2020). The ISI is another important but relatively under-studied parameter that can affect the magnitude and reliability of facilitation (Geertsen et al., 2011; Nielsen et al., 1993; Taube et al., 2017). Several previous studies have used a single, standardized ISI for evaluation of TMS-induced H-reflex facilitation (Gray et al., 2017; Río-Rodríguez et al., 2017), while others determined the ISI on an individual-subject basis (Niemann et al., 2018; Urbin et al., 2017). However, the influence of ISI on the magnitude of TMS-induced modulation of H-reflexes, and inter-individual variability in the timing and magnitude of facilitation across a range of ISIs is poorly understood. In addition to inter-individual differences in conduction velocity and segment length, optimizing or individualizing the ISI for eliciting H-reflex facilitation may be particularly important in neurological conditions (e.g., stroke, spinal cord injury, multiple sclerosis) given the changes in corticospinal excitability and transmission induced by the neurological lesion or injury (Christiansen & Perez, 2018; Knikou, 2017; Li et al., 2018). Thus, as an important first step toward understanding the influence of ISI, here, we varied the ISI between TMS and PNS to systematically manipulate the relative timing of convergence of descending TMS-induced volleys with respect to ascending PNS-induced afferent volleys in the spinal cord to characterize effective connectivity between the primary motor cortex (M1) and spinal circuits, mediated by both direct, fastest-conducting, as well as indirect, relatively slower-conducting descending pathways.

Furthermore, if individualization of ISIs is indeed an important methodological consideration, determining the optimum ISI for eliciting TMS-conditioning of H-reflexes for each study participant can be tedious and time-consuming, potentially limiting or constraining the application of this paired TMS-PNS stimulation technique in clinical trials or experimental studies investigating neuroplasticity (Taube et al., 2017). Therefore, the second objective of this study was to evaluate whether using an individualized, optimal ISI presented an advantage compared to the use of the same or 'standardized' ISI for all participants in the group.

3.3 Methods

3.3.1 Study participants

Ten able-bodied, young individuals (8 females, aged 22-28 years) participated in this study. All participants provided informed consent before study participation. Study procedures were approved by the Emory University Institutional Review Board (IRB).

3.3.2 Experimental design

The soleus muscle of the right leg was tested in all participants. All data-collection procedures were performed with the participants seated in a semi-recumbent position with hips and knees at 30° of flexion, and the ankles secured in rigid boots (**Figure 3-1**). The lower leg, foot, and distal thighs were stabilized with inelastic straps to maintain consistent limb positioning during the experiment.

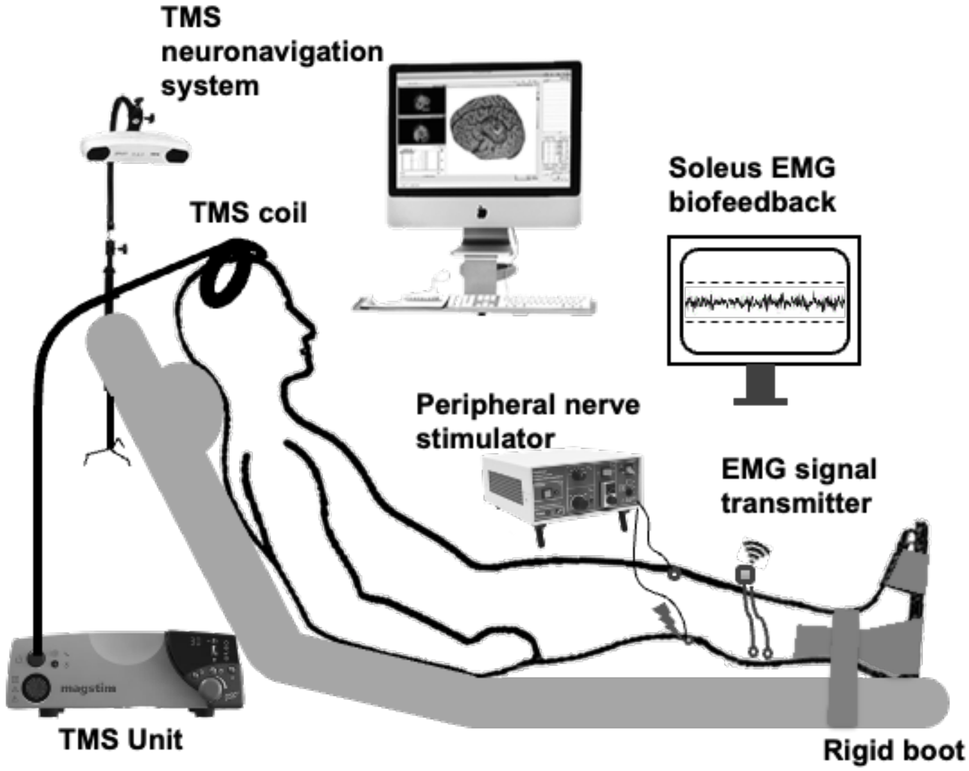

Figure 3-1. Schematic of the experimental setup. Subthreshold transcranial magnetic stimulation (TMS) pulses applied over the soleus motor cortex hotspot were paired with peripheral nerve stimulation (PNS) delivered to the tibial nerve at a range of inter-stimulus intervals (ISIs). The participants were instructed to maintain low-level background soleus muscle EMG activation targeting 10% maximal voluntary contraction (MVC), with ongoing visual biofeedback of ongoing EMG activity was displayed to the participant throughout the session.

3.3.3 Electromyographic recordings

Following standard skin preparation procedures, two surface electrodes (2-cm diameter, EL503, Biopac Systems Inc., Goleta, CA) were placed on the skin overlying the posterolateral aspect of the right soleus and the tibialis anterior (TA) muscle belly. A ground electrode was placed over the ipsilateral lateral malleolus. EMG signals were recorded at a sampling rate of 2000 Hz with a 5-1000 Hz bandpass filter. At the start of the experiment, participants were instructed to perform two isometric maximal voluntary contractions (MVCs) for 3-5 seconds into dorsiflexion and plantarflexion, separated by ~30 seconds of rest. To control for the effects of varying background EMG activation, the participants were requested to maintain the right soleus background EMG activity at a low-level (10% EMG activation obtained during the MVC) during data collection. Throughout the experiment, the participant was provided real-time visual biofeedback on a display screen regarding their ongoing average rectified soleus and tibialis anterior EMG activity, as well as the target activation level (10% MVC) (**Figure 3-1**). If the participant deviated from the 10% MVC EMG activation target, the investigator would pause and instruct the participant to adjust their EMG activation. This EMG visual feedback and experimenter check on the ongoing EMG activation was implemented to ensure consistent soleus background EMG activation during the collection of both unconditioned and conditioned H-reflexes.

3.3.4 Peripheral nerve stimulation

A square electrode (5cm by 5cm, TSYR2020-20, Syrtenty, Titusville, FL) was attached on the anterior aspect of the knee and served as the anode. A pen electrode was used to search for the optimal nerve stimulation site in the popliteal fossa. Electrical stimulation was delivered to the posterior tibial nerve to evoke soleus H-reflexes. The optimal site for nerve stimulation was identified as the location that elicited stable soleus H-reflexes and a visible plantarflexion contraction at higher intensities. After confirming the optimal stimulation site, a self-adherent carbon rubber circular electrode (2.5 cm diameter, TSYR 1000-40 round, Syrtenty, Titusville, FL) was attached, additional pressure was applied using a Styrofoam ball to maintain the electrode's contact with the skin, and the electrode and ball were tightly wrapped. The H-reflex recruitment curve was generated by administering approximately 50-60 single pulses with pulse duration of 1ms, which were delivered using an electrical stimulator controlled with custom-written MATLAB scripts (AcqKnowledge software Version 4.4, Biopac Systems Inc.). To acquire H-reflex and M-wave recruitment curves, electrical stimulation intensity was increased gradually until the maximal muscle response (M_{max}) was reached, as measured by the peak-to-peak amplitude of the raw M-wave responses. Using the H-reflex and M-wave curves, we also obtained the peripheral stimulation intensity required to elicit an H-reflex amplitude of 20% M_{max}.

3.3.5 Transcranial magnetic stimulation

To elicit soleus TMS-evoked motor evoked potentials (MEPs), single TMS pulses were delivered using a custom batwing, figure-of-eight coil with a posterior-anterior current direction connected to a monophasic TMS stimulator (Magstim 200^2) (Gray et al., 2017; Kesar et al., 2018) (**Figure 3-1**). TMS pulses were delivered over the right soleus motor "hot spot" within left M1, defined as the optimal coil position that elicited maximal MEP responses in the soleus at the lowest TMS intensity. A stereotaxic neuronavigation system was used to track and maintain accuracy of TMS coil positioning (Brainsight v. 2.2.14, Rogue Research Inc., Canada). To determine the active motor threshold (AMT), participants were requested to maintain low-level tonic EMG activity in the right soleus at 10% MVC EMG. AMT was determined as the lowest stimulator intensity needed to evoke a soleus MEP of ≥ 100 μV peak-to-peak amplitude in at least 3 out of 5 trials. We were able to elicit measurable MEPs from all study participants. For TMS-conditioning, the TMS intensity was maintained at 90% AMT (sub-threshold).

3.3.6 TMS-conditioning of the soleus H-reflex

To investigate the influence of descending corticomotor projections on spinal reflex excitability, sub-threshold (90% AMT) TMS pulses were delivered at different timing intervals, or ISIs, relative to PNS of the posterior tibial nerve. The ISI between the conditioning TMS pulse (delivered over soleus hotspot on left M1) and the test PNS pulse (delivered in the right popliteal fossa) was varied from -6 to +12ms. We collected conditioned H-reflex data at 24 different ISIs: -6, -5, -4, -3, -2.5, -2, -1.5, -1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 9.5, 10, 10.5, 11, 11.5 and 12ms. Negative ISIs indicate that the PNS was delivered prior to TMS, and positive ISIs indicate that the PNS was delivered after the TMS. At each ISI, 5 conditioned H-reflexes were collected, interspersed with a total of 20 unconditioned (UC) H-reflexes. PNS-evoked responses were collected at a frequency of ≤ 0.25Hz in random order. The intensity of tibial nerve stimulation was set to elicit an unconditioned H-reflex peak-to-peak amplitude equivalent to 20% of M_{max}, as H-reflexes of this size have been shown to be sensitive to inhibitory and facilitatory conditioning in previous publications (Gray et al., 2017; Taube et al., 2015, 2017).

3.3.7 Data processing

Peak-to-peak amplitudes of unconditioned and conditioned H-reflexes at each ISI were extracted from raw EMG data collection files. At each ISI, the magnitude of H-reflex facilitation or modulation was calculated as conditioned H-reflex amplitude as a percentage of the unconditioned H reflex amplitude. The earliest onset of facilitation indicates the ISI at which the fastest descending TMS-induced volleys arrive at the spinal motoneuron pools. The ISI of onset of earliest facilitation was identified for each participant as the first peak of facilitation that was followed by a period of decline before the facilitation curve resumed (Nielsen et al., 1993; Niemann et al., 2018; Taube et al., 2015, 2017) (**Figure 3-2**). We plotted the relationship between ISI (normalized to EFD 0ms) and magnitude of H-reflex facilitation for each participant (**Figure 3-2**). Once we determined the onset of earliest facilitation, the ISI of this time point was redefined as

0ms, and all previous and subsequent ISIs were normalized with reference to the earliest onset of facilitation, described as early facilitation delays (EFDs) (**Figure 3-2**). We statistically compared unconditioned versus conditioned H-reflex amplitudes at EFD 0ms, as well as at EFD -1ms to confirm that a significant facilitation occurred at EFD 0ms (**Figure 3-3**).

In addition to the individualized determination of the onset of earliest facilitation and magnitude of facilitation at a range of EFDs, the individual-specific peak facilitation for each participant was determined at the ISI that generated maximal facilitation. Previous studies have defined standard early and longer interval or late ISIs as -1.5ms and +10ms, respectively. To compare facilitation observed at individualized (determined for each individual using their ISI curve) versus standard ISIs (the same ISI used for all participants), we compared the magnitude of facilitation at (i) individualized ISI where earliest facilitation was observed (EFD=0ms) versus a standardized early ISI of -1.5ms; and (ii) individualized maximal facilitation versus longer interval facilitation at a standardized ISI of +10ms.

3.3.8 Statistical procedures

A repeated-measures analysis of variance (ANOVA) was performed to evaluate the effect of EFD (within-subjects factor with 18 levels, from EFD -2ms to EFD 15ms) on the dependent variable of H-reflex facilitation. We used paired t-tests to compare unconditioned versus conditioned H-reflex amplitudes at EFD 0ms and at EFD -1ms. Additionally, to evaluate conditioning effects at standardized versus individualized ISIs, paired t-tests were used to compare the magnitude of facilitation at (i) the individual-specific ISI where earliest onset of facilitation occurred (EFD 0ms) versus at a standard early ISI of -1.5ms; (ii) the individual specific maximal or peak facilitation versus at a standard longer interval ISI of +10ms. The Shapiro-Wilks test showed normal distribution at all except for one ISI (EFD=1ms). All statistical tests were run in Statistical Package for the Social Sciences (IBM SPSS version 26) and the critical alpha level was set to $p < 0.05$.

3.4 Results

3.4.1 Identification the earliest onset of H-reflex facilitation in individual participants

To determine the influence of TMS-induced fastest descending volleys arriving at the spinal motoneurons, we identified the earliest onset of H-reflex facilitation for each participant. Relationships between ISI and H-reflex facilitation for each participant are shown in **Figure 3-2**. The onset of the earliest facilitation was observed at ISIs ranging from -3 to -5ms (mean= -3.70 ± 0.67ms) across study participants (**Figure 3-3B**). The average magnitude of facilitation at the earliest onset of facilitation was 158.00 ± 28.58% (**Figures 3-2 and 3-4**).

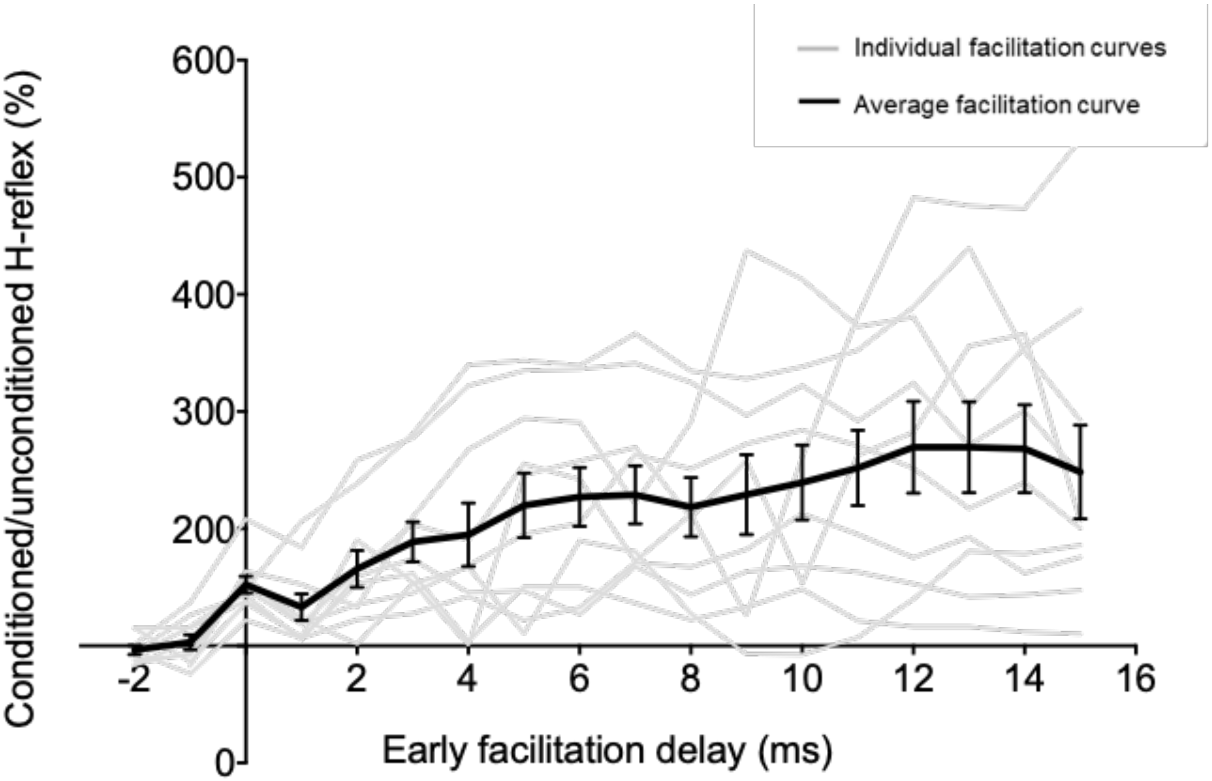

Figure 3-2. The relationship between ISI and TMS-conditioning of Hoffmann's reflex (H-reflex). The graph demonstrates the relationship between different early facilitation delays (EFDs) and H-reflex facilitation (normalized as a percentage of unconditioned H-reflex amplitude), with gray lines representing data from individual participants and the black line representing the group average (error bars represent standard error). The ordinate (y-axis) shows the amplitude of the conditioned H-reflex as a percentage of the control (unconditioned) reflex amplitude. The abscissa (x-axis) shows the inter-stimulus timing between TMS and PNS normalized with reference to delay (in ms) from the ISI at which the earliest onset of facilitation occurred (EFD).

Each individual's data were normalized with reference to the ISI of earliest onset, which was referred to as EFD 0ms. To confirm that EFD 0ms was the first arrival of the fastest descending volley at spinal circuits, we statistically compared the unconditioned and conditioned soleus H-reflex amplitudes at EFD -1ms and 0ms (**Figures 3-3C and 3-3D**). At EFD -1ms, there was no significant difference between the unconditioned and conditioned H-reflex amplitude (p=0.99, paired Cohen's d -0.00056 [95.0%CI -0.262, 0.246]). At EFD 0ms, the conditioned H-reflex amplitude was significantly larger than unconditioned (p=0.0018, paired Cohen's d = 0.709 [95.0%CI 0.471, 1.04]) (**Figure 3-3**).

3.4.2 Influence of ISI on TMS-conditioning of H-reflex facilitation

Overall, sub-threshold TMS conditioning facilitated the H-reflex from EFD 0ms to EFD +15ms (**Figure 3-2**). The repeated measures ANOVA evaluating the effect of EFD on H-reflex facilitation revealed a significant main effect for EFD ($F_{2.815,\ 25.335}$=8.406, p<0.01, η2=0.483).

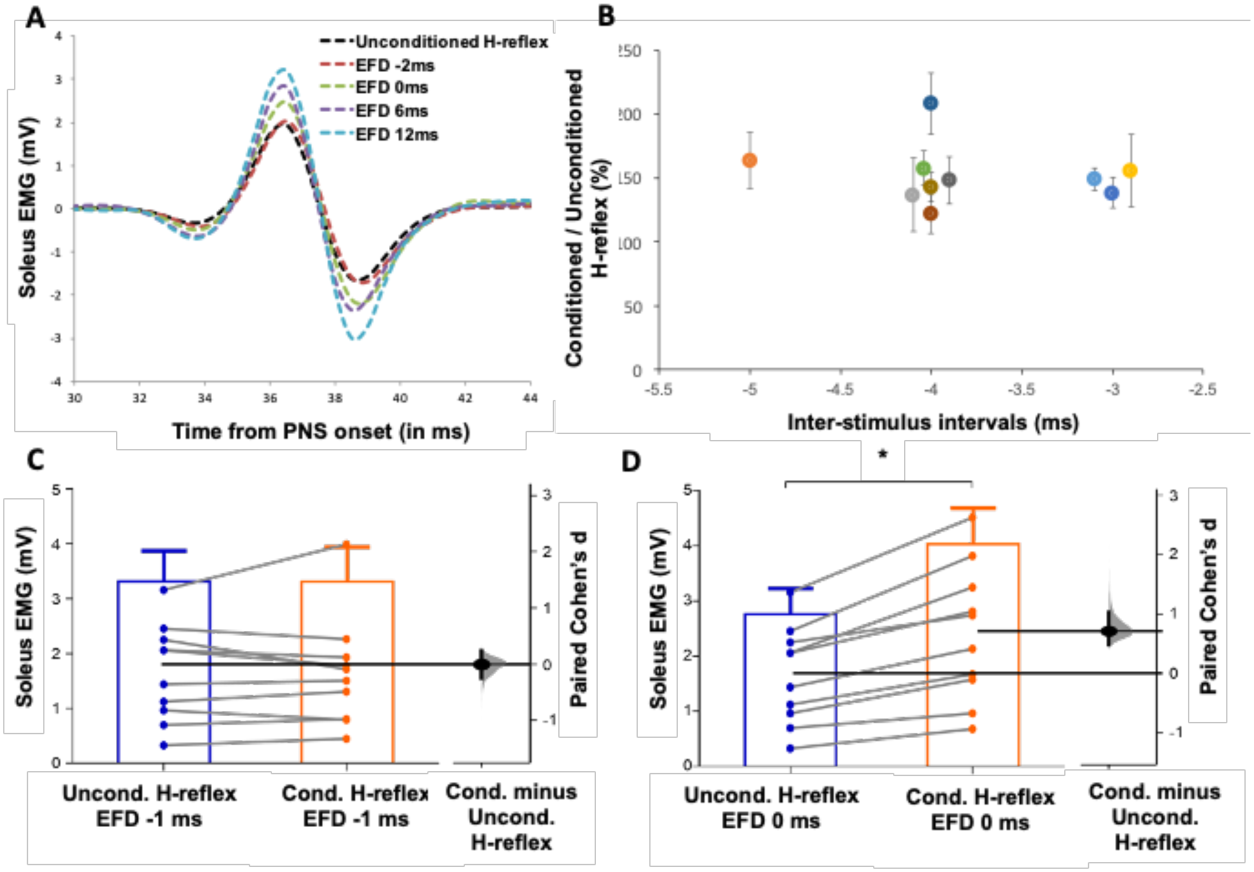

Figure 3-3. (A) Raw H-reflex data. Raw H-reflex traces from a representative participant without (unconditioned H-reflex) and with TMS conditioning at multiple ISIs (conditioned H-reflexes at different delays with respect to the timing of earliest onset of facilitation, i.e., EFD). The conditioned H-reflexes are displayed at EFD −2 ms, 0 ms, 6 ms, and 12 ms. Note that in contrast to the absence of facilitation at EFD −2 ms, H-reflexes at the other EFD ISI intervals are facilitated (larger in amplitude) compared to the unconditioned H-reflex, showing modulation of the spinal reflex by the descending TMS-induced volleys. **(B)** Magnitude and timing of earliest onset of facilitation. The earliest onset of facilitation was observed at ISIs ranging from −3 to −5 ms (mean = −3.70 ± 0.67ms) across study participants (x-axis). The average magnitude of facilitation (y-axis) was 158.00 ± 28.58% but varied across different ISIs. **(C,D)** Graphs with estimation plots showing comparisons between unconditioned vs. conditioned H-reflex amplitudes at EFD −1 ms and EFD 0 ms. Unconditioned and conditioned H-reflexes

(means with standard deviation as well as individual participant data) are shown at EFD −1 ms **(C)** and EFD 0 ms **(D)**. The paired mean difference (Cohen's d) is shown with a Gardner-Altman estimation plot on a floating axis on the right as a bootstrap sampling distribution; the mean difference is depicted as a dot; the 95% confidence interval is indicated by the ends of the vertical error bars. Note that while no significant increase in the conditioned H-reflex amplitudes was observed at EFD −1 ms, a significantly larger amplitude of conditioned vs. unconditioned (with a large effect size) was observed at EFD 0 ms. *Indicates statistically significant difference.

3.4.3 Comparison of early and late facilitation measured at individualized ISI versus standard ISI

To facilitate an individual-specific visualization of our study results, the magnitudes of H-reflex facilitation for each study participant (rows) at each ISI (columns) are demonstrated as a gray-scale gradient map (**Figure 3-4A**), with the different grayscale colors (from black to white) representing the rank of conditioned H-reflex amplitudes (from highest facilitation to lowest facilitation) for each participant. The onset of earliest facilitation and peak facilitation are both demarcated in the map for each participant, showing the inter-individual variability in these ISIs (**Figure 3-4A**). The paired t-test showed no significant difference between the facilitation magnitude at the individualized ISI where earliest onset facilitation was observed (EFD 0ms) versus facilitation at the standard early ISI of -1.5ms (152.20 ± 23.05%, 171.78 ± 68.29%, p=0.30, paired Cohen's d = 0.384 [95.0%CI -0.289, 1.1]) (**Figure 3-4B**). In contrast, the paired t-test showed a significantly greater magnitude of peak H-reflex facilitation when determined using an individualized peak ISI versus the standard longer interval ISI of +10ms (317.51 ± 134.54%, 269.83 ± 122.57%, p<0.01, paired Cohen's d -0.332 [95.0%CI -0.515, -0.204]) (**Figure 3-4B**). The magnitude of peak facilitation at longer interval ISIs (both using individualized peak and standard ISI of +10ms) was significantly greater than the magnitude of earliest onset facilitation observed at EFD 0ms (p<0.01) (**Figure 3-4B**).

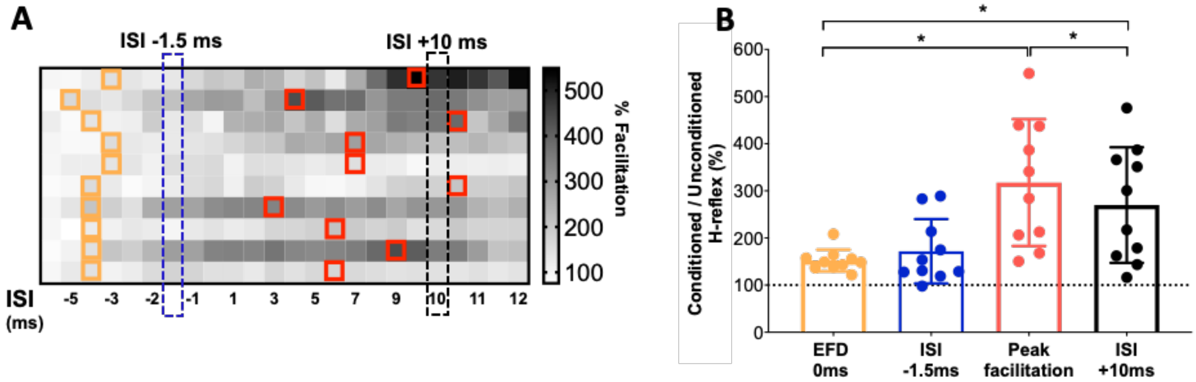

Figure 3-4. Demonstration of individualized earliest onset of and peak magnitude of facilitation in comparison to standard ISIs. (A) The gradient map shows the gray-scale rank of the magnitude of facilitation for each study participant (rows) at each ISI (columns). The cells in the map filled with different grayscale colors (from white to black) represent the rank of conditioned H-reflexes amplitude (from highest facilitation to lowest facilitation) for each participant. For each participant, ISIs at which the earliest onset of facilitation was detected are demarcated with orange outlines. On the same color matrix, individualized ISIs that elicited peak facilitation are demarcated with red outlines. The chart shows the inter-individual variability in the location of ISI that elicits earliest or peak facilitation. **(B)** Comparison of the magnitude of facilitation for early facilitation at individualized EFD 0 ms vs. at a standard early ISI of −1.5 ms; and between individualized peak facilitation vs. at a longer interval standard ISI of +10 ms. *Indicates statistically significant difference.

3.5 Discussion

Here, we studied the temporal dynamics underlying pairing of TMS with PNS, a promising, non-invasive neurophysiologic approach for indexing the excitability of both direct or fast and indirect or relatively slower descending corticomotor projections onto spinal segmental circuitry. We systematically quantified the inter-individual variability in both the magnitude and timing of occurrence (i.e., ISI) of the earliest onset and peak TMS-induced H-reflex facilitation. Our results revealed substantial inter-individual variability in the timing of the earliest onset and the greatest magnitude of H-reflex facilitation and support potential methodological advantages and mechanistic insights gained from utilizing individualized ISIs for measurement of earliest onset and longer-interval facilitation.

3.5.1 Mechanisms and interpretation of earliest onset of facilitation

Our findings show that the earliest onset of H-reflex facilitation occurred at the ISI of -3.70 ± 0.67ms in healthy participants (**Figures 3-2 and 3-3**). The negative values for ISIs of early facilitation indicate that the TMS pulse was delivered after the PNS pulse. This earliest onset of facilitation is posited to represent the excitability of the fastest conducting or direct descending corticomotoneuronal connections (Nielsen et al., 1993; Taube et al., 2015, 2017). In our study, at these ISIs, the TMS-induced input in these fastest or direct descending pathways produced sufficient depolarization in spinal motoneuron pools innervating the soleus, as evidenced by the larger amplitude of the conditioned H-reflex compared to an unconditioned H-reflex (Leukel et al., 2015; Taube et al., 2015). We identified the earliest onset of facilitation from the ISI curve as the first peak of facilitation followed by a decline in facilitation, based on methodology from previous literature (Keller et al., 2018) (**Figure 3-2**). The decline following the first peak, while poorly understood, may be mediated by the activation of spinal inhibitory interneurons (Nielsen et al., 1993). In our study, the ISI at which we observed earliest facilitation is consistent with findings from a previous study that showed that early-onset or short-latency facilitation occurred in the -4 to -2ms (-3.54 ± 0.66ms) ISI range when the soleus was at rest (Taube et al., 2015, 2017). Another study, which used early-onset facilitation to evaluate the effects of ankle joint immobilization, revealed that early facilitation occurred at around the -3ms ISI on average (Leukel et al., 2015). Similar to our current methods, a previous study recalibrated the ISI of early facilitation as 0ms to synchronize the subsequent ISIs with respect to the ISI eliciting earliest onset of facilitation (Aguiar & Baker, 2018). Based on our findings, future studies interested in evaluating the excitability of fastest and/or direct conducting descending projections can test ISIs in the -3 to -4ms range, normalize individual ISI curves to the ISI of onset of earliest facilitation (EFD 0ms), and to improve accuracy, if time permits, individually determine the ISI at which early facilitation is observed.

Although several previous studies used one standard ISI to investigate early H-reflex facilitation in their study cohort (Cortes et al., 2011; Gray et al., 2017; Leukel et al., 2012), early facilitation may show inter-individual variability due to physiological and anatomical differences influencing conduction velocities. In our study participant cohort, if we selected a single or standard ISI in the early facilitation range, a subset of participants may not demonstrate H-reflex facilitation (i.e., facilitation greater than 100%). Thus, in addition to evaluating the magnitude and timing of earliest onset of facilitation, determining the individualized ISI that elicits greatest magnitude of facilitation can be advantageous. For instance, the magnitude of earliest onset facilitation (i.e., % modulation of the conditioned H-reflex amplitude at EFD 0ms) would provide a measure of the strength of effective connectivity of the fastest conducting and/or direct descending pathway between M1 and the spinal motoneuron pool. In conjunction, the ISI at which the earliest onset of facilitation occurs (i.e., ISI at which EFD 0ms occurs) may provide insights into the underlying mechanism of descending cortical influences on spinal excitability. For example, if EFD 0ms occurs at a longer ISI, showing a rightward shift with a neuropathology, it could indicate that the weakened effective connectivity is in part caused by delayed conduction or aberrant transmission in this fastest and/or direct descending pathway. Interestingly, recent work utilized shorter-interval ISIs with greater temporal resolution than our study (up to 0.1ms differences between ISIs), and inferred that EFD 0ms and EFD +0.6ms were informative of changes in the excitability of circuits within infragranular and supragranular cortical layers, respectively (Leukel & Kurz, 2021).

Our results showing no significant difference in the measured magnitude of early facilitation when using a standardized (-1.5ms) versus individualized ISI suggest that at least for younger able-bodied individuals (**Figure 3-4**), the measurement of earliest onset of facilitation is robust and perhaps less susceptible to inter-trial or physiological variability. Early onset facilitation measured at a single, standardized ISI such as -1.5ms may provide a relatively quick and useful index of the overall excitability of relatively fast and direct descending projections. The statistically significant facilitation of the conditioned H-reflex measured at the -1.5ms ISI also suggests that the coincidence of TMS-induced descending and PNS-induced ascending afferent volleys at the spinal motoneurons elicit robust H-reflex facilitation in able-bodied individuals, and probe the overall excitability or effective connectivity in the population of direct and/or fast descending projections (Cortes et al., 2011; Leukel et al., 2012).

3.5.2 Mechanisms and interpretation of longer-interval and maximal facilitation

In addition to evaluating the timing and magnitude of earliest onset of TMS-induced H-reflex facilitation, we also evaluated a wide range of longer interval ISIs and determined the ISI that elicits the greatest facilitation in our young able-bodied participant cohort (**Figure 3-2**). As expected, our results showed that the magnitude of peak facilitation was significantly higher than that of earliest onset facilitation (EFD 0ms). We also showed a high degree of inter-individual variability in the individualized ISIs that elicited peak magnitude of facilitation (**Figure 3-4A**). This inter-individual variability in the magnitude and ISI of occurrence of peak facilitation perhaps reflects the complex and varied array of descending projections that may contribute to the facilitation measured at longer interval ISIs. The longer interval or late facilitation is hypothesized to be mediated by polysynaptic and/or relatively slower conducting, corticofugal descending pathways between M1 and the spinal motoneuron pool. While this method only provides indirect inference and limited anatomic specificity of these descending pathways, previous studies have hypothesized that they include cortico-reticulo-spinal, cortico-vestibular-spinal, cortico-propriospinal, and spinal interneurons that can synapse on multiple populations of spinal interneurons and motoneurons (Nielsen et al., 1993; Serranová et al., 2008).

In our study, similar to some previous work, we utilized the ISI of +10ms as the standardized group ISI to measure longer interval facilitation. The challenge with the paired TMS-PNS technique, especially during the measurement of longer interval facilitation, is that multiple descending volleys may contribute additive EPSPs onto spinal motoneuron membranes, providing a varied spatial and temporal integration of membrane excitability at the spinal motoneuron pool, which could also be a potential explanation for the higher magnitude of facilitation observed at the longer interval ISIs. The conduction velocities and number of synapses in corticomotor projections may vary across individuals (Nielsen et al., 1993; Serranová et al., 2008). Also, the polysynaptic and multi-pathway neural circuit correlates underlying longer interval facilitation may result in greater variability in amplitudes of conditioned soleus H-reflexes (Gray et al., 2017; Nielsen et al., 1993; Nielsen & Petersen, 1995b). The TMS-induced descending volleys within different corticofugal pathways may contribute to the inter-individual differences in both the ISI at which peak facilitation is observed and the magnitude of peak facilitation. Our results that a significantly larger magnitude of peak facilitation was observed at individualized ISIs versus a standard 10ms ISI, as well as the inter-individual variability in the ISI at which peak facilitation was observed, suggest that individualized ISIs may need to be determined for a comprehensive and rigorous measurement of TMS-induced H-reflex facilitation mediated by slower and/or indirect descending pathways.

Based on our current results, we posit that the measurement of longer-interval facilitation (e.g., EFD 0ms to 12ms) at a range of ISIs can help to index the effective connectivity of relatively slower and/or indirect pathways. If the methodological or time constraints of a study necessitate a brief assessment of descending corticomotor effective connectivity, then peak facilitation at longer interval ISIs, even at a single ISI (e.g., +10ms) may be sufficient to index the cumulative strength or excitability of the array of other descending connections between M1 and spinal motoneurons that are distinct from the fastest and/or direct descending pathways probed via earliest-onset facilitation. For a more refined or fine-grained characterization of descending corticomotor effective connectivity, a comprehensive longer-interval ISI curve for individual subjects (similar to **Figure 3-3**) can be collected, and also used to map changes in the ISI curve before versus after an intervention or experimental manipulation. If the ISI versus H-reflex facilitation curve shifts upward at longer intervals, that may suggest an increased effective connectivity in the slower and /or indirect descending pathways. If the peak or the entire curve shifts to the right, this may indicate a greater and perhaps compensatory reliance on a sub-population of relatively slower and /or indirect pathways (e.g., brain stem or propriospinal-mediated descending projections). Finally, while the force of magnetic field induced by TMS can be reduced by extracerebral tissues (scalp, bone, meninges), it is still able to induce an electrical field sufficient to depolarize superficial axons and to activate networks in the cortex (Lefaucheur et al., 2014).

3.5.3 Potential mechanisms and implications of inter-individual variability in magnitude and timing of TMS-induced facilitation

We posit that a neurophysiological assessment battery combining TMS, H-reflexes, and TMS-conditioned H-reflexes can probe site-specific changes in descending corticomotor circuits (Kurz et al., 2019), spinal reflex circuits (Niemann et al., 2018), and interactions between the two. Thus, utilization of paired TMS and PNS can provide more in-depth mechanistic insights into the specific site(s) and magnitude of training-induced plasticity in sensorimotor control circuitry. TMS-derived measures (e.g., MEP amplitude, motor threshold) and H-reflex data can be influenced by testing conditions such as posture, muscle activation, EMG sensor or stimulation electrode position, etc. In the current study, we controlled for, and maintained consistency of, these methodological parameters, manipulating only the relative timing of delivery of TMS with respect to PNS, and collecting unconditioned H-reflexes as a control or reference for the TMS-conditioned H-reflex amplitudes. Inter-individual differences in H-reflex and MEP latencies, nerve conduction velocity, limb length, neuroanatomical structure, and strength of effective functional connectivity may explain the variability observed in the magnitude of facilitation as well as the ISIs eliciting earliest or peak facilitation. Additionally, for the same individual, the trial-to-trial variability in latencies and relative synchronization of TMS-induced descending volleys and PNS-induced ascending volleys may result in physiological variability in the arrival time of orthodromic and antidromic stimuli at the spinal motoneurons (Baudry et al., 2015), which can further contribute to the variability in TMS-induced facilitation. In recent work by Wiegel and Leukel, during TMS-conditioning of H-reflexes, different TMS intensities (e.g., above resting motor threshold) as well as transcranial electrical stimulation were used in upper limb muscles to characterize different cortical pathways

(Leukel & Kurz, 2021). Recent work in monkey and human models also underscores the possibility of measuring the excitability of different cortical circuits by TMS H-reflex conditioning (Kurz et al., 2019; Wiegel et al., 2018).

3.5.4 Limitations and future directions

The limitations of this study include the relatively small sample size, although it is consistent with samples in other similar studies (Capozio et al., 2021; Gray et al., 2017; Lopez et al., 2020). For the current study, a single nerve stimulation intensity was chosen (PNS intensity eliciting an H-reflex amplitude of 20% Mmax) based on previous literature, and facilitation was not measured at a range of intensities across the H-reflex recruitment curve. Similarly, a single subthreshold TMS intensity was used. For instance, in recent studies, H-reflex facilitation at certain EFDs was shown to be differentially influenced by specific movement tasks (Wiegel et al., 2020; Wiegel & Leukel, 2020). The stimulation parameters used here (e.g., 1ms pulse width, separation between consecutive ISIs, sub-threshold TMS according to active motor threshold), while mostly consistent with previous work, have been modified in recent studies, particularly to probe specific neural contributions at the ISIs eliciting early facilitation. Future studies can investigate factors influencing the optimal ISI (e.g., latency of MEPs and H-reflexes) and develop a formula to enable individual-specific estimation of the optimal ISI for measuring earliest onset and peak facilitation.

Although earliest-onset and peak facilitation derived using paired TMS and PNS help to better localize the site of plasticity compared to TMS alone or H-reflexes alone, these techniques do not have the specificity to identify exactly which neural pathway is implicated in eliciting facilitation at different ISIs. As is true for many other non-invasive approaches, especially at longer ISIs, the observed H-reflex facilitation can be caused by many neural sources, spanning spinal, brain stem, and cortical sites, which are challenging to discriminate. Future work can combine TMS-facilitation of H-reflexes with neuroanatomical imaging or complementary neurophysiological techniques to determine relative contributions of specific descending pathways (e.g., cortico-reticulo-spinal, proprio-spinal) to these measures. Further, this method is limited to those muscles from which H-reflexes can be consistently elicited. The soleus and other lower limb muscles may have stronger spinal network contributions, which could influence our findings. Between-muscle differences in neuromotor circuit control can be investigated by applying similar methods in upper limb muscles and other lower limb muscles. We measured H-reflex facilitation in a seated active state for our study (i.e., while participants maintained low-level background EMG of ~10% MVC); future work can compare facilitation in seated versus standing, or during a dynamic postural or walking task (Nielsen et al., 1993; Nielsen & Petersen, 1995a).

Evaluation of the effect of neuropathologies such as stroke or spinal cord injury on the magnitude and temporal profile of TMS-induced H-reflex facilitation is a promising area of future investigation. Understanding how the relationship between ISI and magnitude of H-reflex facilitation (i.e., the ISI curve similar to shown in **Figure 3-2**) is modulated by neuropathological conditions warrants more study. For example, one may postulate that in individuals with a cortical or subcortical lesion affecting the corticospinal pathway, the faster-conducting, direct descending projections may show greater disruption of effective connectivity compared to relatively slower, indirect descending projections that traverse through brain stem centers (Li et al., 2018; Li & Francisco, 2015). In fact, these relatively slower and indirect descending pathways, partly indexed using peak facilitation or area under the curve for longer-latency ISIs, may show a compensatory reorganization to mediate functional recovery following stroke (Hammerbeck et al., 2021; Wilkins et al., 2020). We therefore would hypothesize that post-stroke individuals may show greater or preferential reduction in the magnitude of the earliest onset of TMS-induced facilitation (i.e., EFD 0ms).

3.6 Conclusions

Our study provides further evidence showcasing the advantage of TMS-induced H-reflex facilitation, especially when measured at a range of ISIs, as a unique non-invasive probe to differentially parse out the excitability of the array of direct, fast and indirect, slower descending corticomotor projections onto spinal reflex circuits. Our study findings can guide the methodology for use of the paired TMS-PNS technique in future investigations. Due to variability in conduction latencies of neuronal tracts in neurologically impaired individuals (such as stroke or multiple sclerosis), as well as inter-individual variability in physiological latencies, further development of methods and formulae to estimate the optimal ISI between TMS and PNS for each individual based on their baseline data merits further investigation.

Chapter 4: Influence of Age and Task-Related Activation on Descending Cortical Modulation of Spinal Sensorimotor Circuitry

This chapter is reproduced with minor edits from:

Lopez, AJ; Glover TJ; Mason CF; Ting LH; Borich MR; Kesar TM; "Influence of age and task-related activation on descending cortical modulation of spinal sensorimotor circuitry" (*in preparation*).

4.1 Abstract

Aging-related changes in sensorimotor circuitry can lead to deficits in balance control, resulting in increased risk for falls. Significant knowledge gaps exist in our understanding of aging effects on cortical and spinal sensorimotor neural circuit connectivity. Here, we utilized paired cortical and peripheral nerve stimulation (PNS) to evaluate aging effects on descending modulation of spinal reflexes during rest and active conditions. When a subthreshold transcranial magnetic stimulation (TMS) conditioning pulse is delivered before or after PNS, the resulting modulation of Hoffman's (H-) reflexes probes the influence of direct or faster and indirect or slower descending inputs on spinal motor neurons. We hypothesized that: 1) task-related activation will show greater magnitude of descending modulation of spinal reflexes compared to rest, 2) older adults will show reduced magnitude of descending modulation of spinal reflexes compared to young adults, and 3) descending modulation of spinal reflexes will be related to walking balance performance. Healthy young (YA; n=21; age 27 ± 4 years) and older (OA; n=26; age 63 ± 10 years) adults were evaluated. PNS was delivered to the posterior tibial nerve to generate a soleus H-reflex recruitment curve during sit rest (SR), sit active (SA), and quiet stance (QS) task conditions. Subthreshold TMS to the soleus motor cortex (M1) hotspot was paired with PNS (intensity: 50% Hmax) at three inter-stimulus intervals (ISIs) thought to probe the influence of direct pathways (-1.5ms), indirect pathways (+10ms) and late indirect pathways (+40ms). TMS-conditioned H-reflex amplitudes were used to calculate Conditioned H-reflex % (conditioned H-reflex / unconditioned H-reflex * 100%). The narrowing beam walking test (NBWT) assessed walking balance performance. During the SR task condition, both YA and OA groups showed significantly greater magnitude of Conditioned H-reflex % at the +10ms ISI compared to the -1.5ms ISI ($p<0.0001$) and

the +40ms ISI (p<0.0001). Both groups showed significantly greater magnitude of direct descending pathway modulation of spinal reflexes during task-related activation at the -1.5ms ISI (SR vs. SA: p=0.009; SR vs. QS: p<0.0001). Alternatively, both groups showed significantly decreased magnitude of indirect pathway modulation during task-related activation at the +10ms ISI (SR vs. SA: p<0.001; SR vs. QS: p=0.007). At the +40ms ISI, the OA group showed a significant reduction in late indirect pathway modulation compared to the YA group during both SA (p<0.0001) and QS (p<0.0001) task conditions. Finally, the YA group performed better in the NBWT compared to the OA group (p=0.0003); however, neither group showed a relationship between walking balance performance and descending modulation of spinal reflexes. In conclusion, task-related activation differentially influenced direct (-1.5ms ISI; increased) and indirect (+10ms ISI; decreased) pathway modulation of spinal reflexes, which suggests an activity-dependent effect on direct and indirect descending pathways in a regulatory manner to control spinal reflex gain. Notably, the OA group showed inhibited conditioned H-reflexes during task-related activation at the +40 ms ISI (i.e., late indirect pathways). This novel finding may reflect specific age-related changes to descending inhibitory control of spinal reflex circuitry during performance of motor tasks.

4.2 Introduction

Human movement, whether volitional or reflexive, requires the activation of spinal lower motor neurons (LMNs) and the subsequent command to musculature, known to be the final common path in the motor system (Grosprêtre, 2019; McCrea, 1996; Nielsen, 2004, 2016). However, spinal sensorimotor circuitry is complex, with spinal LMNs receiving varying synaptic inputs from descending supraspinal tracts (Brownstone & Chopek, 2018; Butler et al., 2007; Perreault & Giorgi, 2019; Sayenko et al., 2018), peripheral afferents (Baudry et al., 2014a; Collins et al., 1984), propriospinal neurons (Fisher & Baker, 2021; Iglesias et al., 2008), spinal interneurons (Hayes et al., 2009; Schieppati et al., 1990), and Renshaw cells (Alvarez et al., 2013; Bussel & Pierrot-Deseilligny, 1977; Nielsen & Pierrot-Deseilligny, 1996). The ability to comprehensively and non-invasively probe the influence of these synaptic inputs on spinal LMNs in humans remains challenging and requires rigorous methodologies. In humans, transcranial magnetic stimulation (TMS) (Cavaleri et al., 2017; Hallett, 2007; Kesar et al., 2018) and peripheral nerve stimulation (PNS) (Jimenez et al., 2018; Knikou, 2008; Walsh et al., 1998) have been used to probe the effective connectivity within descending cortical and spinal sensorimotor circuits, respectively. TMS can be used to assess corticospinal excitability by non-invasively stimulating the primary motor cortex (M1) to activate pyramidal neurons and recording the subsequent motor evoked potentials (MEPs) in target muscles using surface EMG (Bestmann & Krakauer, 2015; Brum et al., 2015; Skarabot et al., 2019). PNS can be used to assess the excitability of the monosynaptic spinal reflex pathway by electrically stimulating a mixed peripheral nerve and using electromyographic recording of the subsequent reflex response known as the Hoffmann (H-)reflex (Burke, 2016; Gassel & Diamantopoulos, 1966; Schieppati, 1987). While these independent approaches have provided valuable insights into their respective neural circuitries, significant knowledge gaps exist in our understanding of descending cortical influences on spinal sensorimotor circuits.

To address this gap in knowledge, previous studies from our labs and others have utilized paired non-invasive stimulation (TMS + PNS) to characterize interactions between descending cortical and spinal sensorimotor circuits (Geertsen et al., 2011; Gray et al., 2017; Lopez et al., 2020; Nielsen et al., 1993; Taube et al., 2017; Xu et al., 2022). Common in these studies, a subthreshold TMS conditioning pulse is delivered before or after PNS, and the resulting modulation of soleus H-reflexes provides insight into the influence of direct, faster and indirect, slower descending volleys on the spinal LMN pool (Lopez et al., 2020; Nielsen & Petersen, 1995b; Xu et al., 2022). Additionally, studies have demonstrated that TMS intensity (Andrews et al., 2020; Leukel & Kurz, 2021; Niemann et al., 2018), coil orientation (Niemann et al., 2018), PNS intensity (Lopez et al., 2020), and timing of paired stimulation (Geertsen et al., 2011; Taube et al., 2017; Xu et al., 2022) have effects on the magnitude of descending modulation of spinal reflexes in healthy adults. Of these studies, several have utilized and established standardized inter-stimulus intervals (ISIs) as representative for characterizing the excitability of direct, fast-conducting and indirect, slower-conducting pathways. Direct descending pathway modulation, also referred to as 'short interval facilitation' of the H-reflex occurs when a subthreshold TMS pulse is delivered 1.5 ms after a PNS pulse, allowing the direct, fastest descending volleys to arrive at the spinal LMN pool prior to the afferent signal (Gray et al., 2017; Lopez et al., 2020; Nielsen & Petersen, 1995b). Indirect descending pathway modulation, also referred to as 'long interval facilitation', of the H-reflex occurs when a TMS pulse is delivered before PNS, allowing indirect, slower descending volleys to arrive prior to the afferent signal (Gray et al., 2017; Lopez et al., 2020; Nielsen & Petersen, 1995b). While findings from these methodological studies have established a framework for probing descending cortical influences on spinal sensorimotor circuitry, little is known about the effects of aging and task-related activation on interactions between these circuits.

Previous studies have shown that aging influences corticospinal and spinal reflex excitability using TMS and PNS independently. In previous studies on the effects of aging, older adults demonstrated longer soleus H-reflex latencies (Falco et al., 1994; Klass et al., 2011; Sabbahi & Sedgwick, 1982; Scaglioni et al., 2003), decreased soleus

Hmax/Mmax ratios (Angulo-Kinzler et al., 1998; Baudry et al., 2014a; Scaglioni et al., 2003), and longer MEP latencies (Baudry et al., 2014b, 2015; Shibuya et al., 2016), which all indicated aging-related declines in speed of signal transmission (i.e., conduction velocity) and synaptic efficacy (i.e., spinal LMN membrane excitability). Interestingly, findings from several studies on MEP amplitudes in young and older adults have yielded varied results. Some studies show that older adults have greater corticospinal excitability (i.e., increased MEP amplitude) compared to young adults (Baudry et al., 2014a, 2014b, 2015), while other studies show that the MEP amplitude decreased with age (Oliviero et al., 2006) or was similar to young adults (Rossini et al., 1992). However, these contrasting findings may be due to differences in the task-related activation of the target soleus muscle and warrant further investigation.

While the effects of task-related activation on corticospinal and spinal reflex excitability have been well studied in young adults, few studies have investigated the effects of task-related activation on descending modulation of spinal sensorimotor circuitry in young adults. Previous studies in young adults have reported a decrease in H-reflex amplitude from seated or prone posture to upright stance (Angulo-Kinzler et al., 1998; Koceja et al., 1995; Tsuruike et al., 2003), which is assumed to reduce the risk of unexpected reflex activity that may impair balance control (Diener et al., 1983; Katz et al., 1988). Several studies on subthreshold TMS-conditioned H-reflexes have been done at rest in young adults, while subjects were seated or lying on an examination bed (Andrews et al., 2020; Guzmán-López et al., 2015; Leukel & Kurz, 2021). Other studies in young adults have investigated the magnitude of descending modulation of soleus H-reflexes during different motor tasks: seated voluntary activation (Gray et al., 2017; Lopez et al., 2020; Nielsen & Petersen, 1995b), upright standing (Goulart et al., 2000; Guzmán-López et al., 2015), and antagonist muscle activation (Geertsen et al., 2010, 2011; Nielsen et al., 1993). While these studies have investigated the magnitude of descending modulation of spinal sensorimotor circuitry during different motor tasks, to our knowledge, no prior studies have concurrently investigated the effects of aging-related changes and task-related activation on descending cortical influences on spinal sensorimotor circuits.

The purpose of the current study was to investigate how aging-related changes and task-related activation impact interactions between descending cortical and spinal sensorimotor circuits. Additionally, to determine if any potential relationship exists between descending cortical modulation of spinal reflexes and walking balance performance. We hypothesized that 1) aging-related changes would result in reduced magnitude of descending modulation of spinal reflexes compared to young adults, 2) task-related activation (i.e., seated voluntary activation, upright quiet standing) would show greater magnitude of descending modulation of spinal reflexes compared to a seated rest task, and 3) better walking balance performance would be associated with greater magnitude of descending cortical modulation of spinal reflexes.

4.3 Methods

4.3.1 Study Participants

Twenty-one young (11 females; age 27 ± 4 years) and twenty-six older (14 females; age 63 ± 10 years) neurologically intact adults were recruited to complete the experimental protocol. Each participant provided written informed consent in accordance with the Declaration of Helsinki. Study procedures were approved by the Emory University Institutional Review Board (IRB#00083425).

4.3.2 Experimental Design

Participants completed a single experimental session lasting approximately 3.5 hours. The dominant leg was determined by asking participants their preferred leg when kicking a ball (Promsri et al., 2018, 2020), and data from the dominant leg's soleus and tibialis anterior (TA) muscle were recorded. Measurements were obtained in three positions: while the participant was 1) seated in a chair, hands resting in the lap, feet on the ground, and no volitional activation of target soleus muscle, 2) seated in a chair, hands resting in the lap, feet on the ground, and asked to maintain a low-level volitional activation of target soleus muscle, and 3) in an upright stance with their feet shoulder-width apart, arms resting down at their sides, and facing straight ahead (**Figure 4-1**).

4.3.3 Electromyography (EMG) Procedures

Two surface electromyography (EMG) sensors (2-cm diameter, EL503 Biopac Systems Inc., Goleta, CA) were placed on the skin overlying the tibialis anterior (TA) and soleus muscles, with a common ground electrode placed on the ipsilateral lateral malleolus. To verify proper EMG sensor placement, participants were asked to contract their soleus and TA muscles while an experimenter confirmed EMG activation (Biopac Systems AcqKnowledge Software Version 4.4). After proper EMG placement was confirmed, EMG activity during a maximal voluntary contraction (MVC) of the TA and soleus was recorded, completed through resisted dorsiflexion of the ankle in a seated position for the TA and standing single leg heel raises for the soleus. The participant was asked to maintain maximal contraction of the target muscle for 5 seconds for 2 trials, after which the MVC was determined using the largest EMG amplitude.

Visual biofeedback regarding ongoing TA and soleus EMG (LabVIEW, National Instruments Corporation) was used to enable participants to maintain consistent background EMG activation during the experimental session, and for the experimenters to ensure target level of muscle activation remained consistent during testing. During the seated active task condition, participants were asked to maintain 10% of their soleus MVC, which is a low-level activation that can also be observed during upright quiet stance. EMG activity during quiet stance was verified by having the participant stand quietly for 30 seconds, during which the TA exhibited little-to-no muscle activity and the soleus slightly activated, similar to the 10% soleus MVC during the seated active task condition.

4.3.4 Peripheral Nerve Stimulation (PNS) Procedures

To elicit soleus Hoffmann (H-)reflexes, peripheral nerve stimulation (PNS) was applied to the posterior tibial nerve within the popliteal fossa using a monopolar cathode (round, 2.5 cm), with the anode (square, 5 cm) placed at the midline proximal to the patella (Biopac Systems Inc, Goleta, CA). The stimulating electrodes were self-adhering carbon rubber electrodes. To find the optimal PNS site, the participant was asked to remain in a quiet standing position with equal weight bearing of bilateral lower extremities. The optimal location of the cathode was determined by sending PNS pulses and noting the location that elicited the largest H-reflex amplitude and evoked contraction of the soleus muscle. Once optimal cathode location was determined, inelastic overwrap was used to secure the cathode in place. EMG sensors and stimulating electrodes were also secured to the skin with tape to ensure maximal contact.

Next, the PNS intensities required for threshold for the H-reflex and the maximal M-wave response (Mmax) were obtained during each task condition: sit rest (SR), sit active (SA), and quiet stance (QS). The H-reflex threshold was defined as the lowest PNS intensity where an H-reflex response greater than 0.1mV was visible. The intensity for Mmax was determined after three increasing PNS intensities were sent and the M-wave response plateaued. Soleus H-reflex and M-wave recruitment curves were collected during each task condition (SR, SA, QS). The order of increasing PNS intensities used for the 60 pulses was determined by calculating 20% below the intensity needed to reach H-threshold and 20% above the intensity needed to elicit Mmax; then, the difference between these calculated numbers was dividing by 60. Following these 60 pulses, a H-reflex recruitment curve was plotted, and intensity required to elicit 50% of the maximum H-reflex (Hmax) amplitude was determined for use during TMS-conditioning for each task condition, respectively.

4.3.5 Transcranial Magnetic Stimulation (TMS) Procedures

During TMS evaluation, posture, position of lower and upper limb joints, and background activation state of soleus and TA muscles were monitored. Stereotaxic neuronavigation (Brainsight v. 2.2.14, Rogue Research Inc., Canada) was used to register and scale the participant's head to a standard brain template, and track coil location with respect to the TMS stimulation site. Motor evoked potentials (MEPs) in the target soleus muscle were recorded in response to TMS, using surface EMG sensors. TMS pulses were delivered through a Magstim 200^2 stimulator using a custom 70 mm figure-of-eight batwing coil (Magstim Company Ltd., Dyfed, UK). Soleus hotspot was determined as the location that elicited the largest MEPs from the soleus muscle.

Stereotactic neuronavigation was used to ensure consistent TMS delivery to identified hotspot and accurate coil positioning during the experimental session.

Resting motor threshold (rMT) and active motor threshold (aMT) were determined by stimulating over the hotspot to elicit MEPs. rMT is the lowest TMS intensity that elicits more than 3/5 MEPs of an amplitude greater than 50μV. aMT is the lowest TMS intensity that elicits more than 3/5 MEPs of amplitude greater than 100μV. MEP data were recorded in response to 10 trials at subthreshold intensity (90% MT) and 20 trials at suprathreshold intensity (120% MT). To be considered subthreshold, a maximum of 3/10 TMS pulses elicited MEPs greater than 50μV for rest and 100μV for active conditions. To be considered suprathreshold, a minimum of 14/20 TMS pulses elicited MEPs greater than 50μV for rest and 100 μV for active conditions.

4.3.6 TMS-conditioning of Soleus H-reflexes

To investigate the influence of direct and indirect descending pathways on spinal reflex excitability, a PNS intensity that elicited an H-reflex amplitude at 50% Hmax was paired with a subthreshold (90% rMT or aMT) TMS pulse at three different inter-stimulus intervals, or ISIs (-1.5ms, +10ms, +40ms). The negative ISI (-1.5ms) indicates that the PNS pulse was sent *prior to* the subthreshold TMS pulse, and the two positive ISIs (+10ms, +40ms) indicate the PNS pulse was sent *after* the subthreshold TMS pulse. TMS-conditioned soleus H-reflexes were collected during each task condition: sit rest (SR), sit active (SA), and quiet stance (QS) (**Figure 4-1**). Prior to each session, the order of each set and the order of the ISIs were randomized in Microsoft Excel using the RAND() function. Five blocks of repeating pulses sent approximately 5-6 seconds apart were delivered in the following order: PNS alone to elicit an unconditioned (UC) soleus H-reflex, followed by 3 paired PNS + TMS pulses to condition soleus H-reflexes at 3 predetermined randomized ISIs (i.e., -1.5ms, +10ms, +40ms). The main outcome measure for TMS conditioning of soleus H-reflexes was the Conditioned H-reflex %, calculated using the peak-to-peak amplitude of the conditioned H-reflex response compared to the peak-to-peak amplitude of the unconditioned H-reflex response x 100% (**Figure 4-1**).

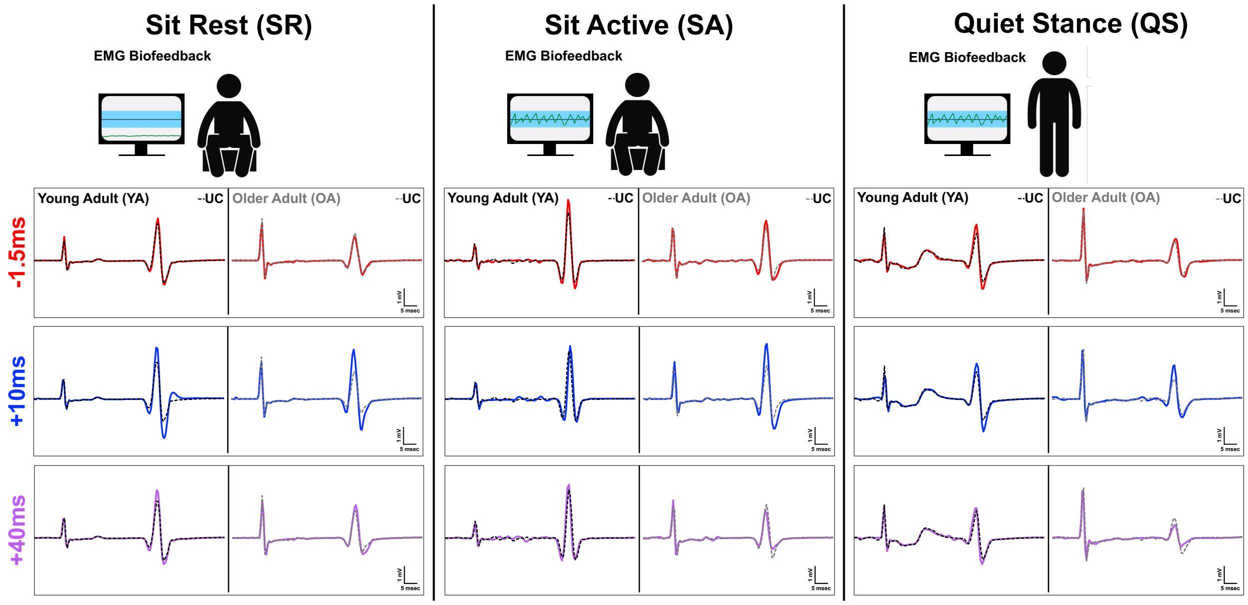

Figure 4-1. Representative H-reflex traces from a young adult (YA) (left) and older adult (OA) (right) participant, respectively, during each task condition (sit rest, sit active, quiet stance) and at the three inter-stimulus intervals, or ISIs (-1.5ms, +10ms, +40ms). The dashed black lines (YA) and dashed gray lines (OA) depict unconditioned (UC) H-reflex responses, while the solid, colored lines depict the conditioned H-reflex responses at each ISI (-1.5ms = blue; +10ms = red; +40ms = purple). During the QS task condition, the YA representative traces show a small 'bump' prior to the H-reflex response, which is beginning formation of the M-wave response. Note the small increases in H-reflex amplitude during task-related activation at the -1.5ms ISI, larger H-reflex amplitude during sit rest at the +10ms ISI and decreases in H-reflex amplitude for OA during task-related activation at the +40ms ISI.

4.3.7 Beam-walking Task

The Narrowing Beam Walking Test (NBWT) is a performance-based test developed to challenge balance control by constraining step width and/or reducing the support surface (Sawers & Hafner, 2018). Consistent with administration procedures, participants were asked to cross their arms over their trunk and walk along the narrowing beam while wearing standardized shoes. Each trial concluded when the participant 1) stepped off the beam, 2) uncrossed their arms, or 3) walked the full length of the beam. The distance walked, divided by the total length of the beam (6.71m), was recorded as the normalized distance. The average normalized distance over trials 3 to 5 was used as the NBWT score.

4.3.8 Statistical Analyses

Normality was assessed using quantile-quantile (Q-Q) plots. Sphericity was assessed using Mauchly's test, and if sphericity could not be assumed, the Greenhouse-Geisser correction was applied to the degrees of freedom. Significant main effects were followed by post hoc contrasts and corrected for multiple comparisons using the Bonferroni method. Due to some participants not completing all task conditions (e.g., several missing samples in the datasets), restricted maximum likelihood (REML) calculations were performed for mixed-effects model analysis. To first confirm the effects of task-related activation (SR, SA, QS) and aging-related changes (YA, OA) on the unconditioned soleus H-reflex normalized to Mmax, a mixed-effects model was fitted. A two-way repeated measures analysis of variance (ANOVA) was then performed to evaluate the effects of timing, or ISIs (-1.5ms, +10ms, +40ms), and aging (YA, OA) on TMS-conditioned soleus H-reflexes during SR. To characterize the effects of task-related activation (SR, SA, QS) and aging (YA, OA) on TMS-conditioned soleus H-reflexes, mixed-effects analyses were performed at each ISI (-1.5ms, +10ms, +40ms). Additionally, at the +40ms ISI during SA, a Pearson correlation coefficient was computed to assess the linear relationship between age in years and the Conditioned H-reflex %. Mixed-effects analyses were completed to compare the change in magnitude of the Conditioned H-reflex % from SA to SR and QS to SR for both groups (YA, OA) and at each ISI (-1.5ms, +10ms, +40ms). Finally, Welch's t test was performed to compare NBWT scores between young and older adult groups, and Pearson correlation coefficients were calculated to assess the linear relationship between NBWT score and Conditioned H-reflex % at the +40ms ISI. All statistical tests were run in GraphPad Prism version 9.4 and the critical alpha level was set to $p < 0.05$.

4.4 Results

Complete datasets for all 3 task conditions were collected for 20 of the 21 young adult (YA) participants and 13 of the 26 older adult (OA) participants. 7 of the 26 OA participants completed the experimental session during SR and SA task conditions, and 3 of 26 OA participants completed the experimental session only during the QS task condition. One YA participant and three OA participants were not included in the analyses due to an inability to elicit stable, consistent soleus H-reflexes during seated and standing task conditions in the experimental session.

4.4.1 Effects of task-related activation and aging on unconditioned soleus H-reflexes

To confirm the effects of task-related activation and aging-related changes, mixed-effects analysis revealed significant main effects of task condition [$F_{(1.956,\ 65.52)} = 9.54$, $p < 0.001$] and group [$F_{(1,\ 39)} = 11.29$, $p = 0.002$] on unconditioned soleus H-reflexes normalized to Mmax. No significant interaction between task condition and group was observed [$F_{(2,\ 67)} = 0.008$, $p = 0.992$]. Across task conditions, OA showed significantly reduced unconditioned soleus H-reflexes compared to YA. Post hoc pairwise comparisons revealed significantly smaller unconditioned soleus H-reflexes during QS compared to SR ($p = 0.013$) and SA ($p = 0.004$) (**Figure 4-2**).

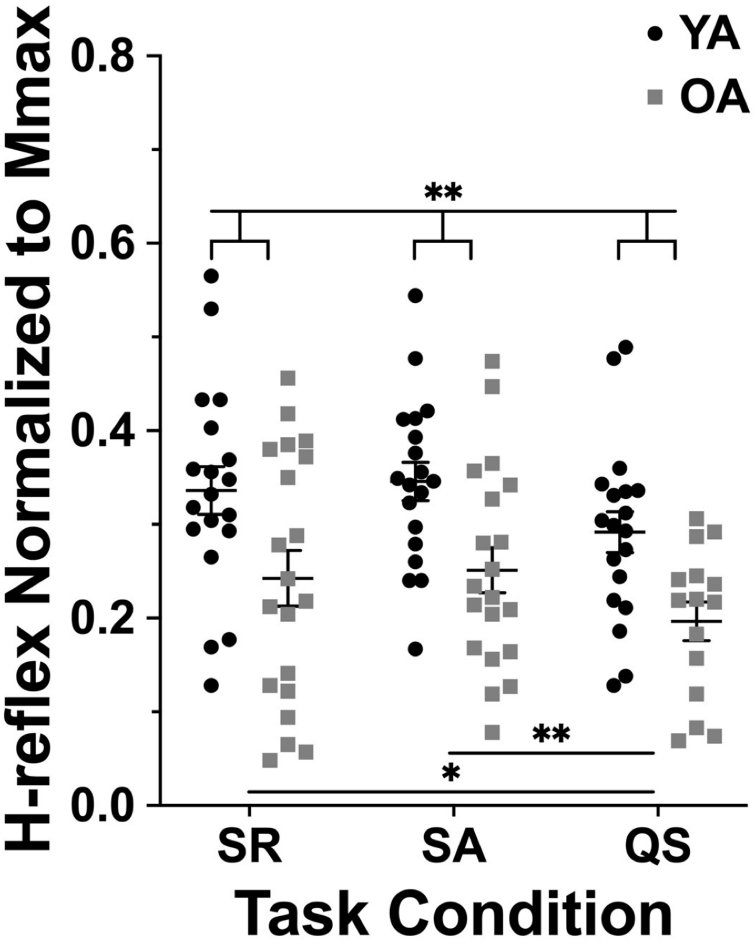

Figure 4-2. Unconditioned (UC) soleus H-reflexes normalized to participant's Mmax during each task condition (SR, SA, QS). Across groups, normalized soleus H-reflexes were significantly lower during QS compared to SA (p=0.004) and SR (p=0.013). OA also showed lower normalized soleus H-reflexes compared to YA (p=0.002). *p<0.05; **p<0.01

4.4.2 Effects of timing and aging on TMS-conditioned soleus H-reflexes at rest

The two-way repeated measures ANOVA revealed a significant main effect of timing on the conditioned H-reflex % [$F_{(1.594, 60.57)} = 37.57$, $p < 0.0001$]. Post hoc pairwise comparisons revealed significantly greater conditioned H-reflex % at the +10ms ISI compared to the -1.5ms ISI ($p < 0.0001$) and the +40ms ISI ($p < 0.0001$), and significantly greater conditioned H-reflex % at the -1.5ms ISI compared to the +40ms ISI ($p = 0.0267$) (**Figure 4-3**).

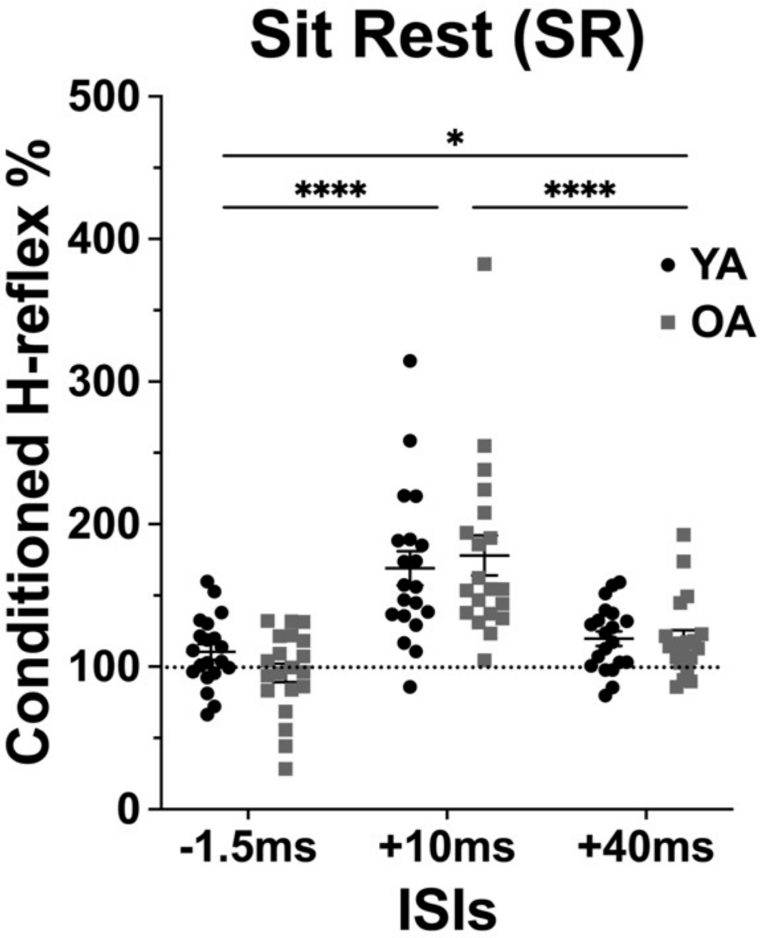

Figure 4-3. Conditioned H-reflex % at each ISI during the sit rest (SR) task condition. During SR task condition, both young adult (YA) and older adult (OA) groups showed significantly greater magnitude of Conditioned H-reflex % at the +10ms ISI compared to the -1.5ms ISI (p<0.0001) and the +40ms ISI (p<0.0001). Additionally, the Conditioned H-reflex % at the 40ms ISI was significantly greater than the -1.5ms ISI (p=0.027). *p<0.05; ****p<0.0001

4.4.3 Effects of task-related activation and aging on TMS-conditioned soleus H-reflexes

To investigate the effects of task-related activation and aging on *direct* pathway modulation of soleus H-reflexes, mixed-effects analysis revealed a significant main effect of task condition [$F_{(1.761,\ 59.86)} = 7.953$, $p = 0.001$] on the conditioned H-reflex % at the -1.5ms ISI. Post hoc pairwise comparisons revealed a significantly larger magnitude of conditioned H-reflex % during SA ($p = 0.009$) and QS ($p < 0.0001$) compared to SR (**Figure 4-4A**). Additionally, to investigate the effects of task-related activation and aging on *indirect* pathway modulation of soleus H-reflexes, mixed-effects analysis revealed a significant main effect of task condition [$F_{(1.758,\ 96.71)} = 11.19$, $p < 0.0001$] on the conditioned H-reflex % at the +10ms ISI. Post hoc pairwise comparisons revealed a significantly reduced magnitude of conditioned H-reflex % during SA ($p = 0.0009$) and QS ($p = 0.0073$) compared to SR (**Figure 4-4B**).

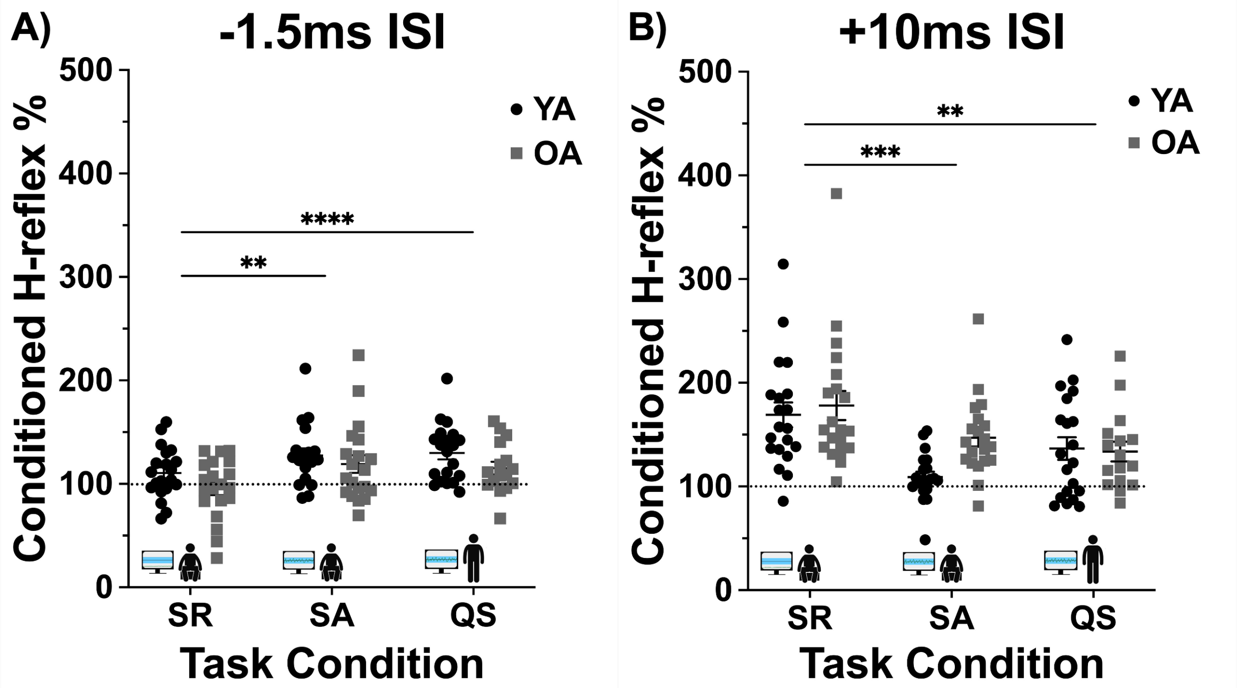

Figure 4-4. Conditioned H-reflex % at the -1.5ms and +10ms ISI during three task conditions (SR, SA, QS). (A) At the -1.5ms ISI, the Conditioned H-reflex % was significantly greater during SA (p=0.009) and QS (p<0.0001) compared to SR. **(B)** At the +10ms ISI, the Conditioned H-reflex % was significantly less during SA (p<0.001) and QS (p=0.007) compared to SR. **p<0.01; ***p<0.001; ****p<0.0001

To investigate the effects of task-related activation and aging on *late indirect* pathway modulation of soleus H-reflexes, mixed-effects analysis revealed a significant main effect for task condition [$F_{(1.775, 97.65)} = 19.12$, $p < 0.0001$], group [$F_{(1, 110)} = 31.54$, $p < 0.0001$], and an interaction between task condition and group [$F_{(2, 110)} = 7.992$, $p = 0.0006$]. Post hoc pairwise comparisons revealed the OA group had significantly reduced magnitude of conditioned H-reflex % during SA ($p < 0.0001$) and QS ($p < 0.0001$) compared to the YA group (**Figure 4-5B**). Additionally, within the OA group, the magnitude of Conditioned H-reflex % was significantly reduced during SA ($p = 0.0002$) and QS ($p = 0.0004$) compared to SR (**Figure 4-5C**). Finally, a Pearson correlation analysis revealed a significant negative correlation between conditioned H-reflex % at the +40ms ISI during SA and age in years ($r = -0.470$, $p = 0.037$) (**Figure 4-5D**).

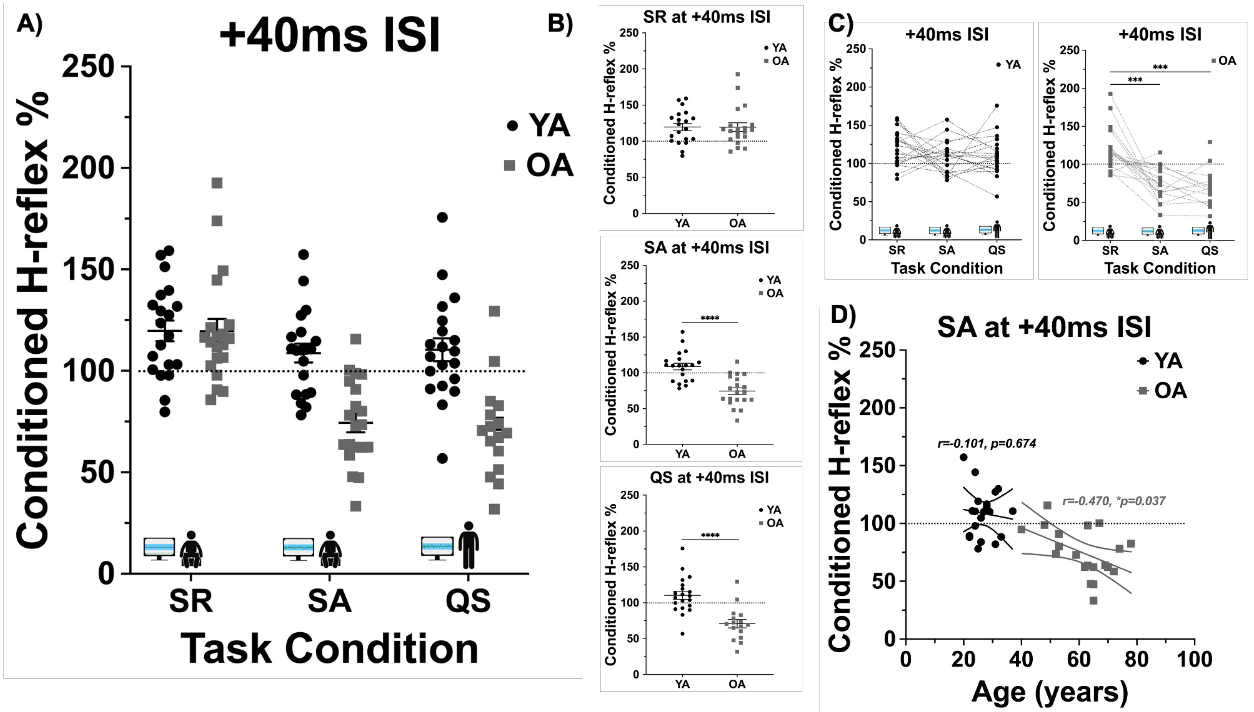

Figure 4-5. Conditioned H-reflex % at the +40ms ISI during all three task conditions for both young adult (YA) and older adult (OA) groups. **(A)** Individual data points at the +40ms ISI for both YA and OA groups with the Conditioned H-reflex % on the y-axis and the three task conditions on the x-axis. **(B)** When compared to YA, OA showed

significantly lower Conditioned H-reflex % at the +40ms ISI during the SA ($p<0.0001$) and QS ($p<0.0001$) task conditions. No significant difference was observed during SR. **(C)** Within the OA group, the magnitude of Conditioned H-reflex % was significantly reduced during the SA ($p=0.0002$) and QS ($p=0.0004$) compared to the SR task condition. No significant differences were observed within the YA group. **(D)** When plotting the Conditioned H-reflex % as a function of age in years, a significant negative correlation was observed for the OA group, where the greater the age of an individual, the more reduced the Conditioned H-reflex % becomes. $^{*}p<0.05$; $^{***}p<0.001$; $^{****}p<0.0001$

4.4.4 Change in conditioned H-reflex % between task-related activation and timing

To explore the change (Δ) in Conditioned H-reflex % between voluntary activation while seated (SA) versus resting while seated (SR) and across ISIs, mixed effects analysis revealed a significant main effect for ISI [$F_{(1.661, 62.28)} = 17.91$, $p < 0.0001$] and an interaction between ISI and group [$F_{(2, 75)} = 4.021$, $p = 0.0219$]. Post hoc pairwise comparisons revealed that when collapsed across groups, Δ Conditioned H-reflex % (SA - SR) at the -1.5ms ISI was significantly greater during voluntary activation compared to the +10ms ISI ($p < 0.0001$) and the +40ms ISI ($p < 0.0001$). Additionally, at the +40ms ISI, Δ conditioned H-reflex % (SA - SR) was significantly lower during voluntary activation for the OA group compared to the YA group ($p = 0.023$) (**Figure 4-6A**).

To explore the change in Conditioned H-reflex % between postural activation while in quiet stance (QS) versus resting while seated (SR) and across ISIs, mixed effects analysis revealed a significant main effect for ISI [$F_{(1.455, 45.10)} = 14.10$, $p < 0.0001$] and a trend towards statistically significant interaction between ISI and group [$F_{(2, 62)} = 2.754$, $p = 0.071$]. Post hoc pairwise comparisons revealed that when collapsed across groups, Δ Conditioned H-reflex % (QS - SR) at the -1.5ms ISI was significantly greater during postural activation compared to the +10ms ISI ($p = 0.0003$) and the +40ms ISI ($p < 0.0001$) (**Figure 4-6B**).

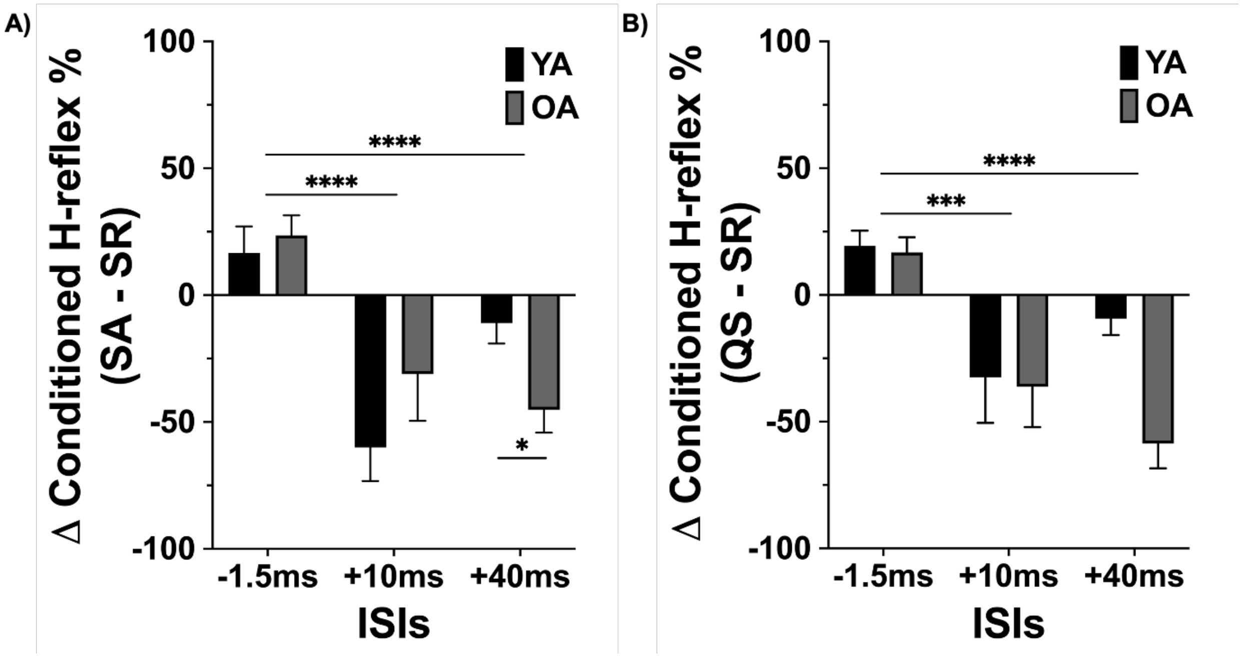

Figure 4-6. The change (Δ) Conditioned H-reflex % between task-related activation conditions (SA, QS) versus the rest condition (SR) at each ISI where the line at 0 indicated no change in the Conditioned H-reflex % between the task conditions. (A) The change from SA to SR indicates that voluntary activation significantly decreased the magnitude of Conditioned H-reflex % at the +10ms (p<0.0001) and +40ms (p<0.0001) ISIs compared to the -1.5ms ISI. Additionally, at the +40ms ISI, OA showed greater decrease in the magnitude of Conditioned H-reflex % during voluntary activation compared to YA (p=0.023). **(B)** The change from QS to SR indicates that postural activation significantly decreased the magnitude of Conditioned H-reflex % at the +10ms (p<0.001) and +40ms (p<0.0001) ISIs compared to the -1.5ms ISI. *p<0.05; ***p<0.001; ****p<0.0001

4.4.5 Walking balance performance and the relation to descending modulation of spinal reflexes

First, to demonstrate the effects of aging on walking balance performance (i.e., NBWT), a Welch's t test revealed that the older adult (OA) group performed significantly worse on the NBWT compared to the young adult (YA) group ($p < 0.0001$) (**Figure 4-7A**). To explore any potential relationship between NBWT scores and Conditioned H-reflex % at the +40ms ISI, Pearson correlation analyses revealed no significant relationship for during SA (YA: $p = 0.57$; OA: $p = 0.93$) and QS (YA: $p = 0.38$; OA: $p = 0.32$) for both groups (**Figure 4-7B**).

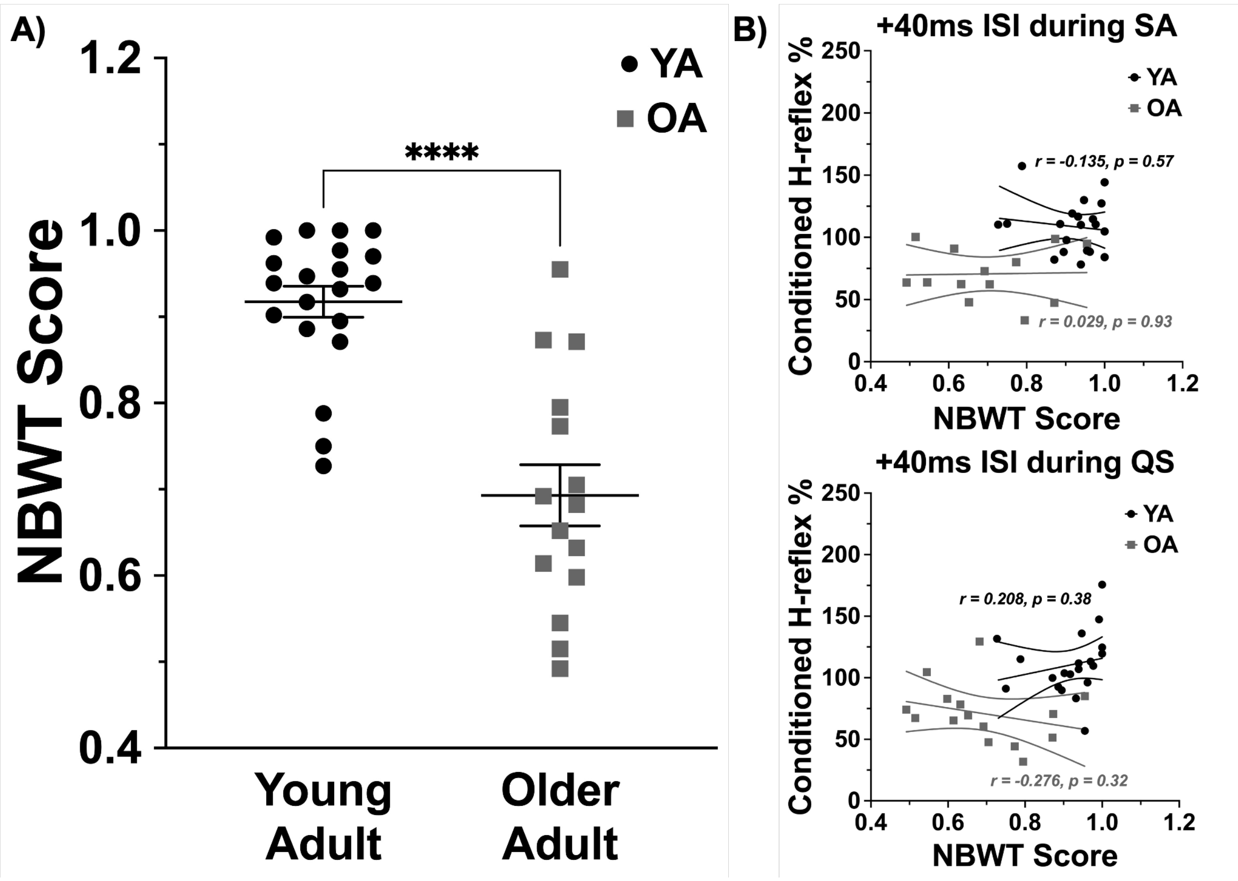

Figure 4-7. Walking balance performance assessed via the narrowing beam walking test (NBWT) and the relation to Conditioned H-reflex % during task-related activation at the +40ms ISI. (A) The YA group performed significantly better on the NBWT assessment compared to the OA group (p<0.0001). **(B)** Pearson correlation analyses revealed that at the +40ms ISI, both YA and OA groups showed no relationship between Conditioned H-reflex (%) and NBWT Score during task-related activation (i.e., SA and QS).

4.5 Discussion

Here, we investigated the effects of aging-related changes and task-related activation on descending cortical modulation of spinal sensorimotor circuits using non-invasive paired stimulation. Our results revealed that across the two groups, during the sit rest (SR) task condition, the magnitude of TMS-conditioned H-reflexes was largest at the +10ms ISI. There were no between-group differences in the magnitude of H-reflex modulation during the SR condition. Both groups also showed increased magnitude of direct pathway (-1.5ms ISI) modulation of H-reflexes and decreased magnitude of indirect pathway (+10ms ISI) modulation of H-reflexes during the sit active (SA) and quiet stance (QS) task conditions compared to the SR task condition. Additionally, for the older adult (OA) group, voluntary (i.e., SA) and postural (i.e., QS) activation resulted in reduced magnitude of late indirect (+40ms ISI) pathway modulation of H-reflexes compared to young adults. Finally, when characterizing the change in the magnitude of descending modulation of H-reflexes between active task conditions (i.e., SA, QS) and the rest task condition (i.e., SR), in both groups, the direct pathway (-1.5ms ISI) showed greater H-reflex modulation during task-related activation compared to rest (positive change scores), while indirect pathways (+10ms ISI; +40ms ISI) showed smaller magnitude of H-reflex modulation during task-related activation compared to rest (negative change scores). Taken together, our findings revealed that task-related activation differentially influenced descending modulation via direct and indirect pathways, and aging resulted in greater descending inhibitory control of spinal reflexes during task-related activation.

4.5.1 Task- and aging-related changes in unconditioned soleus H-reflexes

Previous studies evaluating the effects of task-related activation on soleus H-reflexes have reported decreases in H-reflex amplitude from seated or prone posture to upright standing (Baudry et al., 2015; Koceja et al., 1995). Additionally, several studies have reported an aging-related decrease in the amplitude of soleus H-reflexes (Baudry et al., 2014a, 2014b; Scaglioni et al., 2003). However, these studies typically report the Hmax as a percentage of the Mmax [Hmax (%Mmax)]. Here, we evaluated the unconditioned soleus H-reflex response, calculated from the average H-reflex amplitude at 50% Hmax during each task condition, and normalized the unconditioned H-reflex response to each individual's Mmax. Our results revealed that in both young and older adult groups, the normalized H-reflex response was reduced during upright quiet stance (QS) compared to the sit rest (SR) and sit active (SA) task conditions. Our findings indicated a decrease in the efficacy of synaptic transmission between Ia afferents and spinal LMNs during QS, suggesting a reduced reliance on afferent inputs from muscle spindles to control leg muscles during upright stance. However, across the task conditions, the normalized H-reflex response significantly decreased to a greater extent in older adults, potentially reflecting an age-related reorganization of the spinal control of upright standing (Hortobágyi et al., 2018; Papegaaij et al., 2014). This aging-related depression in the efficacy of the synaptic transmission between Ia afferents and spinal LMNs likely relies, at least in part, on an increase in Ia presynaptic inhibition (Katz et al., 1988) to possibly reduce the risk of unexpected reflex activity that may challenge balance control (Diener et al., 1983). During maintenance of upright stance for older adults, it is possible that increased activation of Ia inhibitory interneurons from an antagonist muscle (i.e., TA), as well as increased descending inhibitory control from brainstem-mediated pathways, may account for increased H-reflex suppression during upright stance. Our findings, in conjunction with previous studies, suggest that down-regulation of soleus H-reflexes during standing reflects the ability of the CNS to reduce the risk of unexpected reflex activity that may impair balance control.

4.5.2 Mechanisms and interpretation of task-related changes in TMS-conditioned soleus H-reflexes

When we combine PNS with a subthreshold TMS pulse using a short timing interval (i.e., -1.5ms ISI), we can generate faster, direct descending volleys that arrive at the spinal cord prior to the arrival of the afferent signal. Alternatively, when combining PNS with subthreshold TMS pulse using longer timing intervals (i.e., +10ms, +40ms

ISIs), we allow for slower-conducting, indirect descending volleys to arrive at the spinal segmental level prior to the afferent signal. Our findings revealed that during a seated rest (SR) task condition, an effect of temporal interval, or timing, was seen across both groups (YA and OA), suggesting no significant aging-related change in descending modulation of spinal reflexes during a seated rest task. However, the timing between paired non-invasive stimulation can differentially impact the magnitude of the TMS-conditioned soleus H-reflex response regardless of age. Previous studies have reported similar findings in healthy adults at the -1.5ms ISI and +10ms ISIs (Andrews et al., 2020; Nielsen et al., 1993; Niemann et al., 2018), and one other study investigated the +40ms ISI while laying at rest in a supine position (Guzmán-López et al., 2015). Here, and previous findings, demonstrate that the largest magnitude of TMS-conditioned soleus H-reflexes occurred at the +10ms ISI (Lopez et al., 2020; Xu et al., 2022), suggesting that a large proportion of excitatory descending inputs to the spinal segmental level contributed to greater facilitation of the soleus H-reflex response. The +10ms ISI typically represents the "peak facilitation" timing interval, possibly mediated by contributions from several different corticofugal pathways (i.e., reticulospinal, vestibulospinal) and may reflect greater opportunity for varied temporal summation of excitatory- and inhibitory-postsynaptic potentials (EPSP-IPSP) in the spinal LMN pool.

However, the effects of task-related activation on each of these ISIs becomes crucially important for understanding the influence of direct and indirect pathway modulation of soleus H-reflexes. Our findings revealed that task-related activation, whether voluntary or postural, has differential effects on TMS-conditioned soleus H-reflexes dependent on the ISI used in the young and older adult groups. Direct pathway modulation (i.e., -1.5ms ISI) is thought to index the direct, faster-conducting descending volleys that arrive at the spinal segment prior to the arrival of the Ia afferent signal

(Lopez et al., 2020; Nielsen et al., 1993; Taube et al., 2017). Our results show that both groups demonstrated increased magnitude of direct pathway modulation of spinal reflexes during task-related activation compared to the SR task condition, which suggests that regardless of the active motor task, voluntary and postural activation preferentially recruited excitatory direct descending pathways that facilitated the TMS-conditioned soleus H-reflex response in both young and older adults. In contrast to direct pathway modulation, indirect pathway modulation (i.e., +10ms ISI) is thought to be mediated by indirect, slower descending volleys arriving prior to the Ia afferent signal (Geertsen et al., 2011; Gray et al., 2017; Lopez et al., 2020). Our results show that both groups demonstrated reduced magnitude of indirect pathway modulation of spinal reflexes during task-related activation compared to the SR task condition, which suggests that voluntary and postural activation may recruit activity-dependent descending inhibitory pathways that suppress the reflex response in both young and older adults.

4.5.3 Mechanisms and interpretation of aging-related changes in TMS-conditioned soleus H-reflexes

Previous studies in young adults have investigated the effects of voluntary activation (i.e., plantarflexing, dorsiflexing, or co-contracting while seated or lying down) (Geertsen et al., 2011; Gray et al., 2017; Lopez et al., 2020; Nielsen et al., 1993) and postural activation (i.e., upright quiet stance) (Goulart et al., 2000; Guzmán-López et al., 2015) on descending cortical modulation of spinal reflexes. Prior work has also demonstrated the importance of timing between the paired non-invasive stimulation, utilizing a range of inter-stimulus intervals to provide in-depth mechanistic insights into the descending cortical and subcortical pathways influencing spinal reflex excitability (Nielsen & Petersen, 1995b; Taube et al., 2017; Xu et al., 2022). Additionally, several studies have explored the effect of varying TMS intensities used to condition H-reflexes (Andrews et al., 2020; Leukel & Kurz, 2021; Niemann et al., 2018). Our results revealed that during the SR task condition, no significant aging-related difference in the TMS-conditioned soleus H-reflex response at each timing interval (i.e., -1.5ms, +10ms, +40ms) was observed. This finding suggests that during the seated rest task, direct and indirect descending pathways responsible for modulation of spinal reflexes are not functionally affected by aging.

When comparing the magnitude of direct and indirect pathway modulation in both the young and older adult groups, our results demonstrated no significant aging-related difference at the -1.5ms ISI (direct pathways) and +10ms ISI (indirect pathways) across all task conditions. We hypothesize that similarities in the magnitude of direct and indirect pathway modulation between young and older adults may suggest that while aging impacts the efficacy of the synaptic transmission between Ia afferents and spinal LMNs, direct and indirect descending pathways have the ability to modulate spinal circuit activity regardless of age. Surprisingly, however, our results revealed that at the +40ms ISI (late indirect pathways), the older adult group had significantly decreased late indirect pathway modulation of H-reflexes during task-related activation compared to the SR task condition. Compared to young adults, older adults also demonstrated significantly decreased late indirect pathway modulation during the SA and QS task conditions. Additionally, the magnitude of late indirect pathway modulation was negatively correlated with age, where the oldest adults also demonstrated the largest magnitude of soleus H-reflex inhibition during the SA task condition. Findings from a previous study revealed that during upright quiet stance (i.e., postural activation) but not during voluntary activation, young adults showed an active inhibition of the soleus H-reflex at a similar timing interval (i.e., +40 - +60ms ISIs) (Guzmán-López et al., 2015). Therefore, we hypothesize that the aging-related changes in late indirect descending pathways are representative of heightened descending inhibitory control of reflex gain during task-related activation, potentially mediated through Ia presynaptic inhibition (Hayes et al., 2009; Katz et al., 1988; Meunier & Pierrot-Deseilligny, 1998) or propriospinal networks that modulate Ia presynaptic inhibition (Islam et al., 2020; Pearcey & Zehr, 2019).

4.5.4 Implications for future research studies

Our results showed that evaluating the effects of task-related activation and aging-related changes on descending cortical modulation of spinal reflexes reveal additional insights into the underlying neural mechanisms of descending cortical and spinal circuit interactions implicated in balance control that may have practical applications for future investigations including individuals with neurologic impairments, such as stroke, spinal cord injury (SCI), or Parkinson's disease. Descending cortical pathways and their influence on spinal sensorimotor circuits are heavily implicated for the control of voluntary movement (Burke et al., 1993; Butler et al., 2007; Sidhu et al., 2012). Current non-invasive neurophysiologic measures, such as standalone single-pulse TMS or H-reflex amplitudes, are unable to specifically evaluate the connections between cortical and spinal circuits in humans, leading to a gap in our understanding of the salient mechanisms underlying recovery of movement associated with rehabilitative interventions. Additionally, evaluating both direct and indirect pathway modulation of spinal reflexes could determine whether rehabilitation interventions differentially modulate the excitability of direct and indirect descending pathways, an area of training-induced neuroplasticity that is not well-understood.

Future studies are needed to examine the effects of neurologic conditions, such as stroke or Parkinson's disease, to parse out any overlap between aging-related changes and neurologic impairment. Taken together, our findings may provide valuable insight into neurophysiologic outcome variables for studying rehabilitation-induced neuroplasticity of direct and indirect descending cortical modulation of spinal sensorimotor circuitry.

4.5.5 Study limitations

In the current study, the same three task conditions (SR, SA, QS) and three ISIs (-1.5ms, +10ms, +40ms) were used for TMS-conditioned soleus H-reflex responses for each participant. The stimulation parameters were also consistent across participants; the subthreshold TMS conditioning intensity and the PNS intensity used to elicit 50% Hmax during each task was determined for each individual at the beginning of each experimental session. Furthermore, the EMG sensor placement location (e.g., over the muscle belly of the TA and Soleus), the PNS stimulation site (e.g., posterior tibial nerve within the popliteal fossa of the participant's dominant leg), and the TMS coil location (e.g., placed over the Soleus M1 'hotspot') were kept the same for each individual across all 3 task conditions. Thus, our experimental design was such that conditioned soleus H-reflexes observed in the -1.5ms, +10ms, and +40ms ISIs compared to unconditioned soleus H-reflexes cannot be ascribed to differences in PNS intensity, TMS intensity, or methodological concerns.

However, the current study has limitations. Throughout the experimental session, we monitored and displayed to the participant real-time visual feedback regarding the ongoing background EMG activation with respect to the target EMG. However, small variations in background EMG may have influenced the study results. While TMS-conditioning of the soleus H-reflex was used to evaluate the influences of descending cortical circuits on spinal reflex excitability, this technique is limited to those muscles from which H-reflexes can be consistently elicited. The soleus and other lower limb muscles may have stronger spinal network contributions, which could have influenced our findings. Previous findings have indicated that some participants may perceive pain in response to a standardized protocol to elicit soleus H-reflexes (Motl et al., 2002, 2004) but participants in the current study did not report discomfort with study procedures. Finally, for female subjects, it is possible that menstrual cycle effects could have influenced both cortical (Smith et al., 1999) and spinal reflex (Rekha & Krishnamurthy, 2017) excitability.

4.6 Conclusions

The current study findings provided the first characterization of the effects of aging-related changes and task-related activation on descending cortical modulation of spinal sensorimotor circuits. Our results suggest that task-related activation resulted in differential modulation of spinal reflexes via direct (-1.5ms ISI) and indirect (+10ms ISI) descending circuits, and at a longer, late interval (+40ms ISI), aging resulted in greater descending inhibition of spinal reflexes during task-related activation. Our findings suggest that aging-related changes in descending cortical and spinal sensorimotor circuit function may provide insight to underlying neural mechanisms that compensate for alternative aspects of these circuits. Thus, it is possible that without these aging-related changes to the underlying neural circuitry, deficits in balance control may be worse. Additionally, our findings further elucidate the roles of varying synaptic inputs within the complex spinal circuit network. Finally, our results offer novel opportunities for understanding and treating aging-related changes in the neural control of balance and movement from subcortical versus cortical mechanisms.

Chapter 5: Discussion, Conclusions, and Future Directions

5.1 Summary of Results

The purpose of this dissertation was to evaluate interactions between descending cortical and spinal sensorimotor circuits across the adult lifespan and determine their implications for balance control. The previous chapters described the ability of descending cortical pathways to modulate the activity of spinal sensorimotor circuits, adding novel insights into how descending pathways modulate the physiologic recruitment order of spinal lower motor neuron (LMN) pools. Additionally, we demonstrated a novel finding that the magnitude of descending modulation of spinal sensorimotor circuits derived using individual-specific timing was significantly larger than the standardized timing approach, providing unique insights into top-down effective connectivity mediated by direct and indirect descending pathways and methodological guidelines to inform future studies on neural correlates of balance control. Further experiments examined the effects of aging and task-related activation on descending cortical modulation of spinal sensorimotor circuits. It was demonstrated that aging-related changes resulted in greater descending inhibition of spinal reflexes during task-related activation via late indirect pathway modulation, which will aid in the identification of translational biomarkers of balance dysfunction and increased falls risk, as well as delineate effects of neurotypical aging versus neurological impairment such as stroke or Parkinson's disease.

Chapter 1 provided background on successful balance control and the underlying neural circuitry implicated in balance control, specifically how the integration of synaptic inputs within the spinal segmental level influences both voluntary and reflexive motor output through a final common path: the alpha (α)-motor neuron. Additionally, a methodological approach (i.e., paired non-invasive stimulation) in humans was introduced to probe the interactions between descending cortical pathways and spinal sensorimotor circuits.

Chapter 2 demonstrated that TMS-conditioning of soleus H-reflexes resulted in greater magnitude of indirect pathway modulation (i.e., +10ms ISI) at multiple PNS intensities on the soleus H-reflex recruitment curve (50%Hmax, Hmax). Additionally, TMS-conditioning resulted in an increased Hmax/Mmax ratio in the indirect pathway modulation condition, inducing a leftward shift of the soleus H-reflex recruitment curve. Our findings suggest that evaluating direct and indirect pathway modulation over a range of PNS intensities offers a non-invasive approach to characterize the ability of descending cortical pathways to modulate spinal circuit activity. Furthermore, our findings elucidate the ability of convergent sensorimotor inputs to modulate the physiologic recruitment profiles of spinal LMN pools. Further optimization of approaches used to characterize integration of ascending sensory and descending motor signals at the spinal segmental level offer novel opportunities to improve our understanding of neurophysiologic mechanisms of abnormal spinal circuit activity and balance control.

Chapter 3 provided further evidence showcasing the advantage of TMS-induced H-reflex modulation, especially when measured at a range of inter-stimulus intervals (ISIs), as a unique non-invasive probe to differentially parse out the excitability of direct, fast and indirect, slower descending corticomotor projections onto spinal reflex circuits. Our study findings can guide the methodology for use of the paired TMS-PNS technique in future investigations. Due to variability in conduction latencies of neuronal tracts in neurologically impaired individuals, such as stroke, multiple sclerosis, or Parkinson’s disease, as well as inter-individual variability in physiological latencies, further development of methods and formulae to estimate the optimal ISI between TMS and PNS for each individual based on their baseline data merits further investigation.

Chapter 4 demonstrated the first comprehensive assessment of how aging-related changes and task-related activation influence descending cortical modulation of spinal sensorimotor circuits. Findings from this study revealed that task-related activation (voluntary activation vs. postural activation) differentially affects the magnitude of conditioned H-reflex responses via direct and indirect descending pathways. Additionally, aging-related changes impact the magnitude of late indirect descending modulation during task-related activation, which may be explained as an aging-related change in descending inhibitory control of the spinal reflex via late indirect pathways, which is meant to decrease the reflex gain during tasks. These results may have broader implications for developing individualized neuromodulation protocols to enhance plasticity within the neural circuits underlying balance control.

5.2 Considerations and Suggestions for Future Studies

While studies from this dissertation investigate interactions between descending cortical and spinal sensorimotor circuits, influences from non-motor cortical regions (i.e., executive or cognitive control centers) onto motor cortical areas (i.e., primary motor cortex, supplementary motor area, premotor cortex) are not well understood. Additionally, parsing out task-dependent influences from these non-motor cortical regions, such as the cognitive attention required to perform a task and involuntary activation of subcortical structures that influence motor output. One methodological approach for investigating the connectivity from a primary cognitive area, such as the dorsolateral prefrontal cortex (dlPFC), to the primary motor cortex (M1) is the utilization of dual-site TMS. Dual-site TMS can be used to evaluate effective connectivity, or causal and directional relationships between dlPFC and M1 circuits during functionally relevant standing balance tasks in an age- and disease- specific manner. The dlPFC area can be targeted with a TMS conditioning pulse to condition, or prime, all pathways downstream from the dlPFC. Then, within a short latency, M1 can be activated by another supra-threshold TMS pulse which will elicit a motor evoked potential (MEP), known to be a measure of corticomotor output. This dual-site TMS paradigm can be utilized to define causal interactions from one cortical region to a separate but connected region during physiologically relevant standing balance tasks. Additionally, paired stimulation techniques can target specific cortical regions of interest and measure effects of cortical manipulation as they occur on a sub-millisecond timescale.

To assess the influence of the vestibular system on interactions between descending cortical and spinal sensorimotor circuits, we suggest the utilization of galvanic vestibular stimulation (GVS). The vestibular system is important for maintaining body and head orientation in space (Wardman & Fitzpatrick, 2002), yet the effects of vestibular afferents on corticospinal motor output are not fully understood. The motor cortex has been implicated in the production of postural responses during standing or following perturbations (T. H. Petersen et al., 2009; Taube et al., 2006), and evidence strongly indicates interactions with the vestibular system. Previous studies have utilized GVS in conjunction with TMS and found that GVS could facilitate motor evoked potentials (MEPs) in arm (Suzuki et al., 2017) and neck (Guzman-Lopez et al., 2011) muscles and suppress MEPs in the soleus muscle (Nepveu et al., 2020). However, vestibular output elicited through GVS did not modify the amplitude of soleus H-reflexes (Nepveu et al., 2020). Nonetheless, incorporating GVS with the paired non-invasive stimulation paradigm highlighted in this dissertation presents an opportunity to probe the influences of the vestibular system on descending cortical and spinal sensorimotor circuits.

Previous studies on healthy humans have used startling acoustic stimulation (SAS) to activate the startle motor response, which has been attributed mainly to subcortical activity of the nuclei within the reticular formation. The brainstem reticular formation also maintains dense anatomical interconnections with the cortical motor system. Previous studies have utilized a combined SAS and TMS approach to examine the contribution of the reticulospinal system and its interactions with the corticospinal system and found that a conditioning SAS has different effects on corticospinal excitability (i.e., MEP amplitude) when at rest (i.e., smaller) or during voluntary activation (i.e., no change) (Y.-T. Chen et al., 2016, 2019). One other study has utilized a combined SAS and PNS approach to condition the size of the soleus H-reflex and found that at ISIs between 75-175ms elicited larger responses compared to test values (Ilic et al., 2011).

To investigate the role of somatosensory inputs to motor cortical regions, electroencephalography (EEG) can be used to measure cortical excitability and connectivity between regions of interest during rest and active task conditions. It is possible that changes in the peripheral afferent state leads to changes in motor output, which could be extrapolated through neurophysiologic measures such as somatosensory evoked potentials, or SEPs.

While findings from this dissertation present novel findings on neurophysiologic outcomes variables, an important next step in future studies would be characterizing the structural integrity of descending cortical circuits and identify any potential relationships to the influences of direct and indirect descending pathways on spinal sensorimotor circuitry. For example, quantifying the white matter integrity of descending pathways implicated in sensorimotor control (e.g., corticospinal tract, reticulospinal tract, vestibulospinal tract) and determining if the integrity of those descending pathways account for greater or lesser magnitude of TMS-conditioned soleus H-reflexes. Additionally, it is known that cortical neurons exhibit structural changes that contribute to dysfunction of the aging sensorimotor system. Previous studies have shown aging-related changes in gray matter volume (McGinnis et al., 2011; Raz et al., 2004; Salat et al., 2004), white matter volume (Ge et al., 2002), and white matter integrity (Ota et al., 2006; Sullivan & Pfefferbaum, 2006). It would be mechanistically insightful to examine aging-related structural changes on the cortical level and determine if any relationship to the magnitude of descending modulation of spinal reflexes exists.

Future studies should consider utilizing additional ISIs past the +40ms ISI. For example, if the three late long-interval ISIs (i.e., +50ms, +60ms, +75ms) were added to the paired non-invasive stimulation protocol, one could 1) characterize any potential shift in the ISI curve with aging (e.g., would the TMS-conditioned soleus H-reflex continue to be inhibited with longer interval ISIs?), and 2) test to see if the TMS-conditioned soleus H-reflexes return to unconditioned H-reflexes amplitudes or if the use of longer late ISIs modulate the conditioned H-reflex further (i.e., facilitation, inhibition). Another simple methodological consideration for future studies would be to utilize additional surface EMG sensors to probe any muscle synergies or inter-segmental influences from bilateral muscle recordings. For example, additional muscles in the dominant, or target, leg such as rectus femoris in the quadriceps, gastrocnemius, or abductor hallucis (AH) could provide insight to synergist muscles that play a role in the maintenance of balance. Alternatively, replicating the EMG sensor placement to the non-dominant, or non-target, leg could provide insight into spinal inter-segmental influences, as well as contralateral and ipsilateral descending tracts that regulate bipedal movements.

5.3 Implications for Clinical Translation

Results from this dissertation establish a methodological approach to characterizing interactions between descending cortical and spinal sensorimotor circuits across the adult lifespan in humans. Findings on the effects of stimulation intensity (Chapter 2), timing intervals between paired stimulation (Chapter 3), and aging and task-related activation (Chapter 4) demonstrate an ability to implement strategic approaches for characterizing the effects of neurologic impairment, such as stroke. As previously mentioned, human movement requires integration of descending motor commands from the brain and ascending sensory signals from the periphery. Neural circuit interactions between descending cortical and spinal sensorimotor structures are important for balance control. However, the effects of stroke on the influence of descending cortical circuits on spinal sensorimotor activity that govern successful balance control are poorly understood. By investigating how stroke impacts neural circuitry responsible for balance control, future work can aid the development of targeted and neurobiologically informed rehabilitative strategies to maximize the recovery of sensorimotor function.

A cortical or subcortical stroke can disrupt the corticospinal tract (CST), the primary motor pathway responsible for controlling volitional movements. Typical motor and locomotor function is dependent on normal functioning of the CST, as well as sensorimotor integration between descending cortical and spinal sensorimotor circuits. Stroke-induced abnormalities in the lesioned hemisphere, CST, and/or spinal circuits have a causal link to post-stroke motor impairments and gait abnormalities.

Thus, future studies that investigate neural correlates of post-stroke balance control and therapies that aim to restore normal functioning in CST circuits have strong potential to enhance post-stroke sensorimotor function and recovery.

Current neuromodulation protocols have shown success in improving post-stroke sensorimotor function and recovery. Paired associative stimulation (PAS) protocols have been shown to modulate corticomotor excitability (Palmer et al., 2018; Poon et al., 2008), motor learning (Sasaki et al., 2018), and spinal excitability (Cortes et al., 2011; Knikou, 2017). Additionally, paired corticospinal-motoneuronal stimulation (PCMS) has been used in humans to enhance plasticity at spinal synapses of lower-limb motor neurons in humans with and without spinal cord injury (SCI) (Bunday et al., 2018; Urbin et al., 2017). Thus, utilizing the protocol for TMS-conditioned soleus H-reflexes, we would recommend developing an adapted paired, repetitive neuromodulation protocol where identification of either the earliest onset ISI (i.e., -1.5ms ISI) and/or peak facilitation ISI (i.e., +10ms ISI) could be used to induce or enhance neural reorganization based on the principles of spike timing-dependent plasticity, within the spinal segmental level. Additionally, combining this potential methodological approach with varying forms of treadmill training and/or balance training could also augment improvements in post-stroke recovery outcomes.

5.4 Limitations

One limitation to the current set of experiments is that we did not collect lifestyle information regarding historical and current physical activity levels, such as prior history with sports and/or physical fitness, the types of physical activities participants have or currently engage in, and a self-report assessment of physical activity level (i.e., sedentary, active) and overall physical health. Future studies should incorporate these suggestions into a pre-session questionnaire to provide insight that may be useful when determining any potential relationships to neurophysiologic outcomes. Throughout all experiments, we monitored and displayed to the participant real-time visual feedback regarding the ongoing background EMG activation with respect to the target EMG. However, small variations in background EMG may have influenced the study results. While TMS-conditioning of the soleus H-reflex was used to evaluate the influences of descending cortical pathways on spinal reflex excitability in all experiments, this technique is limited to those muscles from which H-reflexes can be consistently elicited. The soleus and other lower limb muscles may have stronger spinal network contributions, which could have influenced our findings. Additionally, for all experiments, a single subthreshold TMS conditioning intensity (i.e., 90% RMT, AMT) was used. While other studies have reported the effects of sub- and suprathreshold TMS conditioning intensities (Andrews et al., 2020; Leukel & Kurz, 2021; Niemann et al., 2018), these were typically done during a seated rest task. While we did not collect TMS-conditioned soleus H-reflexes at varying TMS conditioning intensities, it is possible that task-related activation may change the TMS conditioning intensity required to see alternative effects on descending cortical modulation of spinal reflexes. Finally, we did not objectively assess pain perception during the experiment. Previous findings have indicated that some participants may perceive pain in response to a standardized protocol to elicit soleus H-reflexes (Motl et al., 2002, 2004) but participants in the current study did not report significant discomfort with study procedures.

In **Chapter 2**, it is possible that an order effect in the delivery of stimulation pulses during collection of unconditioned and conditioned H-reflex recruitment curves may influence our findings. For example, an effect of delivering pulses at the +10ms ISI (i.e., LIF) prior to the unconditioned (UC) and the -1.5ms ISI (i.e., SIF) may artificially increase the magnitude of H-reflex modulation. Thus, future studies should consider randomizing the order of stimulation delivery to avoid any potential order effects. During collection of unconditioned and TMS-conditioned H-reflex recruitment curves, soleus H-reflex responses were recorded once at each increasing PNS intensity for all conditions (UC, SIF, LIF) due to experimental time limitations. Thus, evaluating SIF and LIF using a greater number of trials throughout the H-reflex recruitment curve, as well as collecting a wider range of PNS intensities across the H-reflex recruitment curve (i.e., prior to H-reflex onset), could establish reliability and reproducibility, and may be an important methodological consideration for future studies. Additionally, in **Chapter 2**, we assessed SIF and LIF at a single standardized ISI respectively for all participants that does not account for inter-individual variability in conduction velocities, limb length, or other individual characteristics. Individualizing the ISI for each participant to optimize the magnitude of facilitation (Nielsen et al., 1993; Taube et al., 2017), and accounting for each individuals' body or limb length parameters, as well as nerve conduction velocities, would be valuable directions for future study. In **Chapters 2 and 3**, both studies included a relatively small sample size, although it is consistent with samples in other similar studies (Capozio et al., 2021; Gray et al., 2017; Guzmán-López et al., 2015; Nielsen et al., 1993).

In **Chapter 3**, a single nerve stimulation intensity was chosen (PNS intensity eliciting an H-reflex amplitude of 20% Mmax) based on previous literature, and modulation of the soleus H-reflex was not measured at a range of intensities across the H-reflex recruitment curve. For instance, in recent studies, H-reflex facilitation at certain EFDs was shown to be differentially influenced by specific movement tasks (Wiegel et al., 2020; Wiegel & Leukel, 2020). The stimulation parameters used here (e.g., 1ms pulse width, separation between consecutive ISIs, sub-threshold TMS according to active motor threshold), while mostly consistent with previous work, have been modified in recent studies, particularly to probe specific neural contributions at the ISIs eliciting early facilitation. Future studies can investigate factors influencing the optimal ISI (e.g., latency of MEPs and H-reflexes) and develop a formula to enable individual-specific estimation of the optimal ISI for measuring earliest onset and peak facilitation. Although earliest-onset and peak facilitation derived using paired TMS and PNS help to better localize the site of plasticity compared to TMS alone or H-reflexes alone, these techniques do not have the specificity to identify exactly which neural pathway is implicated in eliciting facilitation at different ISIs. As is true for many other non-invasive approaches, especially at longer ISIs, the observed H-reflex modulation can be caused by many neural sources, spanning spinal, brain stem, subcortical, and cortical sites, which are challenging to discriminate. Future work can combine TMS-facilitation of H-reflexes with neuroanatomical imaging or complementary neurophysiological techniques to determine relative contributions of specific descending pathways (e.g., reticulospinal, propriospinal) to these measures. Between-muscle differences in neuromotor circuit control can be investigated by applying similar methods in upper limb muscles and other lower limb muscles.

In **Chapter 4**, we categorized participants as “Man,” “Woman,” “Non-binary/non-conforming,” and left a fillable option titled “Other”. In order to fully assess the effects of sex and gender identity on outcome measures, it would be helpful to ask participants to indicate sex assigned at birth as well as gender identity. However, researchers would need to ensure that the sample size is large enough to have the statistical power required to assess biological sex differences in outcome measures.

5.5 Conclusions

In summary, findings from this dissertation suggest that descending cortical and spinal sensorimotor circuits have differentially important roles throughout the adult lifespan in the regulation of motor output, and interactions between these circuits remain highly implicated in and important for the neural control of balance. The aging process gradually endures structural and functional changes to the neuromotor system, which can be linked to declines in balance ability and movement. Future research will benefit from the methodological toolkit provided from the novel findings in young and older adults presented throughout this dissertation. Understanding how aging affects neural circuitry responsible for sensorimotor function and its relation to balance control is a crucial prerequisite to (1) developing neurobiology-informed treatments to improve balance and reduce falls risk in older individuals; and (2) future work that aims to parse out the effects of aging versus the effects of neuropathology such as stroke or Parkinson’s disease.

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