The Long-Latency Reflex: A Biomarker for Functional Impairment Following Stroke

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Abstract

Walking is often impaired after a stroke, yet current motor rehabilitation strategies fail to produce meaningful improvements in walking function. Lack of functional improvement may be a consequence of providing rehabilitation to individuals that lack the neural substrate for the therapy to provide an effect. There is a critical need for non-invasive biomarkers that can predict potential for recovery of walking ability following stroke. This dissertation proposes the long-latency reflex (LLR) as a functional biomarker that relates to walking and other lower extremity function. To evaluate the potential for LLR presence as a biomarker, we utilize muscle stretch and electromyography to quantify the health of neural circuitry; probe motor and sensory contributions to the LLR response; and examine the relationship between LLRs and lower extremity function. We find that LLRs are absent in some individuals with chronic stroke, and across two independent samples these individuals are the lowest functioning. Data generated in this dissertation confirm a transcortical component in the tibialis anterior LLR and further suggest that many individuals retain the residual substrate necessary to elicit an LLR response in the context of concurrently timed transcranial magnetic stimulation. Altogether, the LLR shows promise as an unambiguous, clinically accessible biomarker of lower extremity dysfunction in chronic stroke. The specific mechanisms of LLR absence remain to be systematically investigated in future research.

Table of Contents

Abstract iii

List of Tables viii

List of Figures ix

Chapter 1: Introduction and Development of the Research Question 1

Biomarkers 4

Lower-Extremity Reflexes 6

The Long-Latency Reflex 8

Sensory Function in Stroke 13

Scope of This Dissertation 16

Chapter 2: Comprehensive Methods 18

Clinical and Functional Assessments 18

Fugl-Meyer Assessment of Physical Performance 18

Short Physical Performance Battery 19

Dynamic Gait Index 19

Maximal Voluntary Contractions 20

Gait Assessment 20

Three-Dimensional Gait Analysis 22

Sensory Assessments 23

Light Touch 23

Vibration 24

Proprioception 25

Assessment of Long-Latency Reflexes 26

Assessment of Cortical Contribution to Long-Latency Reflexes 27

Chapter 3: Lower Extremity Long-Latency Reflexes and Walking Function in Chronic Stroke 29

Note 29

Introduction 29

Methods 31

Subjects 31

Instrumentation 32

Protocol 34

Data Analysis 37

Study Variables 37

Statistical Analysis 39

Results 40

Discussion 44

Chapter 4: Characterization of Cortical Contribution to LLRs Post-Stroke 49

Introduction 49

Methods 52

Subjects 52

Instrumentation 53

Protocol 54

Data Analysis 58

Study Variables 59

Statistical Analysis 60

Results 62
Subjects 62
LLR Presence/Absence 63
LLR Characteristics 64
CST Efficacy 65
Dynamic Facilitation of LLRs 67
Clinical and Functional Tests 71
Discussion 73
Chapter 5: Influence of Sensorimotor Function on LLRs 78
Introduction 78
Methods 80
Subjects 80
Instrumentation 82
Sensory Testing Protocol 83
Study Variables 86
Statistical Analysis 87
Results 88
Experiment 1 88
Experiment 2 90
Experiment 3 93
Discussion 95
Chapter 6: The Relationship Between Sensory Impairment and Gait Performance 100

Introduction 100
Methods 102
Subjects 102
Protocol and Instrumentation 104
Data Analysis 105
Study Variables 105
Statistical Analysis 107
Results 108
Joint Kinematics 108
Joint Kinetics 113
Discussion 115
Chapter 7: Conclusions and Future Directions 120
Future Directions 122
Chronicity 122
Relationship between LLRs and CST Integrity 123
Sensorimotor Integration 123
Mutability of the LLR and Response to Treatment 125
Clinical and Translational Research Impact 125
Chapter 8: References 127

List of Tables

Table 3.1. 33

Table 4.1 63

Table 5.1 81

Table 5.2 90

Table 6.1 103

Table 6.2 110

Table 6.3 110

Table 6.5 114

List of Figures

Figure 1.1 10

Figure 2.1 26

Figure 3.1 36

Figure 3.1 38

Figure 3.2 40

Figure 3.3 41

Figure 3.4 42

Figure 3.5 43

Figure 4.1 65

Figure 4.2 66

Figure 4.3 68

Figure 4.4 69

Figure 4.5 70

Figure 4.6 71

Figure 4.7 72

Figure 5.1 91

Figure 5.2 92

Figure 5.3 94

Figure 5.3 94

Figure 5.4 95

Figure 6.1 107

Figure 6.2 109

Figure 6.3 111

Figure 6.4 115

Chapter 1: Introduction and Development of the Research Question

Stroke is the most common cause of adult long-term physical disability in the United States (1). This disability decreases quality of life for survivors and caregivers by limiting activity and reducing community participation. Motor impairment affects most people who have had a stroke, and gait and balance impairments are often the focus of lower extremity rehabilitation interventions (2). Although improved walking is a common goal of rehabilitation, only about 50% of patients improve in response to treatment (3,4). A potential contributor to this limited treatment response is the inability to understand intrinsic pathophysiology at the individual level. By first understanding the vast heterogeneity of impairments present after stroke, we can better inform the development of effective and efficient treatment strategies.

The Institute of Medicine defined the vision of rehabilitation science as understanding the factors that contribute to disability and developing treatments and technology to improve the quality of life for individuals with disabilities (5). Several conceptual models were then developed to create a unified basis of understanding of disability and disabling conditions, including the Institute of Medicine's Enabling-Disabling Process (5,6) and the World Health Organization's International Classification of Impairment, Disability and Handicaps (7), later renamed the International Classification of Functioning, Disability and Health (ICF). The ICF model and its associated coding scheme are currently employed in many health-related settings (8). More recently, the NIH's National Center for Medical Rehabilitation Research released an updated Research Plan on Rehabilitation which outlines goals for research and plans for NIH infrastructure to accommodate those goals (9). Translational science is among the six primary goals for

the future of medical rehabilitation research. Two specific objectives from the NIH plan that I will address in this proposal include: (1) understanding the physiologic impairments associated with disabling conditions and the rehabilitation thereof, and (2) characterization of potential biomarkers that may be prognostic for individuals with specific conditions.

Much of the rehabilitation science behind walking interventions to date has focused on characterizing and/or improving the biomechanical aspects of walking, with less emphasis on understanding and improving the physiologic mechanisms that underlie walking impairment. The only major multisite clinical trial for walking rehabilitation post-stroke was the Locomotor Experience Applied Post-Stroke (LEAPS) study (10). This study compared locomotor training at two different time points after stroke to a home-based exercise program, finding no significant differences between the three treatment groups, with an average response rate of 52% for improvements in walking speed category across all groups (10). All primary and secondary outcomes assessed in this study were standardized clinical measures such as walking speed, functional speed category, six-minute walk test distance, and number of steps taken per day (11). Because physiologic measures were not recorded, is not possible to ascribe any true physiologic differences among study responders and non-responders. Although this was the largest and most scrutinized walking rehabilitation study to date, the approximate response rate of 50% is a common finding across many lower extremity treatment studies, regardless of treatment modality or outcome measured (12–15). This response rate is alarming, and likely relates to heterogeneity in the underlying physiology of these individuals, which in turn produces a heterogeneity in motor behavior (10,16). Any progress that can contribute

to understanding of this physiologic heterogeneity will drive progress in personalizing treatments, and ultimately improve treatment outcomes for individuals with stroke.

Most rehabilitation approaches, including the LEAPS trial, are theoretically rooted in the idea that the central nervous system is capable of changing in response to experience, otherwise known as neuroplasticity (17). Neuroplasticity must be measured either at the individual neuron or neuronal population level; therefore, it can only be inferred from changes in behavior (18). Animal models confirmed the presence of plasticity in response to learning and injury, and similarities in behavior between human and animal studies led many to suggest that neuroplasticity is the foundation for neurorehabilitation (19–21). However, direct evidence of rehabilitation-induced plasticity in humans is lacking (22). This is partially due to technological limitations of recording from neurons in awake, behaving humans. However, techniques such as electroencephalography (EEG) and transcranial magnetic stimulation (TMS), as well as indirect measurements like functional magnetic resonance imaging (fMRI) and electromyography (EMG), are increasingly allowing for better approximation of population-level neuroplasticity (20,23,24). Beyond showing evidence that plasticity occurs, there is little evidence that plasticity links to function. The field of neurorehabilitation appears to be on the verge of discovering markers of functional plasticity in humans, but we must complete this vital step before it will be possible to create informed treatment strategies and actualize the NIH's vision for translational rehabilitation research (25–27).

Biomarkers

Biomarkers are essential tools for gaining insights about functional status or approximation of population-level plasticity. The Biomarkers Definitions Working Group defines a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (28). Various types of biomarkers are currently under investigation for their role in neuroplasticity and recovery after stroke, and most of these are extracted from saliva or blood. Several plasma microRNAs appear to be indicative of post-stroke plasticity in rodent models and early work indicates they may also relate to recovery of upper extremity function in humans (29). Genetic polymorphisms under investigation include the CCR5-Δ32 mutation which may be neuroprotective (30). Other polymorphisms, like the BDNF $Val^{66}Met$ polymorphism, may impair learning and recovery (31). Most common biomarkers utilized in medicine are invasive and require laboratory tests, but they do offer the advantage that they can be measured early after stroke and they can be passively measured, making them ideal for individuals with severe stroke in an inpatient setting. Additionally, non-invasive biomarkers of physiologic function do exist in standard clinical practice (e.g., blood pressure, temperature). We will refer to these herein as functional biomarkers.

Promising evidence indicates that neurophysiologic biomarkers have the capacity to accurately predict motor function post-stroke. According to Stinear et al., a good predictive marker has five needs: 1) ability to predict the future; 2) measurement in the subacute stage; 3) prediction of a specific timepoint, not to include discharge; 4) predictive value for individual patients; and 5) ease of clinical use (32). The prediction of recovery

potential (PREP) algorithm and its newer iteration, PREP2, are likely the most advanced biomarkers in development for functional recovery after stroke (33,34). PREP utilizes the presence or absence of motor evoked responses (MEPs) in combination with a functional evaluation of hand and shoulder movement and diffusion-weighted MRI to sort patients into categories of complete, notable, limited, or no predicted upper limb recovery potential. Implementation of the PREP algorithm in a public hospital in New Zealand increased physical therapist confidence in therapy content and decreased inpatient rehabilitation stays by one week on average, saving time and money for both hospitals and patients (35). A recent feasibility study in a United States-based cohort had worse predictive accuracy than anticipated, however they did not include MEPs in their dataset, thus validating the need for a TMS-based marker for this particular algorithm (36). This research group is also in the process of developing an algorithm for recovery of independent walking early after stroke (37). This algorithm does not assess walking mechanics, merely the ability of a person to walk without assistance or supervision. Markers like these are necessary tools for understanding pathophysiology and quantifying individual differences, both of which could be vital to improving walking function after stroke. Once a biomarker is developed and proof-of-concept has been established, large sample studies can be undertaken to ensure that the marker has good positive predictive value, negative predictive value, sensitivity, and specificity (38). The field of neurorehabilitation biomarkers is still young, and much work needs to be done to bring these markers into the clinic.

MRI and TMS provide promising avenues for biomarker development and scientific discovery, however they are not cost-effective or feasible within many clinical settings.

While not every clinic currently possesses an EMG system, there are neurology, orthopedics, and physical medicine & rehabilitation clinics that routinely examine EMG markers. For example, electrodiagnosticians may assess nerve conduction velocity, neurologists can perform EMG-guided injections, and some physical therapists and kinesiologists use EMG to measure muscle strength and functional task performance. While each of these types of clinicians has a different aim and scope of practice, there is potential to bring interdisciplinary approaches together if the benefit to the patient proves significant. In that case it may be reasonable to assume a clinic could expand the functionality of their services with the use of existing hardware and non-invasive approaches. Potential EMG biomarkers include M-waves, h-reflexes, and f-wave amplitude in response to nerve stimulation; burst superimposition using nerve or muscle stimulation; measures of volitional contraction (e.g., maximal voluntary contractions); or assessment of stretch reflexes. The latter two methods to not require electrical stimulation. Because EMG is a measurement of the final common pathway, some of these metrics can provide behavioral insights without clear evidence of the neural pathways responsible for the output. However, h-reflexes and stretch reflexes can provide information about spinal and supraspinal pathway function in a non-invasive, functionally relevant, and clinically accessible manner.

Lower-Extremity Reflexes

There are a variety of stretch reflex responses that are relevant to walking and lower extremity movement. The most well-known lower extremity reflex is the monosynaptic spinal reflex, which can be observed in response to muscle stretch or a tendon tap (39). This reflex also has an electrical analogue, the Hoffman reflex or h-reflex

(40). However, there are additional reflex responses with varying origins and functions. In specific contexts, a muscle stretch can produce three distinct reflex responses within a single muscle, sometimes called M1, M2, and M3, or the short-, medium-, and long-latency reflexes, named for the relative timing of responses (41). There are additional reflexes that are relevant to the legs, including cutaneous reflexes and reflexes in response to noxious stimuli. We will focus here on stretch reflexes and what is known about the impact of stroke on reflexes.

A short-latency reflex, occurring around 45 milliseconds after stretch in the tibialis anterior, causes excitation of a sensory nerve (42,43). This sensory nerve travels through the dorsal root ganglion into the dorsal horn of the spinal cord and synapses on the alpha motor neuron in the ventral horn (44). The alpha motor neuron is then excited, causing an evoked response in the homonymous muscle that can be recorded with EMG. This reflex is often but not exclusively monosynaptic, as there is evidence indicating that tendon tap excitatory post-synaptic potentials arrive later than direct monosynaptic excitation (45). Whether the response is monosynaptic or oligosynaptic, the reflex itself is purely spinal. However, spinal reflexes are not free from supraspinal influence. Descending pathways can synapse directly on the alpha motor neuron or on spinal interneurons and affect excitability of the short-latency reflex circuitry (39). We see this in numerous ways, such as modulation by attention or the impact of supraspinal lesions on the short-latency reflex. Hyperreflexia, or an increase in gain of short-latency reflexes, is a common observation in individuals with stroke and other central nervous system lesions. Work in reflex coupling during volitional multi-joint movement of the upper

extremity indicates that increased reflex excitability is associated with greater levels of impairment, and may prove useful as a biomarker, in chronic stroke (46).

The medium-latency reflex occurs around 70 ms following stretch in the plantar- and dorsiflexors (43,47). The origins and specific circuitry of this reflex are still under debate, and very likely are under the control of multiple mechanisms. The absence of medium-latency reflexes during tendon vibration suggest the involvement of group II muscle spindle afferents, which are slower conducting than the group Ia afferents involved in short-latency reflexes (48). Group II conduction velocities and central delay estimates suggest that the reflex follows an oligosynaptic spinal pathway (47). Some studies suggest that medium-latency reflexes may involve structures as high as the midbrain, although they do not exhibit latencies long enough for cortical involvement (49).

Reports vary on whether medium-latency reflex timing or amplitude are affected by stroke. This could be due, in part, to the differing criteria for reflex responses employed across studies. In a postural study by Nardone et al., participants with stroke had paretic medium-latency reflexes that were smaller in amplitude than healthy controls and the responses in their non-paretic leg, however the time window investigated for medium-latency reflexes in this study (65ms after onset in the TA) may have been long enough to capture long-latency reflexes as well (42).

The Long-Latency Reflex

The long-latency stretch reflex, also known as the transcortical reflex or the functional stretch reflex, is an oligosynaptic reflex that is observable in response to muscle stretch (50,51). The healthy adult LLR response has a supraspinal component and presumably travels from somatosensory to motor areas, hence the name transcortical

reflex (50). The specific neural circuitry involved in the transcortical reflex pathway is entirely not known, however there are candidate cells that likely contribute to the LLR. Corticomotoneuronal cells are neurons with direct, monosynaptic connections between motor cortex and alpha motoneurons (52). These cells contribute to LLRs in the primate upper extremity (53). Corticomotoneuronal cells also exist in humans and are presumed to be involved in motor learning and contribute to LLRs (54,55). Figure 1 shows a simplified schematic representation of the transcortical reflex pathway. The reflex has a sensory component (blue), a cortical integratory component (mixed blue and gold), and a motor component (gold). In health, this reflex is robust and has been observed in various upper- and lower extremity muscles (55,56).

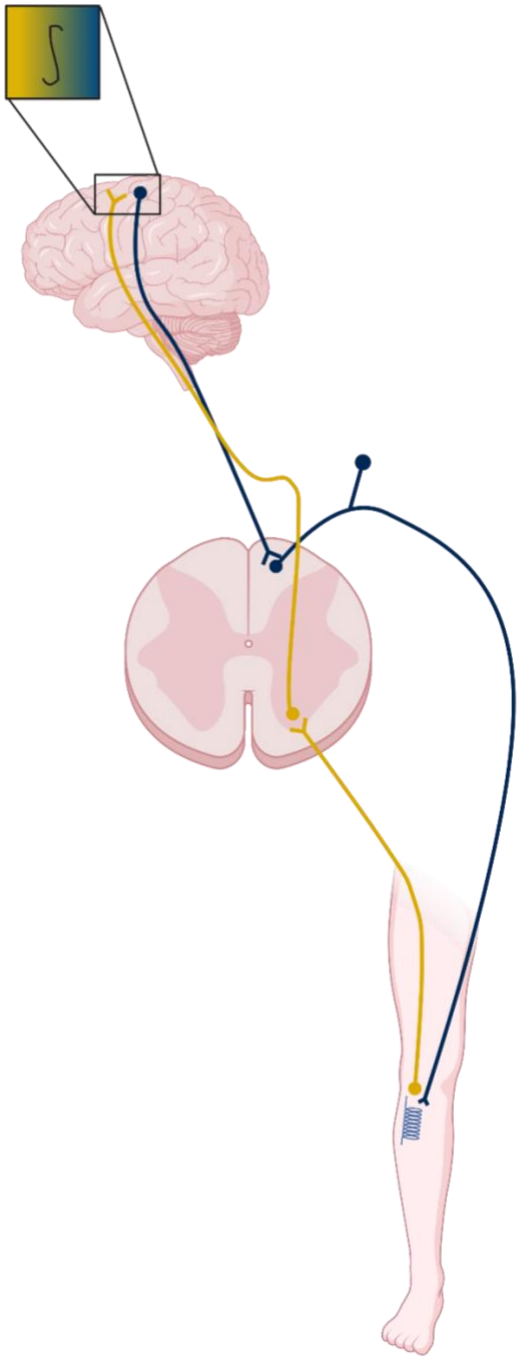

Figure 1.1. Schematic of transcortical reflex pathway. Muscle stretch activates sensory fibers (blue), information is integrated in cortex (mixed color integration symbol), and responses are generated in motor tracts (gold). Created with BioRender.com.

The long-latency reflex is flexible. It is easily modulated by perceptual set, background muscle activity, movement velocity, and experimental task (57–59). Following first observations, there was a heavy research emphasis on LLRs, including characterization in healthy individuals and animal models (51,53,60,61) and demonstration of changes in LLRs with varying neurologic pathologies (62–64). The flexibility of the LLR comes with both advantages and disadvantages. Some theories indicate that the LLR is similar in complexity to volitional movement, thus providing a model system that can give insights into the control of volitional movement (59). The disadvantage of this flexibility is that LLRs are less stereotyped than most reflexes, and study results are difficult to generalize. Mixed results and varying terminology across the literature sparked a major debate regarding the mechanism and functional implication of this reflex response. Klippel-Feil syndrome, a rare disorder that produces mirror movements due to a bifurcation of corticospinal projections, produces LLRs in both hands in response to a unilateral stretch (65). Diminished or absent LLRs have been described previously in some individuals following cortical stroke (64). These and other studies led many to conclude that LLRs, at least those in the distal upper extremity, were cortical in origin (66,67). However, other studies indicated otherwise. One study of lower extremity LLRs showed similar behavior to the short-latency monosynaptic reflex and persistence of all responses after anaesthesia of the foot (68). Another showed that selective cooling or ablation of cortical, thalamic, and cerebellar regions failed to abolish the LLR in 20 Cebus monkeys (69). These two studies offered the hypothesis that LLRs are oligosynaptic spinal responses. Technological advancements and a growing body of evidence indicate that LLRs in both the upper and lower extremities have a cortical

component, but most lower extremity responses are likely to have mixed cortical and spinal contributions (70,71). These theoretical debates create confusion in the early lower extremity literature, with some groups characterizing only short-latency reflexes and anything after the duration of the short latency reflex, which likely includes a mix of medium- and long-latency reflexes depending on the study methods employed. Taken together, the flexibility of the reflex and variation across the extant literature necessitate thorough study of a given response before conclusions can be drawn regarding mechanisms and implications.

Upper extremity LLRs are altered with in some individuals with neuropathologies. Parkinsonian rigidity is associated with an increase in LLR amplitude relative to healthy adults (72,73). Some individuals with multiple sclerosis reveal diminished or even absent LLRs, which sometimes coincide with abnormal somatosensory or motor evoked responses (63,74). Diminished or absent LLRs were described in some individuals following cortical stroke (64,75), while later onset and prolonged duration LLRs were present on the contralesional side of one individual with a focal right supplementary motor cortex lesion (76). In a sample of individuals with only thalamic stroke, LLRs were attenuated or absent, and these differences appeared to detect the presence of pathology better than somatosensory evoked potentials alone (77).

There are fewer studies of lower extremity LLRs in neurologic populations. Most of the extant research is unable to explain differences between individuals with normal and abnormal LLRs, and research interests in the clinical significance of the LLR have waned over time. Some studies assessed LLRs in parallel with other evoked potentials such as motor or somatosensory evoked potentials, but none discuss the impact of the

presence or absence of the LLR on an individual's sensorimotor function. One study aimed to find an association between reflex function and postural sway in individuals with spastic paraparesis, amyotrophic lateral sclerosis, and stroke (42). Although this study technically focused on only short- and medium-latency reflexes the example EMG and findings of diminished reflex amplitude in the paretic leg of individuals with stroke suggest that they may be quantifying both medium- and long-latency reflexes under their label of medium-latency reflex. The authors of this study did not find associations between postural sway and reflex function. Another study used a standing platform paradigm that induced LLRs in the antagonist muscle rather than the stretched muscle itself (78). This study found that individuals with stroke in the internal capsule or sensorimotor cortex had intact but longer duration LLRs than individuals with stroke in other brain regions or healthy adults. They also found larger LLR areas in both stroke groups than healthy individuals. The mixed LLR responses present in the literature likely relate to both heterogeneity in experimental methods and within the population of chronic stroke. The presence or absence, or other features of the LLR (e.g., latency, amplitude, duration) could represent biomarkers of sensorimotor function that are relevant to walking impairment. We propose that the long-latency reflex (LLR) may be a functional biomarker of walking impairment following stroke.

Sensory Function in Stroke

Although stroke is known to cause somatosensory impairment for many individuals, the extent to which sensory impairment directly impacts motor function is not well acknowledged in the clinic. Additionally, somatosensation is often not the focus of standard stroke rehabilitation. Like motor deficits, somatosensory deficits can arise from

a lesion to cortical area directly involved in somatosensory function (e.g., primary and secondary somatosensory cortex) or from areas where somatosensory information is integrated to provide valuable, functionally relevant information. A unique aspect of somatosensory function that contributes to its underrepresentation in the stroke literature is the relative difficulty in its measurement. Somatosensation is often subjective, and its measurement can be further complicated in the presence of aphasia (79). While objective measures of somatosensory function do exist, they are sometimes difficult to measure in the presence of motor deficits. Here, I will discuss the current state of the research in lower extremity somatosensory impairment and its impact on motor function, including both subjective and objective measures, as each have their merits and their limitations. Because this dissertation focuses on stretch reflexes and sensorimotor control of the lower extremities, we will focus here on tactile, vibratory, and proprioceptive somatosensation, which we will herein refer to as simply ‘sensation’.

A 1988 study classified 95 individuals entering subacute rehabilitation and unable to ambulate independently as having: only motor deficits (n=27, 28.4%); motor and sensory deficits (n=32, 33.7%); motor, sensory, and visual deficits (n=32, 33.7%); or other (n=4, 4.2%) (80). Individuals with only motor deficits had higher initial functional status and were likely to return to independent ambulation and self-care within 14 weeks after their stroke. Individuals with motor and sensory deficits, the latter classified based on a finger proprioception test, were unlikely to recover independent mobility but most could ambulate with assistance by week 16. Those with motor, sensory, and visual deficits were also able to recover assisted ambulation, but later than the less affected groups, after approximately 28 weeks. This study shows both a clear link between sensory deficits and

poor functional outcomes after inpatient rehabilitation, and the high prevalence of somatosensory deficits present after unilateral hemispheric stroke. Despite these results, progress in measuring and focus on improving sensory deficits has remained in the minority among stroke rehabilitation research studies.

There are many methods to quantify sensory deficits and little agreement among researchers on which tools to use or which modalities to assess. Validated clinical batteries for stroke that include lower extremity sensory measurement include the sensory sub-score of the Fugl-Meyer Assessment of Physical Performance (FMA) and the Nottingham Sensory Assessment. The FMA sensory sub-score measures light touch and proprioception in the limbs, and each item is scored as absent, impaired relative to the nonparetic limb, or intact (81). Light touch and proprioception are two of several sensations attributed to the dorsal column-medial lemniscus tract, and the remaining sensations (vibration, two-point discrimination, and pressure) are not assessed in the FMA (82). The sensory sub-score has better intra-rater reliability than inter-rater reliability among experienced stroke physical therapists, with intra-class correlation coefficients classified as good (83). The Nottingham Sensory Assessment measures touch, pinprick, temperature, and proprioception through seven different assessments across many body areas (i.e., face, trunk, arms, and legs), classifying some domains on a 5-point scale and others as impaired or normal (79). This assessment measures sensation in both the dorsal column-medial lemniscus tract (touch, proprioception) and the spinothalamic tract (pinprick, temperature). This metric has good intra-rater reliability but poor inter-rater reliability, with a revised scale available that has marginally improved reliability (84). The tradeoff between these two metrics is higher reliability with a more gross measurement in

the FMA, versus a more comprehensive assessment across multiple pathways but less reliable measure with the Nottingham Sensory Assessment.

Because reflex pathways involve both sensory and motor components, they provide an efficient assay of multiple, functionally relevant tracts. An impaired reflex could be due to sensory dysfunction, motor dysfunction, or integration of the full reflex loop, and it is therefore important to consider the impact of sensory dysfunction in individuals with chronic stroke.

Scope of This Dissertation

We propose that the LLR may be a functional biomarker of walking impairment following stroke. The overall project provides proof-of-concept for use of the LLR to characterize walking impairment in individuals with chronic stroke.

This dissertation is structured with an introduction and development of the research question (Chapter 1), an overview of the common methods employed in the research (Chapter 2), three chapters consisting of data from two experimental studies (Chapters 3-5), and a summary chapter (Chapter 6).

The aim of Chapter 3 is to investigate antagonist control of plantarflexion in chronic stroke. This will be done via a secondary analysis of a dataset collected to assess corticospinal efficacy to the plantarflexors in individuals with stroke and a group of healthy older adults.

Chapter 4 will characterize the cortical component of the LLR during isolated plantarflexion. Through pairing transcranial magnetic stimulation (TMS) with stretch, we aim to confirm that the presence of LLRs in the context of this experiment represents a functional transcortical reflex pathway. By timing TMS to arrive at the same time as the

LLR, the combined response should be augmented beyond the simple arithmetic sum of the two responses, indicating increased cortical contribution (43). This paradigm will be used to characterize LLRs in healthy individuals and individuals with chronic stroke.

Chapter 5 will seek to understand the relationship of somatosensory function to the LLR by characterizing clinical scores and measures of tactile sensation, vibratory sensation, and proprioception in individuals with both intact and impaired LLRs. I will assess the relationship between these measures of sensory function and LLR timing and amplitude.

Chapter 6 will expand on the findings of Chapter 5 regarding vibratory thresholds, with a focus on the relationship between vibratory thresholds and gait biomechanics.

I will then integrate my findings and the state of the field in Chapter 7, along with a discussion of the potential for modulation of the LLR and the functional implications for biomarker-informed walking rehabilitation.

Chapter 2: Comprehensive Methods

Clinical and Functional Assessments

Clinical measures are the most common primary outcomes used in gait rehabilitation research with human subjects. Clinical tests are often short and relatively easy to administer. These measures can vary from simple assessments of walking speed and endurance to test batteries that assess impairment, gait and balance performance, and surveys that incorporate patient perspectives. With a multitude of measures to choose from and a host of literature regarding the validity of these measures, these outcomes provide a solid foundation for comparing gross functional status across study populations. However, clinical measurements are limited by subjectivity, floor and ceiling effects, and a lack of sensitivity to differentiate between individuals with differing clinical presentations; thus, it is necessary to assess measures that capture individual physiologic differences in addition to traditional clinical and functional measures.

Fugl-Meyer Assessment of Physical Performance

The Fugl-Meyer Assessment of Physical Performance (FMA) was designed to assess motor recovery after a stroke (81). There are five domains within this assessment battery that can be administered separately or together, as well as upper- and lower-extremity-specific assessments. The lower-extremity assessment has a total maximal score of 100 points. The domains include: motor function, sensory function, balance, joint range of motion, and joint pain, and these can be further divided into upper and lower extremity subscales. Scoring is performed on a three-point ordinal scale, where 0 corresponds to an inability to perform the task, 1 corresponds to partial performance, and 2 corresponds to full performance. The lower extremity motor function sub-score is

commonly reported in the literature (maximum value, 34 points) for studies assessing walking function (85). Another sub-score that can be reported is the synergy score, which assesses the patient's ability to perform isolated movements in- and outside of whole-limb activation patterns and has a maximal value of 22 points (Bowden et al 2010; Brunnstrom 1970; Kautz and Patten 2005). Overall, the FMA provides high interrater reliability (Sanford et al 1993), content validity (Fugl-Meyer et al 1975), and construct validity (Badke and Duncan 1983).

Short Physical Performance Battery

The Short Physical Performance Battery (SPPB) is a lower extremity functional assessment that was designed to assess the safety of community dwelling older adults (86). The SPPB involves three components, including: repeated chair stands, standing balance, and time to walk eight feet. These three easy-to-administer tests are highly correlated with self-reported disability and prediction of mortality in older adults. Early reports indicate that the SPPB may be valuable in assessing functional capacity after stroke, but large-scale validity assessments in this population have not yet been published (87).

Dynamic Gait Index

The DGI was developed to assess the likelihood of falls in older adults (88). It evaluates functional stability during walking through an eight-item battery each scored on a 0-3 scale, for a maximum score of 24 points. Most items are intended to present common challenging walking conditions, including head turns, stepping over obstacles, and climbing stairs. Published cutoffs suggest that a score of 19 or below is predictive of falls in the older adult population, while greater than 22 indicates a safe ambulator (89).

The DGI is validated for use in chronic stroke, and like the SPPB it is quick and easy to administer in a clinical or research setting.

Maximal Voluntary Contractions

Maximal voluntary contractions (MVCs) are measures of volitional muscle strength. Although the term 'maximal' resides in the name, the amount of force generated in a static contraction depends on many factors, including: intrinsic muscle strength; mechanical arrangement of the joint (i.e., length and position of the joint relative to measurement device); and environmental conditions (90). The experimental environment in which MVC force is measured includes factors such as feedback of results, instructions on how to produce the desired contraction, level of arousal, competition, goal setting, and verbal encouragement all impacting force output (91). Peak volitional force production can be increased by approximately 5% when participants are provided verbal encouragement (92). In the experiments that follow, participants are provided visual feedback of smoothed dynamometer torques and verbal encouragement to "push" or "pull" depending on the direction of intended force. MVCs are measured within the context of our experiments to provide an individualized torque target for assessment of TMS-evoked MEPs and LLRs. However, MVCs are also useful for assessing general strength of a given body segment, and declines in strength due to aging or pathology (93).

Gait Assessment

There are a multitude of ways in which gait can be assessed, ranging across equipment needed and intended outcomes. Instrumented walkways provide a quick and straightforward opportunity to assess functional capacity in a clinically accessible manner (94). For this dissertation we will assess spatiotemporal aspects of both self-selected and

fastest comfortable walking using either a GaitRite mat (Chapters 3 & 5) or a Stepscan Pedway (Chapters 4 & 5). Here, I will discuss each in turn and then provide information regarding the outcomes of interest.

The GaitRite Electronic Walkway is a portable floor mat with pressure-sensitive switches (GaitRite Platinum Plus System, Version 3.9, Havertown, PA). Each 2'x2' length of the mat contains 2,304 sensors, and each walkway arrives at a fixed length, ranging from 14-26 feet. The GaitRite Walkway is easily the most common pressure-sensitive walkway used in gait research, with over 5,000 publications citing its use across a variety of age groups and health conditions. An advantage of using a GaitRite with impaired populations is that the mat is only ¼ inch in depth and can be taped down to create a flush seam with the floor, minimizing trip hazards. A disadvantage of the GaitRite is that the sensors do not measure pressure directly, only relative pressure, which does not allow for estimates of ground reaction force. The software offers easy exporting of average values or individual footfalls across individual tests or groups of tests.

A Stepscan Pedway (Stepscan Technologies, Prince Edward Island, CA) consists of an array of 2'x2' pressure-sensing tiles that can be configured into a walkway of varying lengths. Each tile contains 14,400 sensors and has a sensing pressure range of 0-700 kPa. The Pedway is modular and portable, offering the opportunity to change walkway length to fit a given space. For the studies detailed in Chapters 4 and 5, we used a 12-foot active walkway. Inactive tiles can be connected to either end of an active tile walkway, and the experiments that follow use either four or six feet of inactive tiles at either end to allow comfortable acceleration and deceleration. The software provides gait reports consisting of average spatiotemporal measures. Other capabilities include reporting of

pressure distributions across each foot and estimates of vertical ground reaction force. We also have access to metadata, including individual footfall and individual sensor-level measurements.

Self-selected walking speed is often referenced as the ‘sixth vital sign’ or the ‘functional vital sign’ (95). Walking speed is a valid measurement that is indicative of functional status in various populations, including older adults and individuals with neurologic deficits (96). A study by Perry et al. that categorizes people with stroke by level of walking function and community mobility is often cited as a categorization scheme by walking speed alone (97). Although walking speed was a significant predictor of placement within one of six walking categories, their data and conclusions reflect that walking speed should not be the sole predictor of walking function in people with stroke. Self-selected walking speed is a good and necessary measure of walking function but may work best as a component within a larger classification scheme, especially in individuals with walking deficits (98). It has been suggested that measures of endurance (e.g., six-minute walk test of maximal VO_2) or specific biomechanical attributes of walking (i.e., kinematic and kinetic trajectories) form a more complete assessment of walking function.

Three-Dimensional Gait Analysis

Chapter 6 uses three-dimensional motion capture to assess the role of somatosensation on biomechanical aspects of treadmill walking. A series of cameras are set up around the treadmill to capture body motions in all planes. Kinematics are metrics of limb motion such as joint angles or segment velocities (99). These can be derived by reconstructing a model of the human skeleton that moves in agreement with the

trajectories of reflective markers placed on body landmarks. Kinetics also take forces into consideration to derive metrics like joint moments and powers. We record ground reaction forces via force plates embedded in a split-belt treadmill and use inverse dynamics to derive joint kinetics from the joint trajectory and ground reaction force data.

Sensory Assessments

Light Touch

Mechanical sensation of light touch can be quantitatively assessed using Semmes Weinstein monofilaments, also called von Frey hairs. These filaments are calibrated to bend when a precise amount of force is applied to a test site, and participants provide verbal responses when they sense the touch of a filament (100) or when they can discriminate between two time intervals where one included application of a monofilament (101). In rehabilitation and podiatric clinics, monofilament testing is commonly used in patients with diabetic peripheral neuropathy to assess for loss of protective sensation, or the ability to sense 10 grams of force (the 5.07 Semmes Weinstein filament) (102).

Although researchers have since expanded to wider populations of interest, there are few high-quality studies that report thresholds of normal sensation. These studies vary by testing protocol, test sites, and participant demographics. One study that included 20 healthy controls from ages 47-76 assessed ten test sites on the foot using an interval comparison method (101). Normative values from this study overlapping with test sites in this dissertation include 4.28±0.09 at the hallux and 5.12±0.13 at the heel. Another study assessed the same ten test sites, reporting the threshold as the filament that could be sensed at all ten sites (‘sensed’ in this case indicates an affirmative response from the participant in at least one out of three sequential trials with a given filament) in three

different groups of healthy individuals stratified by age: 3.61 (0.4g) for ages 18-34, 4.31 (2g) for 35-64, and 4.74 (6g) for 65 and older (103). In these studies with diabetic participants, test sites were chosen based on assessing the major foot dermatomes and locations where patients commonly present with foot ulcers (101). In stroke, where the peripheral nerves are likely intact, there is less of a need to assess every foot dermatome to build a picture of the impact of stroke on sensory thresholds. For consistency across measures, both tactile and vibratory sensation assessments will take place on the same five landmarks of the paretic or test leg: hallux; heel; medial malleolus; and proximal tibia.

Vibration

Vibration is a useful technique in sensory assessment because it travels along the same tract as light touch but provides the additional complexity of a dynamic sensation (82). One early study reported normative vibratory thresholds in 110 healthy males (ages 10-74) of 3.208μm for the hallux and 3.940μm for the proximal tibia (104). A study that segmented participants by ten-year age brackets found increasing thresholds with age and reported an average vibration perception threshold of 18.3V (approximately 3.3μm) at the medial malleolus in the 60-year age bracket (105). A small sample of older adults had a perception threshold of 10V (approximately 1μm) at the heel (106).

An important consideration when comparing vibratory thresholds between studies is the device used and the characteristics of the vibration it emits. One commonly used device, the Bio-Thesiometer (Bio-Medical Instrument Co, Newbury, OH, USA), has a 13mm diameter plastic probe that vibrates at a rate of two times the AC frequency. In this device's case, studies conducted in the United Kingdom use devices that vibrate at 100Hz, while studies in the United States use a vibration frequency of 120Hz (104). To

control for some of the variation this introduces, threshold values are first recorded in volts and then converted to microns according to a device-specific calibration table.

There are multiple thresholds that can be assessed for vibratory sensation. Using the method-of-limits, vibration perception threshold (VPT) is the lowest amount of vibration a person can feel at a given site. Vibration disappearance threshold (VDT) is the point at which the sensation of vibration disappears, measured by starting the device well above the VPT and gradually turning the vibration intensity down. Vibratory threshold (VT) is the mean of the VPT and VDT, and some claim it is more robust than either VPT or VDT alone (104).

Proprioception

Proprioception, or the ability to sense body position in space, is another important aspect of somatosensation (107). Proprioception can be measured in multiple ways, including by sensing static or dynamic positions or sensing movement itself. For this dissertation, we chose to measure ipsilateral joint position sense via a position matching task built into the functionality of the Biodex System 4 Pro dynamometer (108). Joint position sense is quick to assess and is easy for a participant to understand the instructions. Because the participants indicate when the position is matched using a button press, there is also no need for participants to verbalize responses, making assessment valid in aphasic patients. This task measures passive proprioception in isolation, so there are arguments that it may not be ecologically valid since it is so far removed from how the proprioceptive system acts during normal movement (109). There are other methods to assess proprioception, including contralateral position matching, threshold to detection of passive motion, and active movement extent discrimination

assessment (109). These measures vary in terms of task complexity and measurement apparatus necessary for the assessment.

Assessment of Long-Latency Reflexes

As mentioned in Chapter 1, LLRs occur in response to an active stretch. In this dissertation, we achieve stretch in the TA by having seated participants hold a low level of static torque prior to stretching the ankle into active plantarflexion. We record the response using surface EMG and LLRs are identifiable in the raw data in real-time. Aspects of the LLR of interest in this dissertation include response presence or absence, response amplitude, and response latency. We combine dynamic stretches with TMS, and Figure 2.1 illustrates our experimental setup from two different perspectives.

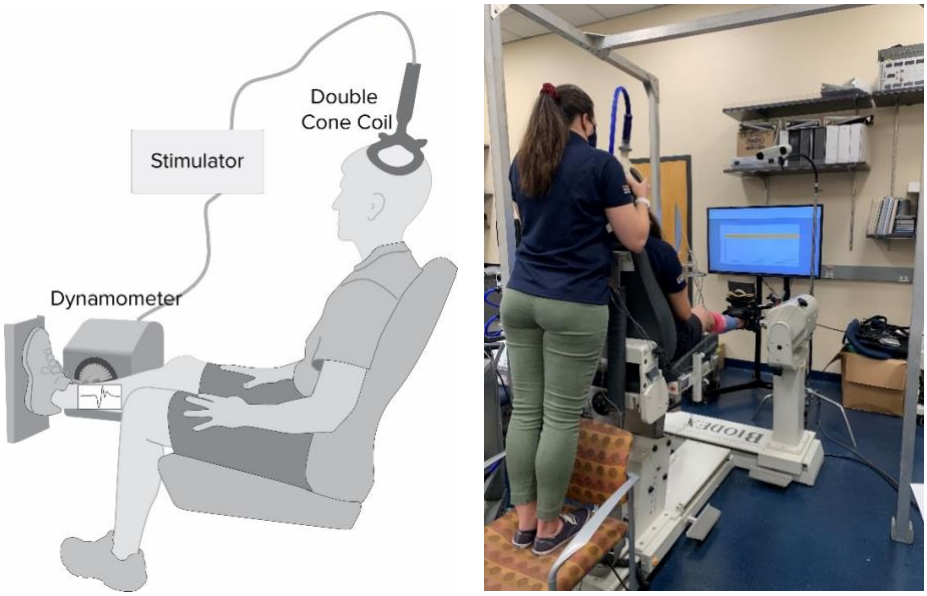

Figure 2.1. Illustration (left) and photograph (right) of experimental setup. The participant is seated with one leg extended and resting against the footplate of a Biodex dynamometer. The photograph on the right shows the visual feedback of a torque target (orange bar) on the monitor screen. Left image reprinted by permissions from Springer Nature Customer Service Centre GmbH: Springer Nature, Exp Brain Res. Banks, C.L., Little, V.L., Walker, E.R., Patten, C. Lower extremity long-latency reflexes differentiate walking function after stroke, © 2019 (doi: 10.1007/s00221-019-05614-y).

We acknowledge that, given the opportunity to plantarflex at their maximal speed, the healthy participants would likely be receiving stretches at an appreciably higher speed than some individuals with stroke. To control for that, we cap the maximal dynamometer speed to 90deg/s, a speed achievable by all participants. This is based on previous experiments with dynamic plantarflexion in participants with chronic stroke (12). With these advantages and disadvantages in mind, this makes the task of stretching the TA unique to our specific setup, and a variation from other lower extremity reflex tasks recorded in the literature.

During the experiment, we approximate LLR latency in real-time for parameterization of TMS timing. After each LLR trial, a cursor marks the location in time when rectified TA EMG exceeds a threshold of the mean + 2.5 standard deviations of the pre-movement (100ms) signal. This threshold was determined from measurements on healthy individuals. Cursor location, visual identification of LLRs from a trained experimenter, and the online signal average of approximately 10 trials are used simultaneously to approximate an individual's LLR latency. This latency approximation is later used to time TMS stimulation when we assess the cortical contributions to LLRs.

Assessment of Cortical Contribution to Long-Latency Reflexes

Superposition of TMS and stretch-evoked potentials is the gold standard for determining the cortical contribution of a reflex response. Because late responses can be due to delayed spinal reflex transmission, oligosynaptic reflex pathways, or transcortical reflexes (50), it is necessary to determine which part of the recorded reflex is transcortical for a given paradigm. One technique is to stimulate at a variety of latencies after stretch and determine which time points have evoked responses larger than the arithmetic sum

of the stretch response and a non-stretch-related motor evoked potential (43,66). Because we can calculate the latency of the LLR online, we can stimulate at a variety of latencies relative to the LLR, including before, during, and after response onset. This is done in our custom software by calculating the average LLR and MEP latencies, then subtracting the MEP latency from the LLR latency. For the individuals where LLRs cannot not be evoked by stretch alone and thus LLR latency cannot be determined, we can stimulate according to timings from the literature and early experiments.

Chapter 3: Lower Extremity Long-Latency Reflexes and Walking Function in Chronic Stroke

Note

Most of the material presented in this chapter is published in Experimental Brain Research, 237: 2595-2605 (2019). https://doi.org/10.1007/s00221-019-05614-y.

Introduction

Clinically accessible biomarkers of sensorimotor function need to be rooted in the underlying pathophysiology of stroke and relevant to common biomechanical deficits. Unlike the underlying physiology, the biomechanical deficits in walking following stroke are better characterized (110). Here, we investigated the association between known clinical and biomechanical deficits and two neurophysiologic markers in the paretic lower extremity of individuals with chronic stroke.

One key biomechanical deficit present in many individuals with chronic stroke is impaired plantarflexor power generation in late stance (111,112). In normal walking, the ankle is the primary energy generator, producing the necessary propulsion to advance the limb during swing (113,114). It is currently unclear what limits the ability of individuals with stroke to produce plantarflexion, but three potential contributors include weakness, excessive co-contraction, and spasticity. Weakness, one of the cardinal sequelae of stroke, arises from central factors and prevents sufficient and appropriate muscle activation patterns (115,116). Excessive co-contraction increases joint stiffness, is energetically inefficient, and is assumed to be a common manifestation in post-stroke gait impairment (117,118). Spasticity manifests in the forms of hypertonia and hyperreflexia, and is a common treatment target in the paretic ankle musculature (119,120). Impaired

plantarflexion during gait could arise from any combination of these factors, but all are worth exploring to understand walking impairment within this population.

We originally designed an experiment to assess the neurophysiologic correlates of plantarflexor dysfunction following stroke (121). This is a secondary analysis of that study. Because paretic leg propulsion requires coordination of both plantar- and dorsiflexor muscle activity (122), the role of the antagonist dorsiflexors also remains to be explored. Assessing the dorsiflexors can provide insights into the roles of co-contraction and, potentially, spasticity during plantarflexion. This is possible because plantarflexor power generation stretches the dorsiflexors, producing stretch-mediated reflex activity. Here, a reflex is defined as an electromyographic (EMG) response of a consistent duration exceeding background activity occurring at a consistent delay after stretch (123). Muscle stretch produces reflex responses of varying latencies, but here we will focus on the long-latency reflex (LLR) because evidence suggests that it has a strong cortical component (43,124). There are several common characteristics of the LLR between the upper and lower limbs and across muscles. The latency of this response is too long for a monosynaptic spinal pathway, but too short to be volitionally mediated (41). This time window is ideal for integration with sensory information from all modalities and modification in response to a perturbation (125). These stretch-mediated responses can be highly informative regarding central nervous system gating and integration of information, leading to new understanding of the control of walking and lower extremity movement (59).

The goal of this secondary analysis is to investigate the association between neurophysiologic responses in the antagonist muscle during plantarflexion and lower

extremity function following stroke. We hypothesize that these neurophysiologic markers will allow differentiation of a clinically heterogeneous group of individuals with chronic stroke. Specifically, the presence or absence of the LLR will differentiate high- and low-functioning individuals, allowing for further study of the underlying mechanisms of dysfunction within low-functioning individuals.

Methods

Subjects

Of the 39 individuals that participated in the larger study, 14 individuals with chronic stroke (age 63±8 years, 12 male, 2 female) and 13 healthy age-matched controls (age 61±8 years, 6 male, 7 female) met the criteria for inclusion in this analysis. Demographic data for the stroke cohort, reported in Table 3.1, illustrate a functionally diverse group of individuals with mild-to-moderate motor impairment. Study inclusion criteria for the stroke group included: a diagnosis of unilateral cortical or subcortical stroke at least six months prior to date of enrollment, ability to follow three-step commands, and ability to walk at least ten meters without assistance. CT or MR imaging results in medical records confirmed stroke diagnosis. All participants were free of any contraindications for transcranial magnetic stimulation, including implanted metal above the chest, seizure disorders, or pregnancy (126). In addition to study inclusion criteria, this secondary analysis was restricted to participants with adequate ankle range of motion and volitional movement velocities to measure LLRs during the dynamic plantarflexion task, as well as measurable motor evoked responses (MEPs) to transcranial magnetic stimulation in the tibialis anterior. We excluded nine individuals due to insufficient range of motion during

the task, two for lack of measurable MEPs, and one control who was determined neurologically unhealthy.

Testing occurred at the Brain Rehabilitation Research Center in the Malcom Randall VA Medical Center in Gainesville, FL. Isolated plantarflexion, instrumented gait analysis, and clinical assessments spanned 2-3 days for each participant. The University of Florida Health Science Center Institutional Review Board approved all procedures, and all participants gave written informed consent prior to participation. Testing was conducted in accordance with the Declaration of Helsinki.

Instrumentation

Isolated plantarflexion movements were tested using a commercially available dynamometer (Biodex System 3.2, Shirley, NY) and controlled by a Power1401 data acquisition system (Cambridge Electronic Design (CED) Limited, Cambridge, England). We collected surface electromyography (EMG) using a commercially available system (MA300-28, Motion Lab Systems, Baton Rouge, LA) from the medial gastrocnemius (MG), soleus (SOL), and tibialis anterior (TA) muscles using gel surface electrodes (Cleartrace 2, ConMed, Utica, NY) and snap-on preamplifiers (MA-420, Motion Lab Systems, Baton Rouge, LA). Electrodes were placed according to SENIAM guidelines (127). We applied single pulse transcranial magnetic stimulation (TMS) using a Magstim 200^2 with a 110 mm double cone coil (Whitland, UK). A Brainsight TMS neuronagivation system (Rogue Resolutions Ltd, Montreal, CA) was used to maintain coil placement.

Torque, position, and velocity signals from the dynamometer were low-pass filtered using an analog hardware filter (100 Hz cutoff). All data were recorded in CED Signal 6.0 at a sampling rate of 2000 Hz.

Table 3.1 Subject Demographics.

Subjects	Age (yrs)	Sex	Paretic Side	Chronicity (mos)	Stroke Type	Lesion Location	LE FMA	SPPB	DGI	AFO/Device	LLR Status
Stroke 01	80	M	R	97	Hemorrhagic	L temporal lobe	32	11	23	--	LLR+
Stroke 02	53	M	R	25	Ischemic	L parietal lobe	34	12	24	--	LLR+
Stroke 03	61	M	R	35	Ischemic	L ICA occlusion with striatocapsular involvement	24	10	16	Aircast®	LLR+
Stroke 04	61	M	R	87	Ischemic	L temporal, parietal, and frontal lobes	12	5	8	Custom AFO	LLR-
Stroke 05	73	M	R	62	Ischemic	L parietal lobe	34	11	22	--	LLR+
Stroke 06	67	M	R	86	Ischemic	L parietal with basal ganglia involvement	34	8	22	--	LLR+
Stroke 07	69	M	L	267	Hemorrhagic	R internal capsule	17	6	10	Walk Aide®	LLR-
Stroke 08	73	M	L	93	Ischemic	R posterior limb of internal capsule	26	8	15	Custom AFO	LLR-
Stroke 09	55	F	L	25	Ischemic	R temporal lobe	34	12	23	--	LLR+
Stroke 10	57	M	R	59	Ischemic	L frontal and temporal lobes with basal ganglia involvement	13	5	10	Custom AFO	LLR-
Stroke 11	62	M	L	65	Ischemic	R parietal and temporal lobes	34	12	21	--	LLR+
Stroke 12	56	F	R	131	Ischemic	L occipital and parietal lobes	19	7	9	Custom AFO	LLR+
Stroke 13	54	M	L	7	Hemorrhagic	R putamen	22	7	14	Custom AFO	LLR-
Stroke 14	66	M	R	131	Ischemic	L insula and basal ganglia	34	12	24	--	LLR+

yrs years, *M* male, *F* female, *L* left, *R* right, *mos* months, *ICA* internal carotid artery, *LE FMA* Lower Extremity Fugl-Meyer Motor Score, *SPPB* Short Physical Performance Battery, *DGI* Dynamic Gait Index, *AFO* ankle-foot orthosis, *LLR* long-latency reflex. Reprinted by permissions from Springer Nature Customer Service Centre GmbH: Springer Nature, Exp Brain Res. Banks, C.L., Little, V.L., Walker, E.R., Patten, C. Lower extremity long-latency reflexes differentiate walking function after stroke, © 2019 (doi: 10.1007/s00221-019-05614-y).

Protocol

Dynamometer testing took place in a single session. Each participant was seated with the seatback fully upright and the paretic leg (or randomly assigned test leg in healthy controls) extended, with approximately 90 degrees of hip flexion, 20 degrees of knee flexion, and the ankle positioned against the footplate at neutral plantar/dorsiflexion, as shown in Fig. 2.1 and detailed in the Assessment of Long-Latency Reflexes section. All joints were positioned so movement could occur only through the sagittal plane at the ankle. This configuration minimizes contributions of the hip muscles to plantarflexion while simultaneously positioning the medial gastrocnemius and soleus muscles within the optimal operating ranges (128,129). We assessed maximum voluntary contractions (MVCs) at the beginning of the session in both neutral and dorsiflexed (approximately 5 degrees) ankle positions. MVCs were determined in real-time as the best of 3-5 trials involving 2-4 second contractions, with at least 60 seconds of rest between trials. Participants received visual torque feedback and verbal encouragement from study personnel. Trials in which participants contracted thigh muscles in addition to ankle musculature were excluded. The test leg was randomized across healthy control participants. The original study assessed corticospinal efficacy to the plantarflexors, so TMS was localized to generate MEPs in the MG and SOL. Resting motor threshold (rMT) was the minimum stimulus level required to elicit a response $\geq$50 μV peak-to-peak amplitude in at least 50% of trials (126). Due to the close relationship between the ankle musculature within the cerebral architecture, TA MEPs are almost always elicited when targeting the plantarflexors, a phenomenon we observed during our study (55,130).

During testing, participants were instructed to generate and hold 10-20% of their measured maximum voluntary contraction (MVC) torque against the dynamometer footplate. Participants were provided real-time visual feedback of torque output to ensure consistent effort and task attention. Following a one-second hold of 10-20% measured MVC torque, a magnetic stimulus was applied at 120% of SOL rMT. Stimulus intensity was 63±8% of maximum stimulator output for healthy controls and 84±17% for individuals with chronic stroke. rMT could not be determined for eight individuals with stroke, so they were stimulated at 100% maximum stimulator output. Two individuals had discernable thresholds, but 120% rMT was unable to be achieved due to thresholds being higher than 80%, and these individuals were stimulated at 100% as well. In the isometric condition, the footplate remained stationary in the neutral position during stimulation. In the dynamic condition, the footplate started in approximately five degrees of dorsiflexion. Following the one-second hold, the footplate released, allowing the participant to plantarflex, “as hard and as fast as possible,” up to a maximum velocity of 90 degrees per second through their available range of motion (Figure 3.1). This rate is comparable to, or slower than, angular velocities that occur at the ankle during normal walking, and comparable to stretch velocities employed in another lower extremity reflex study (56). Magnetic stimulation was triggered when the ankle moved through the neutral position. After each trial, the participant had 2-3 seconds of rest before the footplate was passively returned to the starting position and the next trial began. A minimum of six trials were performed in each test condition.

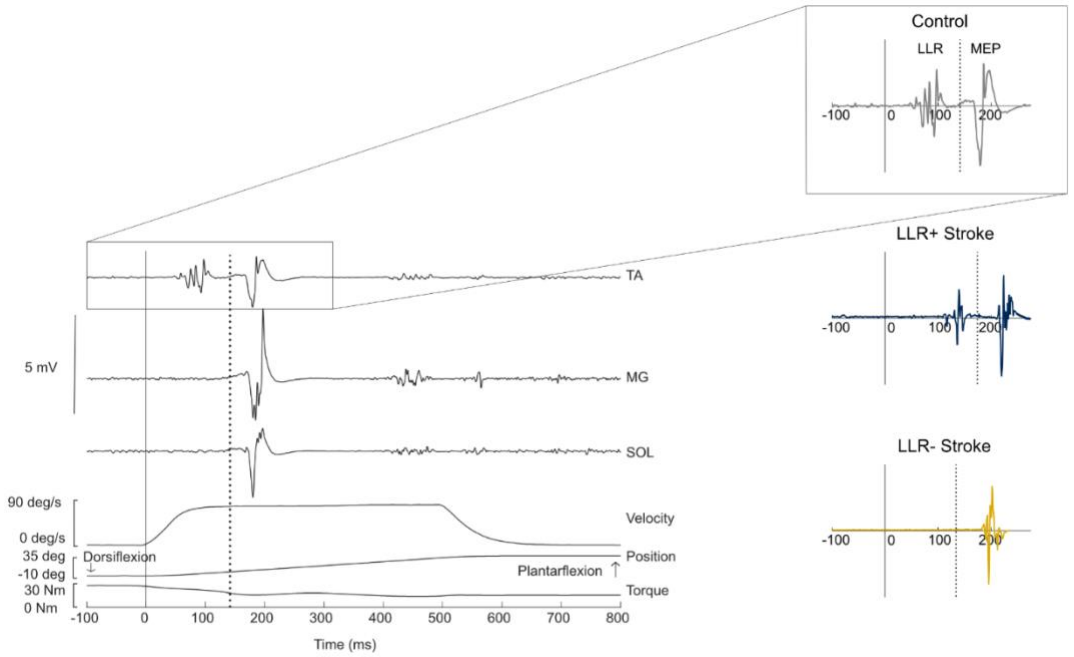

Figure 3.1. Experimental data from a representative healthy control (main panel) and tibialis anterior (TA) electromyography (EMG) from the same control and two representative individuals post-stroke (right panel). EMG from TA, medial gastrocnemius (MG), and soleus (SOL) are shown in the top three traces of the left image. The bottom three traces display dynamometer velocity, position, and torque. The solid line indicates the time when dynamometer movement began (time=0), while the dotted line indicates delivery of the TMS pulse. In the right panel, TA EMG from the same control, an individual post-stroke with long-latency reflex (LLR) activity (LLR+) and an individual with absent LLR responses (LLR-) are shown. Following delivery of TMS, a motor-evoked response (MEP) is seen in all individuals.

Data Analysis

Data were processed offline using Matlab R2015a (The MathWorks, Natick, MA, USA). TA EMG was filtered using a 4^{th} order bandpass filter (10-450 Hz cutoff range). In the isometric condition, background EMG was measured 100 milliseconds prior to the magnetic stimulus to determine an activity threshold for each trial (mean ± 1 standard deviation). In the dynamic condition, the length and position of the activity threshold window was adjusted manually for each participant to include only the period prior to movement onset (range of 50-100ms). This difference in establishing duration of the activity threshold window for the dynamic condition was to exclude LLR activity from the background EMG calculation.

Study Variables

Primary variables of interest include LLR presence and TA MEP_{area} change.

LLR presence was quantified as the percentage of trials in which the amplitude of an EMG burst in TA within 100-170 milliseconds after movement initiation exceeded 2.5 times the average background EMG (131). Given that all controls showed LLRs, these criteria were determined using a detection algorithm developed on the healthy control data (Figure 3.1). All healthy controls revealed LLRs in at least 50% of dynamic trials. Using the distribution of the healthy individuals as the standard, individuals were classified as LLR present (LLR+) if LLRs were present in $\geq$ 50% of trials and LLR absent (LLR-) otherwise. LLR presence was measured only during dynamic plantarflexion.

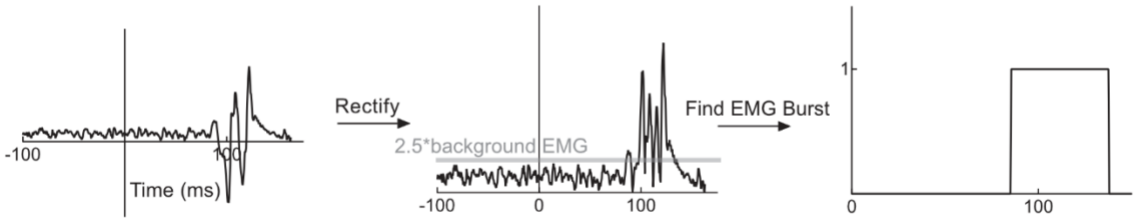

Figure 3.1. LLR detection algorithm. Developed using tibialis anterior electromyography (EMG) from healthy individuals, long-latency reflexes (LLRs) were detected by rectifying the EMG and then searching for a burst that exceeded 2.5 times the background EMG prior to movement onset. Once this threshold was achieved for a minimum of 10ms, the burst was transformed into a step function, allowing for consistent, objective identification of LLRs.

TA MEP_{area} is the area under the rectified and background-normalized motor evoked response elicited by TMS measured in the tibialis anterior muscle. We have expressed TA MEP_{area} change as the ratio between the isometric and dynamic conditions using the following equation:

$$MEP_{area}\ \text{change}\ (\%) = \frac{\text{Dynamic}\ MEP_{area} - \text{Isometric}\ MEP_{area}}{\text{Isometric}\ MEP_{area}} \times 100$$

Secondary clinical variables for this analysis include: Lower Extremity Fugl-Meyer Motor Score, Short Physical Performance Battery (SPPB) score, Dynamic Gait Index (DGI), self-selected walking speed (SSWS), and fastest comfortable walking speed (FCWS). SSWS and FCWS were measured as the average speed from 3-5 passes over a 16-foot pressure-sensitive walkway (GaitRite Platinum Plus System, Version 3.9, Havertown, PA). For SSWS, participants walked at their casual, comfortable pace. FCWS

was assessed as the fastest speed the participant could safely attain when walking, “as if you are crossing the street and the walk signal changes to a red hand.” Clinical measures were administered within a single session by a licensed physical therapist (VLL).

Our secondary biomechanical variable for this analysis is peak ankle plantarflexor power (A2). A2, the second peak in the sagittal plane ankle power profile, corresponds to plantarflexor power generation (99). A2 was derived from inverse dynamics using motion analysis performed while participants walked at their self-selected speed on an instrumented split-belt treadmill (Bertec, Columbus, OH). Marker data were obtained with a 12-camera Vicon motion capture system (Vicon MX, Vicon Motion Systems Ltd., Oxford, UK) using a modified Helen Hayes marker set sampled at 200 Hz. One healthy control and one individual post-stroke did not complete the instrumented gait assessment. Ankle power data during gait are not available for one additional stroke participant due to dependence on a rigid ankle foot orthosis during gait assessments. All other participants walked on the treadmill with either an Aircast® or without a brace and produced valid kinetics.

Statistical Analysis

Data were assessed for normality using the Shapiro-Wilk W test and were not normally distributed (p's<0.05). Therefore, TA MEP_{area} change and walking speeds were assessed for group differences using Kruskal-Wallis ANOVA and a significance level of $\alpha=0.05$. Post-hoc analyses were carried out using the Steel-Dwass method to correct for multiple comparisons. Clinical assessments were compared between subgroups of individuals stratified by LLR presence using Mann-Whitney U tests with Bonferroni

correction, and significance assessed using α=0.017. One-tailed Spearman correlations assessed the relationships between MEP_{area} change and A2, using α=0.05. All tests were carried out in JMP Pro 11 (SAS Institute, Cary, NC).

Results

All thirteen healthy controls revealed LLRs in response to rapid stretch of the TA during voluntary plantarflexion. Nine individuals post-stroke, herein referred to as LLR present (LLR+), also showed this stretch-mediated EMG. Five individuals post-stroke, referred to herein as LLR absent (LLR-), lacked long-latency EMG activity in response to TA stretch. The frequency distribution of LLR presence is shown in Figure 3.2. All LLR+ individuals showed LLRs in at least 60% of trials, while LLR- individuals revealed LLRs in 40% of trials or less, making an unambiguous distinction between presence and absence of this robust phenomenon. These three patterns are exemplified on the right-hand side of Figure 3.1.

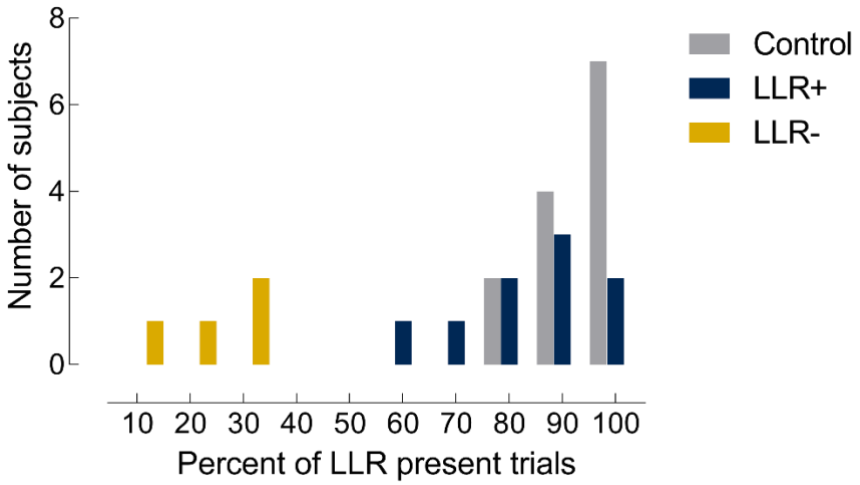

Figure 3.2. Frequency distribution of long-latency reflex (LLR) responses. Healthy individuals revealed LLRs in nearly all trials (grey). Individuals with stroke with present LLRs in ≥50% of

trials were classified as LLR+, and this distribution does not overlap with LLR frequency in individuals classified as LLR absent (LLR-).

Twenty-one of the 27 individuals tested showed facilitation of TA MEP_{area} in the dynamic, relative to the isometric, condition (Figure 3.3). There was a significant effect of group (p=0.017) on TA MEP_{area} change. Post-hoc tests revealed significant facilitation of TA MEP_{area} (p=0.012) in LLR- individuals, with a median (IQR) of 295% (156—757) relative to controls, with a median of 49% (-10—108).

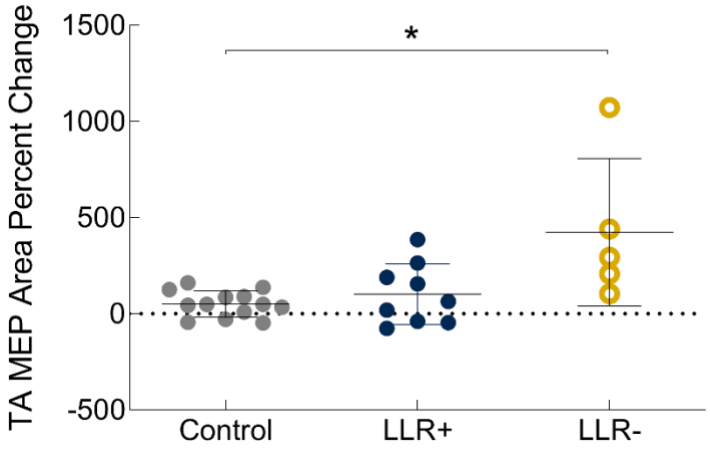

Figure 3.3. LLR- individuals (*n*=5) show exaggerated TA MEP_{area} change relative to healthy controls (*n*=13). LLR+ individuals (*n*=9) are not different from healthy controls or LLR- individuals. Bars represent median ±interquartile range. Aterisk indicates significance at $p<0.05$ using Kruskal-Wallis ANOVA and Steel-Dwass post hoc analysis.

LLR- individuals revealed lower clinical scores and walking speeds than LLR+ individuals and healthy controls (Figure 3.4a). Fugl-Meyer motor score was lower in LLR-

individuals, with a median (IQR) score of 17 (12.5—24), than LLR+ individuals with a median score of 34 (28—34; p = 0.007). SPPB score was also lower in LLR- individuals, with a median score of 6 (5-7.5), than LLR+ individuals with a median score of 11 (9—12; p = 0.006). DGI score was lower in LLR- individuals, with a median score of 10 (9—14.5), than LLR+ individuals, with a median score of 22 (18.5—23.5; p=0.013). Kruskal-Wallis ANOVA detected differences across groups for SSWS (p=0.0002) and FCWS (p=0.001, Figure 3.4b). Post-hoc analyses revealed that SSWS was higher in healthy controls, with a median speed of 1.3 m/s (1.2—1.5), than both LLR+, with a median speed of 1.1 m/s (0.96—1.2; p=0.01), and LLR-, with a median speed of 0.37 m/s (0.24—0.64; p=0.005), and higher in LLR+ than LLR- individuals (p=0.021). FCWS was higher in healthy controls, with a median speed of 2.0 m/s (1.7—2.3) than LLR+, with a median speed of 1.6 m/s (1.2—1.9, p=0.03), and LLR-, with a median speed of 0.48 m/s (0.34—0.95; p=0.01) but was not significantly different between LLR+ and LLR- individuals (p=0.053).

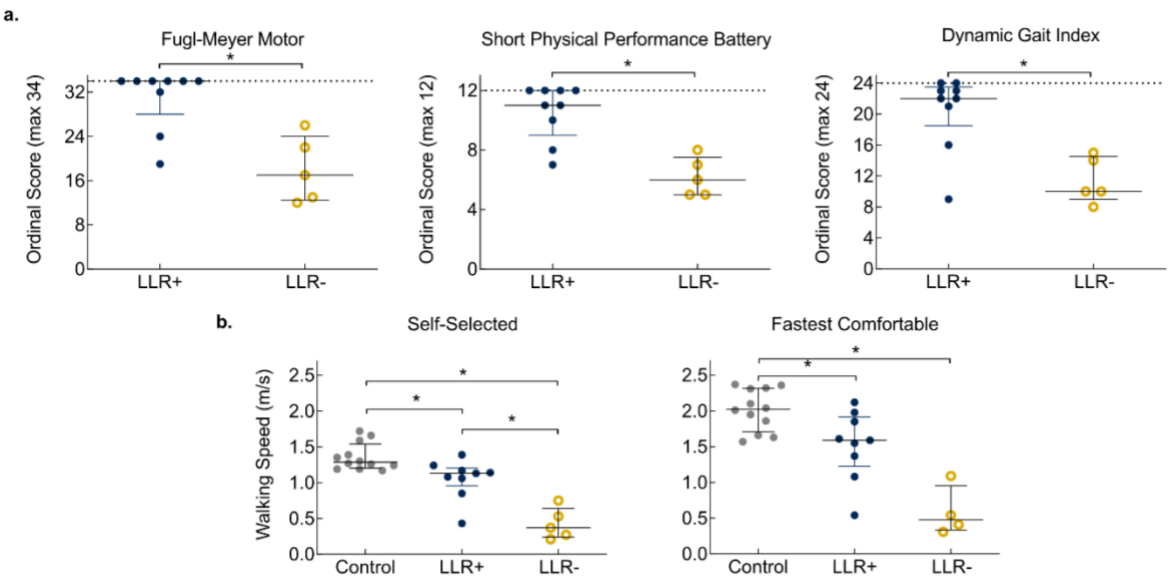

Figure 3.4. Clinical scores are markedly lower for LLR- individuals than LLR+ individuals post-stroke. a. From left to right, clinical scores include: Lower Extremity Fugl-Meyer Motor score, Short

Physical Performance Battery, and Dynamic Gait Index. **b.** Walking speed was different between all groups for self-selected walking speed (left) and between controls and each LLR group for fastest comfortable walking speed (right). One healthy control did not complete the walking speed assessment, while one LLR- individual completed only the self-selected walking speed measurement. Bars represent median±interquartile range. Asterisk indicates significance at $p<0.017$ for clinical scores or $p<0.05$ for walking speeds. Reprinted by permissions from Springer Nature Customer Service Centre GmbH: Springer Nature, Exp Brain Res. Banks, C.L., Little, V.L., Walker, E.R., Patten, C. Lower extremity long-latency reflexes differentiate walking function after stroke, © 2019 (doi: 10.1007/s00221-019-05614-y).

TA MEP_{area} change was not associated with A2 magnitude in healthy controls (p=0.29, Figure 3.5), however, the stroke group revealed a significant correlation (p=0.03). The scatterplot in Figure 3.6 illustrates an unambiguous gap between low and high ankle power. It is worth noting that all LLR- individuals produce low ankle power, although not all individuals that produce low power are LLR-.

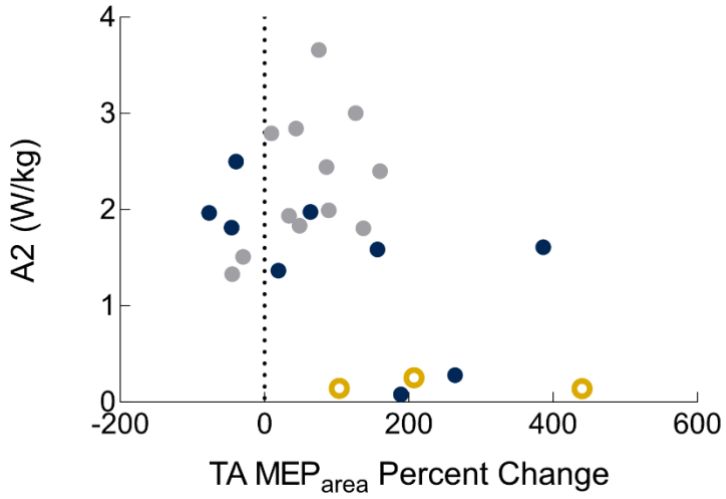

Figure 3.5. MEP_{area} change predicts ankle plantarflexor power (A2) post-stroke, but not in healthy individuals. In healthy controls (n=12, gray), the two variables are not correlated (Spearman ρ=0.34, $p>0.05$), however in individuals post-stroke, there is a significant negative correlation between tibialis anterior (TA) MEP_{area} change and A2 ρ=-0.64, p=0.03). LLR+ individuals are indicated in blue closed

circles (*n*=9), while LLR- individuals are indicated with gold open circles (*n*=3), for illustrative purposes only. One LLR- individual relied on a rigid AFO for safe ambulation, and one LLR- individual and one healthy control did not complete the instrumented gait analysis, therefore their A2 could not be calculated.

Discussion

Our primary findings are that the most impaired individuals show a dysregulation of TA MEPs and lack LLRs in TA during voluntary, dynamic plantarflexion. Healthy individuals had relatively unchanged TA responses to dynamic plantarflexion, and all showed LLRs. Because the higher-functioning individuals with chronic stroke in this sample were not physiologically distinct from the healthy controls, our discussion will focus primarily on the subset of lower-functioning individuals. These individuals appear to have difficulty integrating appropriate information, including afferent signals, within the context of this plantarflexion task. Importantly, there is not one-to-one correspondence between the dysregulation of TA MEPs and lack of LLRs. This, in combination with a review of the literature, leads us to conclude that these phenomena arise from distinct mechanisms.

The lower extremity LLR may serve as a first line of defense in response to a perturbation (123,132). Although we are unable to mechanistically confirm that our observations represent the same LLR recorded in other muscles and tasks, the latency of the measured response and the absence of LLRs within a subset of individuals with central nervous system injury leads us to believe that the measured LLR arises from a transcortical pathway (43,64). The detection algorithm we designed assessed responses

within 100-170 milliseconds of movement initiation, a window that is comfortably late enough for transcortical involvement (133). With that said, we cannot conclude that we are exclusively measuring a transcortical reflex within this dataset. There is a convergence of pathways that can contribute to the response at this latency, and we do not have the experimental control necessary to ascribe a particular mechanism to the data from this secondary analysis (41,71). Overall, the idea that sensory information alters motor output dates back to the work of Sherrington, Evarts, and others (134,135). However, the LLR could represent a simple, clinically accessible probe of sensorimotor function for individuals with chronic stroke. Individuals who lack LLRs could be missing key components of normal motor control, but further study is necessary to draw conclusions about the functional consequences of this phenomenon.

The underlying mechanism responsible for the exaggerated facilitation of TA MEPs also remains unclear. Given the dynamic condition instructions, it is not unreasonable that the controls exhibited a small facilitation in TA MEP size due to a generalized increase in motor excitability during volitional plantarflexion. However, the excessive facilitation present in some individuals post-stroke warrants further consideration. Diminished reciprocal inhibition, and even a reversal pattern termed reciprocal facilitation, have been observed in some neuropathologies, including: cerebral palsy, spinal cord injury, and stroke (136–138). The appearance of reflex reversals is inconsistent and poorly understood, but may be attributable to the disynaptic reciprocal inhibitory circuit (138–140). In addition to spinal circuitry, supraspinal inputs to inhibitory interneurons contribute to the reciprocal inhibitory pathway (141). Lesions in the motor cortex may, therefore, interrupt normal patterns of inhibitory control, allowing for pathologic

disinhibition with dynamic movement. Our observation that the magnitude of TA MEP facilitation during plantarflexion is negatively correlated with the magnitude of ankle plantarflexor power may be indicative of over-excitable dorsiflexor activity, inhibiting plantarflexion vital to gait. The mechanism of this dysregulation warrants further investigation. The finding that all LLR- individuals and some LLR+ individuals within this sample exhibited excessive facilitation indicates that this facilitation is likely driven by a different mechanism than the LLR; the interaction between these two responses would require further investigation within a larger sample.

The three potential contributors to plantarflexor dysfunction mentioned earlier are weakness, excessive co-contraction, and spasticity. The clinical outcomes and the LLR- group, coupled with their reduced ankle power, demonstrate that these individuals are weak (Table 1). The dysregulation of dynamic dorsiflexor MEPs in some individuals points to impaired antagonist control, another indicator of central nervous system impairment. Whether in the control or stroke groups, there was no evidence of excessive co-contraction during the isolated plantarflexion movements in this sample (Figure 3.1). Previously, we assessed co-contraction during gait in most of the individuals from this sample and found that co-contraction is not a common strategy employed by individuals with chronic stroke (16). Spasticity is also an unlikely contributor to impairments in dynamic plantarflexion within this sample. Although a small portion of individuals within this sample had measurable hyperreflexia in the fast passive stretches of the Modified Ashworth Assessment, reflex responses were *absent* during active stretches of the dorsiflexors, the opposite of an expected finding in the case of spasticity. Although definitive conclusions cannot be drawn from this small dataset, weakness and impaired

reciprocal inhibition from the tibialis anterior appears to be the most likely sources of these impairments in motor control. Strength training can improve both central and peripheral weakness, and it produces no exacerbation of spasticity in the process, thus these LLR- individuals have the potential to benefit from strength training (12,142).

The primary limitation of this study was that the methods were not designed to assess LLRs. All plantarflexor stretches employed in this study were followed by TMS pulses. For some individuals in the larger sample, there was insufficient time for the LLR to occur prior to the TMS response, and these cases were therefore excluded from this secondary analysis. Despite our focus on dorsiflexor excitability in this study, TMS was targeted to the plantarflexors. Two individuals who were unable to produce measurable soleus MEPs had consistent TA MEPs in both conditions, a finding that is not surprising due to the relative ease of eliciting TA MEPs when either the TA or SOL is the primary target (143). One control and one LLR- individual did not complete the gait analysis portion of the study, and therefore we were unable to calculate A2 for these individuals and compare between LLR subgroups. The remaining sample size and methods employed preclude the ability to draw definitive conclusions regarding the role of these neurophysiologic outcomes in walking deficits. However, these preliminary findings offer the opportunity for theoretical discussion. Future work is indicated to replicate and elucidate the mechanisms and functional significance of LLR absence in chronic stroke.

The two neurophysiologic phenomena assessed in this study are both associated with impaired walking function following stroke, but likely stem from different mechanisms. Not all individuals who exhibited exaggerated dorsiflexor excitability were LLR-, but the majority of LLR- individuals showed exaggerated TA MEPs in the dynamic condition. It is

possible that the LLR+ individuals with exaggerated TA MEPs possess a more segmental deficit, while LLR- individuals possess a supraspinal impairment, but it is too early to draw definitive conclusions from a mechanistic perspective. What can be concluded is that the LLR- individuals were clinically and biomechanically lower functioning than LLR+ individuals and healthy controls. Work must be done to gain a better approximation of the prevalence of these deficits among individuals with stroke, including assessment of supraspinal contributions to LLR presence or absence and measurement of individuals in across all stages of recovery. Due to the ease of measurement and the unambiguous presence or absence of response, the LLR appears to be a promising physiologic marker of motor dysfunction in chronic stroke.

Chapter 4: Characterization of Cortical Contribution to LLRs Post-Stroke

Introduction

In Chapter 3, we found that some individuals with chronic stroke lack LLRs, and that those LLR- individuals were lower functioning than LLR+ individuals or age-matched controls. However, we can only speculate by the timing of the response and the apparent lack of responses in individuals with hemispheric lesions that the response we measured is transcortical. Here, we will replicate our experiment from Chapter 3 while adding methods to confirm that the measured response has a cortical component. We will also add methods to further characterize individuals' motor function according to LLR status. These steps will contribute further evidence to our working hypothesis that the LLR is a functional biomarker offering relevant physiological information for clinicians to use in rehabilitation practice.

One reason it is insufficient to conclude that the responses reported in Chapter 3 are transcortical by comparison to the extant literature is that transcortical reflexes are task specific. When instructing a subject to resist when their muscle is stretched rather than let go, LLR amplitude increases (60). Varying task constraints, such as switching a task from controlling for position to controlling for torque, can change LLR amplitude (57). A 2015 review by Kurtzer compiles evidence that the LLR accounts for the biomechanical properties of the arm and the task, rather than merely providing a stereotyped response to the stretch (144). Since our dynamic plantarflexion task is one that has not been explored previously from an LLR perspective, we must gather more evidence that the response measured has a cortical component.

Most reflex studies are conducted with stretch speeds upwards of 200 deg/s (133,145,146). When our plantarflexion experiment was designed, we desired a task that involved a plantarflexion movement that was like late stance phase of the gait cycle. The ankle reaches a maximum plantarflexion velocity of 100-300deg/s at this phase for typical adults and scales with gait speed (147). We also know from previous single joint experiments that 90deg/s was an achievable rate of speed for individuals with chronic stroke (12). Although these are not the only experiments in the literature at lower stretch speeds (68), to our knowledge they are the only plantarflexion stretches at speeds this low.

Confirming that the lack of LLRs described in Chapter 3 involves a cortical component would contribute important new evidence for the neurorehabilitation literature. Our data to date suggest LLR presence or absence is an unambiguous metric that is currently informative from a behavioral perspective. If this LLR is transcortical, it also contributes neurophysiologic understanding of this metric, making it more powerful as a standalone metric than most other biomarkers utilized in clinical trials or rehabilitation practice. Further characterizing the amplitude and latency of the LLR in LLR+ individuals as well as its relationship to corticospinal efficacy measured by single-pulse transcranial magnetic stimulation (TMS) will provide insight about the motor portion of the transcortical reflex pathway. It is possible that this step could differentiate LLR+ individuals with chronic stroke from healthy individuals, or alternatively LLRs could be a probe of healthy corticospinal tract (CST) function in individuals with intact LLRs. Insights from this chapter will not, however, allow us to draw conclusions about the sensory or integratory

components of the response, or specific brain areas that are involved beyond the broad scope of the CST.

The objectives of this chapter are to: (1) provide convincing evidence that the LLR is a valid indicator of the dysfunction of the transcortical reflex pathway serving the distal lower limb, and (2) characterize the relationship between LLRs and clinical measures of motor function. The gold standard for confirming the presence of a supraspinal component within a response is to augment the afferent information with TMS (43,51,66). We will deliver single-pulse TMS timed to generate a motor evoked potential (MEP) timed to occur at the same latency as the LLR. If the responses to stretch and TMS arose from separate pathways, under this condition there would either be cancellation or simple summation of the two responses. However, if TMS facilitates the LLR beyond the arithmetic sum of the two responses measured separately, this extra facilitation indicates presence of a transcortical component within the LLR (43,66).

Next we will assess the ability of LLRs to differentiate functional status by evaluating the relationship between LLR presence/absence and commonly used clinical measures of impairment and walking ability. Although we have shown this differentiation in Chapter 3, our early experiments were not specifically designed to measure the LLR phenomenon, thus limiting the size of our sample and the conclusions that could be drawn. Here we revise our methods to specifically induce the LLR, allowing for more conclusive associations to be made between the LLR and a participant's level of motor function.

Methods

Subjects

Participants from the greater Sacramento region were recruited through a voluntary online registry and through UC Davis Health, with the assistance of the Neurology and Physical Medicine & Rehabilitation patient care teams. Efforts were made to draw a racial, ethnic, and gender diverse population that represented the broader demographics of the area.

Potential participants were screened by telephone or email to ensure they met the study inclusion criteria. All participants were at least 18 years of age, able to follow three-step commands, and able to ambulate independently for at least 25 feet with or without an assistive device. To undergo TMS, a screening survey drawn from the safety recommendations of Rossi et al. ensured that participants had no implanted magnetic or electronic devices above the chest and low risk of seizure (148,149). Healthy individuals additionally needed to be free of any central nervous system impairments or major orthopedic impairments that affected their ability to use their ankle. Participants with stroke had a history of cortical or subcortical stroke that was confirmed by CT or MRI imaging six or more months prior to enrollment in the study. This time window ensured that participants' neurologic function was relatively stable and they were unlikely to be undergoing intensive rehabilitation (150).

All experimental procedures were approved by the University of California, Davis Institutional Review Board. Participants gave written informed consent prior to participation. The Montreal Cognitive Assessment (MoCA) was used as a screening tool

to assess ability to follow three-step commands and sufficient cognitive function to provide informed consent. Testing was conducted in accordance with the Declaration of Helsinki.

Instrumentation

EMG was collected using a Motion Lab Systems MA-420 system with wired snap-on preamplifiers (Motion Lab Systems, Baton Rouge, LA, USA). This EMG system has 10-position gain control, which allowed for increased signal-to-noise ratio when looking for relatively brief events like motor evoked potentials. Electrodes were placed bilaterally on the medial head of the gastrocnemius (MG), soleus (SOL), and tibialis anterior (TA) using ConMed Cleartrace^2 Ag/AgCl adult ECG electrodes trimmed to accommodate a 2cm interelectrode distance (ConMed, Utica, NY, USA). Electrodes were placed following SENIAM guidelines (127).

LLR testing utilized a Biodex System 4 Pro™ isokinetic dynamometer with the Combination Ankle Attachment configured for plantar/dorsiflexion (Biodex Medical Systems, Inc., Shirley, NY, USA). Torque, position, and velocity signals from the dynamometer were isolated using a custom analogue hardware filter (100 Hz low-pass cutoff) and fed into the data acquisition system using coaxial cables with Bayonet Neill-Concelman (BNC) connectors. During the experiment, the dynamometer was controlled via computer serial commands.

Maximal M-waves were assessed using peripheral nerve stimulation to the tibial nerve (MG and SOL) and common peroneal nerve (TA). Stimulation was provided through a probe electrode and a Digitimer DS8R bipolar constant current stimulator (Hertfordshire, UK). Stimulation pulses were monophasic, with a pulse width of 1000 μs, with device pulse amplitude ranging from 2-1000 mA of current.

Transcranial magnetic stimulation (TMS) utilized a Magstim 200^2 monophasic stimulator (pulse width 1ms, 100μs rise time) with a double cone coil (Magstim, Plymouth, MN, USA). The double cone coil has two coils of wire with a diameter of 110 mm, inductance of 17.85μH, and maximum field strength of 1.4T. This coil configuration is ideal for stimulating the lower extremity region because it is highly focal and has a half-depth of approximately 2cm (151). This coil typically provides a high enough field strength to handle the relatively high motor thresholds that are common in people with stroke (152). A Brainsight® neuronavigation system with a generic configuration was used to maintain coil placement and track target errors (v2.4, Rogue Research, Montréal, Québec, CA).

Data acquisition was performed using a Power1401 interface and Signal 7.0 software (Cambridge Electronic Design Ltd, Cambridge, England). Data were collected at a rate of 2000 Hz. Stimulation levels and timing and Biodex pedal movement could be carefully controlled via a custom-written script. Triggering using torque thresholds for the TMS and LLR measurements was accomplished using output sequencer control running directly on the Power1401 device.

Protocol

The participant was seated in the Biodex chair in the same position illustrated in Figure 2.1 and detailed in Chapter 3 (approximately 90 degrees of hip flexion, 20 degrees of knee flexion, and the ankle positioned against the footplate at neutral plantar/dorsiflexion).

Plantarflexor maximum voluntary contractions (MVCs) were assessed at neutral and at the end of the participant's dorsiflexion range of motion. Dorsiflexor MVCs were also assessed in the neutral position. Participants were provided visual feedback of root

mean squared dynamometer torques (time constant 0.5). For each MVC, participants were provided verbal encouragement from study staff to push or pull as hard as they could against the static foot plate (92). After holding a steady maximal contraction for 3-5 seconds, the participant was instructed to relax. At least one minute of rest was taken between trials. At least three MVC trials, with no apparent compensatory movements from the hip or knee, were collected in each position and the highest torque was taken as the experimental MVC torque. MVC torques were measured for the purpose of setting torque targets later in the experiment, as well as giving a measure of each participant's static voluntary torque generating capacity.

Maximal M-waves were assessed next, for the purpose of normalizing EMG data in post-processing. The TA muscle was targeted first by stimulating the common peroneal nerve just distal to the fibular head (153). Then we moved the electrode to the popliteal fossa to target the tibial nerve innervating the MG and SOL muscles (40). The target electrode position for each nerve was the spot where the largest M-wave could be evoked at a low but measurable level of stimulation. The maximal M-wave (M-max) was then determined by progressively increasing stimulus intensity by 5-10 mA until the M-wave amplitude was saturated. This maximal stimulation intensity was repeated five times for each muscle.

Next, the TMS hotspot was determined for the target leg starting at the vertex and angling and/or translating toward the lesioned hemisphere. The double cone coil was positioned so current flowed in an anterior-to-posterior direction (154). Because the cortical areas serving the plantar- and dorsiflexor muscles overlap and we were interested

in plantarflexion (143), we prioritized selecting a spot where MEPs could be elicited in all three lower leg muscles. After the hotspot was located, we evaluated motor thresholds.

Resting motor threshold (rMT) was the stimulation level where MEPs were ≥50μV in at least three out of five trials Active motor threshold (aMT) was assessed while participants were holding isometric contractions at a level from 10-20% MVC. We assessed aMT for all three muscles during plantarflexion. We also assessed TA aMT during dorsiflexion, but technical difficulties prevented collection of this measure in some subjects. Participants were provided visual feedback of their torque generation and a colored target bar showing 10-20% of their MVC was displayed on screen. Once the desired contraction level was held for one second, the data acquisition system triggered a stimulation. We systematically varied stimulus intensity, starting below rMT, until we identified aMT as the lowest intensity necessary to detect an MEP ≥100μV and distinct from background EMG in at least three out of five trials.

After aMTs were determined for each muscle, all further TMS testing took place at approximately 110% TA aMT. This value is approximate because we have threshold measurements during active dorsiflexion in some, but not all, individuals, and because we targeted a stimulation level where MEPs were reliably visible. If the TA aMT during dorsiflexion was sizably lower than the TA aMT during plantarflexion, we typically increased stimulation intensity to a level where MEPs were more frequently elicited. Ten static MEPs at 110% TA aMT and 10-20% MVC contraction were recorded prior to testing LLRs, and the latency of these MEPs was recorded in real-time. Ten more MEPs were assessed at the end of the session to verify that no changes in TMS excitability or instrumentation occurred during dynamic testing.

LLRs were assessed using dynamic stretches that began from an isometric preload. Participants started at their maximum dorsiflexed position and were provided a visual target showing 10-20% of their dorsiflexed position MVC torque. After a one-second hold within the torque window, the footplate would release, allowing the participant to rapidly plantarflex. Instructions provided to each participant were to: “push as hard and as fast as possible when the foot pedal releases.” One to three practice trials were completed to familiarize the participants with the task, then 10 trials were recorded. LLR latency was measured during data collection for the purpose of parameterizing triggering in the next block. Preload limits were adjusted to 10-30% MVC for one participant with stroke due to low MVC torques. This correction was applied through the remainder of this participant’s experiment.

The main experiment, or LLR + TMS block, combined most of the previous block’s protocols. TMS was combined with dynamic stretches to elicit LLRs, using the same protocol as described in the previous paragraph. We used six stimulation states, each defined by the time interval between stretch and stimulation. States were block randomized to ensure an approximately equal number of trials in each state. The first state did not include stimulation; it was merely a replication of the stretch only condition described in the previous paragraph. For the remaining five states, stimulation time was calculated using the following equation:

$$\text{Stimulation Time} = \text{LLR latency} - \text{MEP latency} + \text{Delay}$$

Using timing delays of -20ms, -10ms, 0ms, 10ms, and 20ms, we were able to assess MEPs arriving before the LLR (negative delays), at the same time as the LLR (0ms delay),

and after LLR onset (positive delays). Eight trials of each state, for a total of approximately 48 total trials, were collected for each participant.

For LLR- participants, we were not able to use the same timing structure as above because there was no LLR latency available for stimulation time calculation. Instead, we varied stimulation time so MEPs would arrive between 80-120ms in steps of 10ms. This time window was informed by the LLR latencies observed in Chapter 3 as well as other LLR latencies in the literature, and is a similar protocol to the one applied by Petersen et al. (43).

Data Analysis

Data were exported to .mat files and processed offline using Matlab 2020a (The Mathworks, Natick, MA, USA). EMG data were bandpass filtered (4^{th} order Butterworth, cutoff frequency 10-450Hz). Torque, position, and velocity data were lowpass filtered (2^{nd} order Butterworth) using a 20Hz cutoff for torque and position and a 50Hz cutoff for velocity. Position data were corrected relative to the recorded neutral position and right leg data were directionally flipped so that right and left leg positions were positive in plantarflexion. Static data (i.e., M-waves and pre- and post-MEPs) were aligned relative to stimulation time. Dynamic data were aligned relative to movement onset using a velocity threshold of 2deg/s because the noise profile of the velocity data was ±1deg/s in pilot experiments.

A custom Matlab user interface was utilized for analyzing M-waves, MEPs, and LLRs. We first calculated an activity threshold, or the mean±1 standard deviation of EMG over a 100ms period, typically from 5-105ms before stimulation to avoid stimulus artifact. Then, using the activity threshold and knowledge of typical shape and timing of responses

as a guide, the M-wave or MEP start time was manually identified as the zero crossing prior to the evoked response exceeding the activity threshold. This value will be referred to as M-wave or MEP latency moving forward. M-wave and MEP stop time was then identified as the time when the rectified evoked response passed back under the activity threshold. The LLR was identified as a response greater than 80ms after stretch, distinct from background, and exceeded 2.5 times the activity threshold in amplitude. LLR latency was placed at the first activity threshold crossing of the rectified response. Using the start and stop times identified, the user interface calculated peak-to-peak amplitude and area under the curve for each evoked response.

Study Variables

The main variables of interest in this analysis are LLR presence/absence, LLR characteristics and corticospinal tract (CST) efficacy, and dynamic facilitation of evoked responses. Secondary variables of interest include clinical measures of impairment and function.

As described in Chapter 3, we defined LLR presence as having discernable LLRs in at least 50% of trials collected. This status was assessed during the experiment to use for calculating stimulation time in the LLR+TMS block, and then confirmed in post-processing.

Baseline LLR characteristics and CST efficacy measured include LLR latency, duration, amplitude, and, area as well as MEP latency, amplitude, and area.

Dynamic evoked responses, as measured in the LLR+TMS block, were expressed as a percent facilitation. As in Petersen et al. (1998) and Christensen et al. (2001), the combined response from stretch and TMS must be larger than the arithmetic sum of the

individual responses alone (43,155). To quantify this, we added the mean peak-to-peak value from the static pre TMS block to the mean peak-to-peak value from the dynamic LLR block. Dynamic facilitation in the LLR+TMS block was then calculated as follows:

$$\text{Dynamic Facilitation } (\%) = \frac{\text{Dynamic evoked response} - (\text{MEP} + \text{LLR})}{\text{MEP} + \text{LLR}} * 100$$

Where a facilitation value of 0% would indicate that the evoked response is equal to the sum of the responses alone, and a value of 50% indicates that the evoked response is 1.5 times the two responses alone.

Clinical and functional measures were assessed by the same rater in all individuals [CLB]. For individuals with stroke, we assessed impairment via the Fugl-Meyer Assessment of Physical Performance (FMA). Functional measures assessed in all individuals include the Short Physical Performance Battery (SPPB), Dynamic Gait Index (DGI), and walking speeds. Self-selected and fastest comfortable walking speeds were assessed using a 12-foot Stepscan Pedway with either four feet or six feet of inactive walkway at either end (Stepsan Technologies, Inc., Charlottetown, PEI, CA).

Statistical Analysis

LLR characteristics and CST efficacy are continuous variables that were compared both between groups (control/stroke) and across LLR status (control/LLR+/LLR-). For the group comparisons, one-tailed t-tests were calculated for each metric, with Bonferroni corrections for multiple comparisons projecting a significance level of α=0.0125. Comparisons across LLR status were made using one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) for post-hoc testing, also using a Bonferroni corrected significance level of α=0.0125.

Dynamic evoked responses were compared between groups using an LLR Status*Timing two-factor ANOVA. Tukey's HSD was carried out for significant main effects or interactions. Additionally, we sought to compare the maximum level of facilitation within and across groups. We computed one-tailed one sample t-tests within each group with a null hypothesis of 0% facilitation and an alternate hypothesis of greater than 0% facilitation. Prior to conducting the experiments, a power analysis based on data from Petersen et al (43) using α=0.05. and 1-β=0.8 indicated that three samples per group would achieve an estimated power of 0.98.

Most of the clinical and functional scores assessed here are ordinal scores and sample sizes are relatively small for this type of data, so we utilized non-parametric statistics. Prior to the experiment, samples were estimated from Chapter 3 data using bootstrapping with α=0.05. and 1-β=0.8. The FMA was compared between LLR+ and LLR- subjects using a one-tailed Mann-Whitney U test. SPPB and DGI scores, as well as walking speeds, were compared across Status (control/LLR+/LLR-) using Kruskall-Wallis ANOVAs with Bonferroni-corrected pairwise Wilcoxon tests for post-hoc analysis. Power analysis suggested that at least four individuals were necessary per group.

Data were analyzed using custom written scripts in R version 4.1.2 (156) via the RStudio platform version 2021.09.1 (157). R packages utilized include tidyverse, readxl, psych, and rstatix (158–161).

Results

Subjects

Eight individuals with chronic stroke and seven age-matched healthy controls participated in this study. One healthy individual did not tolerate TMS, so procedures through MVCs and LLRs were completed but data are missing for all TMS-related variables and clinical tests. Summary demographic data are provided in Table 4.1. One individual with stroke has an unknown chronicity, the participant and medical records estimate chronicity between 4-6 years prior to enrollment. Lesion location and mechanism varied widely across the stroke sample. Two individuals had cortical lesions, two subcortical, and four had mixed cortical and subcortical involvement. Four individuals had ischemic strokes, three had hemorrhages, and one had multiple embolic strokes. Due to the wide variety of etiologies, no comparisons will be made based on lesion location across this small sample. One individual was taking anti-spasmodic medication and had been on a stable dose at the time of the study.

Table 4.1. Study Demographics

	Control	Stroke
Demographics		
n	7	8
Sex (m/f)	3/4	6/2
Race (white/Black/Asian/not stated)	7/0/0/0	5/1/1/1
Ethnicity (Hispanic or Latino/NHL/not stated)	0/7/0	1/6/1
Age (yrs)	56±14	56±18
Chronicity* (yrs)	---	6.5±3.3
Lesion location (cortical/subcortical/mixed)	---	2/2/4
Paretic/test leg (r/l)	2/5	3/5
Self-selected walking speed (m/s)	1.3±0.15	0.80±0.49

*Date of stroke is unknown for one participant, chronicity values calculated with n=7

m male, *f* female, *NHL* not Hispanic or Latino, *yrs* years, *L* left, *R* right, *m/s* meters per second

LLR Presence/Absence

All seven healthy individuals revealed LLRs upon initial assessment. Consistent with our findings in Chapter 3 and using the same amplitude threshold of 2.5*background EMG, LLRs were present in 91.4±10% of trials for these healthy individuals. Of the eight individuals with stroke, four were classified as LLR+ and four were LLR-. LLR+ individuals had responses in 80.0±25% of trials. One LLR- individual showed a response in three trials that gradually diminished throughout testing and the other three LLR- individuals showed no LLR activity in the expected time window. All subjects maintained their in-experiment LLR status after post-processing.

LLR Characteristics

Figure 4.1 shows LLR characteristics related to timing (i.e., latency, duration), and size (i.e., amplitude, area) across groups. We performed Student's t-tests to compare LLR latency, duration, and area between the Control and LLR+ groups because they had reliable LLR start and stop times with enough trials for averaging. LLR amplitude was calculated for all individuals, with a time window of approximately 80-120ms used for calculating amplitude in trials without LLRs. LLR latency was not different between controls (M=111ms, SE=6.7ms) and LLR+ individuals (M=119ms, SE=19.6ms), $t(3.7)$=-0.384, p=0.36. LLR duration was also not different between controls (M=52.5ms, SE=3.0ms) and LLR+ individuals (M=41.3ms, SE=4.6ms), $t(5.5)$=2.029, p=0.95. When comparing LLR amplitude across all three LLR statuses via one-way ANOVA, there were significant differences, $F(2,12)$=7.273, p=0.009, ω^2=0.55. Tukey HSD *post-hoc* tests revealed that controls (M=579μV, SE=99.4μV) had significantly larger LLRs than the LLR- individuals (M=102μV, SE=33.8μV), with a Bonferroni adjusted *p-value* of 0.007. LLR+ individuals (M=383μV, SE=65.3μV) were not different from either controls (p_{adj}=0.30) or LLR- individuals (p_{adj}=0.16). Regarding LLR area, there were no differences between controls (M=4436μV*ms, SE=795μV*ms) and LLR+ individuals (M=2560μV*ms, SE=623μV*ms), $t(8.9)$=1.86, p=0.95.

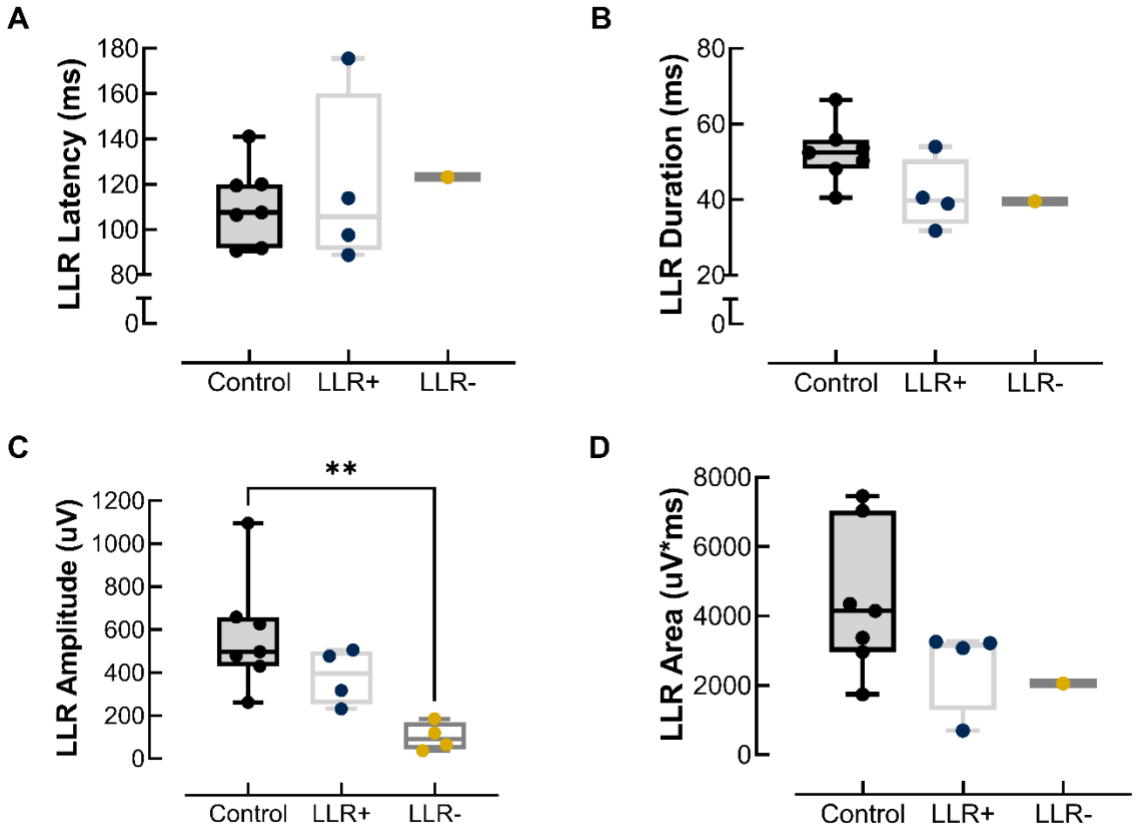

Figure 4.1. LLR characteristics do not differ between LLR+ individuals and controls, but LLR amplitude is lower in LLR- individuals than healthy controls. A) LLR latency, or time of onset after movement in milliseconds (ms). **B)** Duration of LLR in ms. **C)** Peak-to-peak amplitude of the LLR response, in μV. **D)** Area under the curve of LLR response, in μV*ms. Statistical comparisons for plots A, B, and D were only made between control and LLR+ groups due to the presence of only one LLR- individual with any distinct responses for those metrics. All boxplots represent median ± range. ** indicates significance at a level of $p<0.01$.

CST Efficacy

Stimulation intensity was planned to be 110% TA aMT. We assessed aMT during both dorsiflexion and plantarflexion because we wanted to ensure that MEPs were present during our plantarflexion task. We could not determine rMTs or aMTs for one participant, so they were stimulated at 100% maximum stimulator output (MSO). One

participant did not tolerate the calculated stimulus intensity of 99% MSO, so their testing was conducted at 95% MSO, which was 105% of their TA aMT. Stimulation intensities were 53±3% MSO (mean ± standard error) for the Control group, 44±6% MSO for the LLR+ group, and 77±15% MSO for the LLR- group, with no statistically significant differences across groups.

MEP data were analyzed for test leg MG, SOL, and TA during static 10-20% MVC contractions, as shown in Figure 4.2. We assessed two-factor mixed effects ANOVAs (Status*Muscle) for each of MEP latency, amplitude, and area. There was no main effect of Status on LLR latency, $F(2,8)=3.34$, $p=0.088$, $\omega^2=0.34$. There was also no main effect of Muscle on MEP latency, $F(2,18)=2.77$, $p=0.089$, $\omega^2=0.23$, or a significant Status*Muscle interaction, $F(4,18)=0.086$, $p=0.99$, $\omega^2=0.014$. There were also no significant effects on MEP amplitude (Status: $F(2,8)=2.48$, $p=0.15$, $\omega^2=0.37$; Muscle: $F(2,18)=0.722$, $p=0.50$, $\omega^2=0.06$; Status*Muscle: $F(4,18)=1.01$, $p=0.43$, $\omega^2=0.17$). The same pattern of no difference is observed in MEP area (Status: $F(2,8)=2.58$, $p=0.14$, $\omega^2=0.38$; Muscle: $F(2,18)=0.92$, $p=0.42$, $\omega^2=0.075$; Status*Muscle: $F(4,18)=1.12$, $p=0.38$, $\omega^2=0.18$).

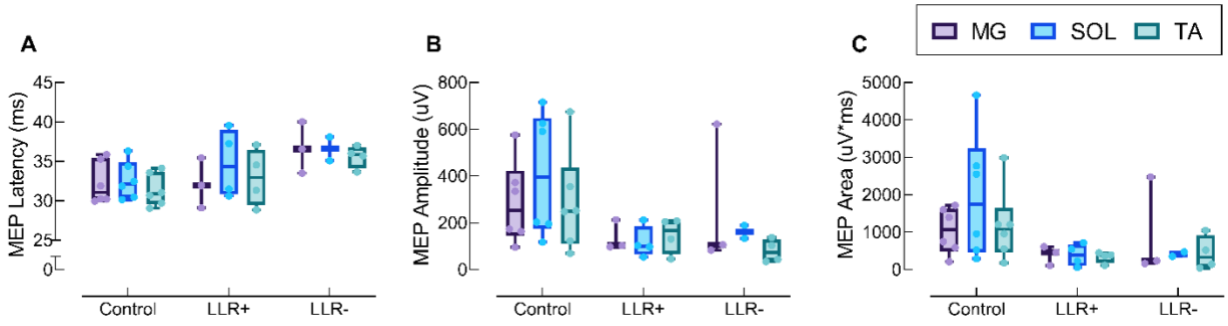

Figure 4.2. There are no statistical differences in MEP latency (A), amplitude (B), or area (C) between groups or muscles assessed. A) There are no statistically significant differences across LLR status or muscle (*medial gastrocnemius (MG):* purple; *soleus (SOL):* blue; *tibialis anterior (TA)*: green). **B)**

MEP amplitude and **C)** MEP area have high variability within the healthy control and LLR- groups. Boxplots illustrate full range of data, with points displaying individual subjects. All subjects are present in TA plots, but two subjects (one LLR+, one LLR-) are missing from MG plots and two subjects (both LLR-) are missing from SOL plots due to lack of measurable MEPs at the active stimulation intensity.

Dynamic Facilitation of LLRs

Figure 4.3 depicts responses across the different experimental conditions for three representative individuals. As a group, all subjects exhibited facilitation of LLR responses that exceeded the sum of the MEP and LLR (Figure 4.4). We computed a series of one sample t-tests to test whether facilitation was greater than 0% within the whole sample and by group. In healthy individuals and LLR+ individuals, the 0ms delay condition (i.e., TMS arriving at the same time as the LLR latency) was significantly greater than 0%, M=97.8%, SE=35.1%, $t(9)$=2.79, p=0.011. We also compared the state in which each participant achieved maximum facilitation to see if the sample and group means were greater than 0%. The overall sample statistics are as follows: M=178.7%, SE=36.1%, $t(13)$=4.96, p=0.0001. The control group achieved significant facilitation (M=126.6%, SE=27.4%, $t(5)$=4.63, p=0.003), while the LLR+ (M=202.6%, SE=85.4%, $t(3)$=2.83, p=0.049) and LLR- groups (M=232.6%, SE=89.7%, $t(3)$=2.59, p=0.04) are not significantly different from 0% when correcting for multiple comparisons (α=0.0125).

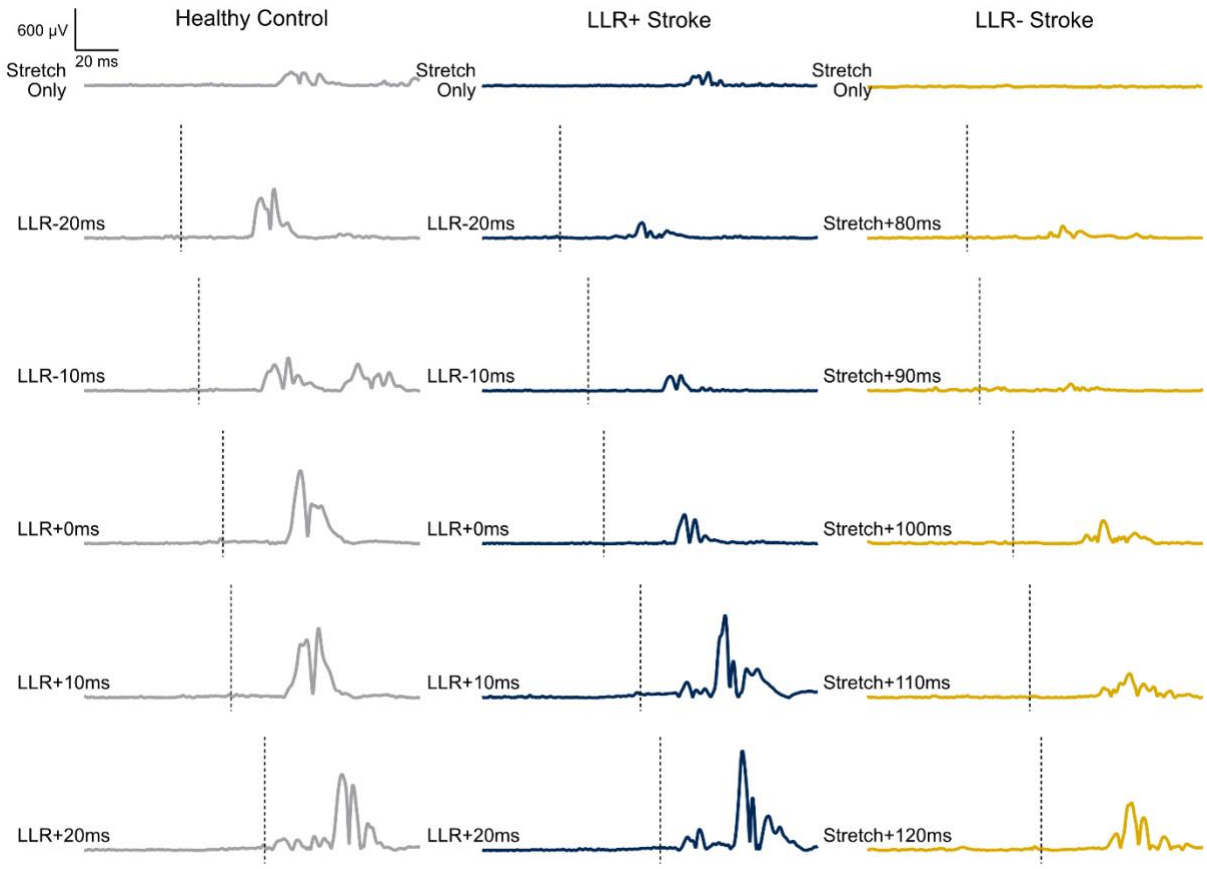

Figure 4.3. Responses to the TMS+LLR paradigm from three representative subjects. Each image starts at the time of stretch, and trials with transcranial magnetic stimulation (TMS) included have a dotted line marking the time of stimulation. A representative healthy control is depicted in the left-hand column in grey, an individual with stroke classified as long-latency response present (LLR+) is depicted in the center panel in navy blue, and an individual classified as LLR- is depicted in the right-hand column in gold. The top row of graphs shows the LLR in response to stretch alone. The five rows that follow depict stimulations occurring at 10ms intervals from before the expected LLR arrival (LLR-20ms and LLR-10ms), at the time of LLR onset (LLR+0ms), and in 10ms windows after the LLR (LLR+10ms and LLR+20ms) In the Healthy Control and LLR+ individual, there is a facilitation of the response greater than the sum of the

Stretch Only and the MEP alone (seen in LLR-20ms row), occurring in the time intervals after LLR onset.

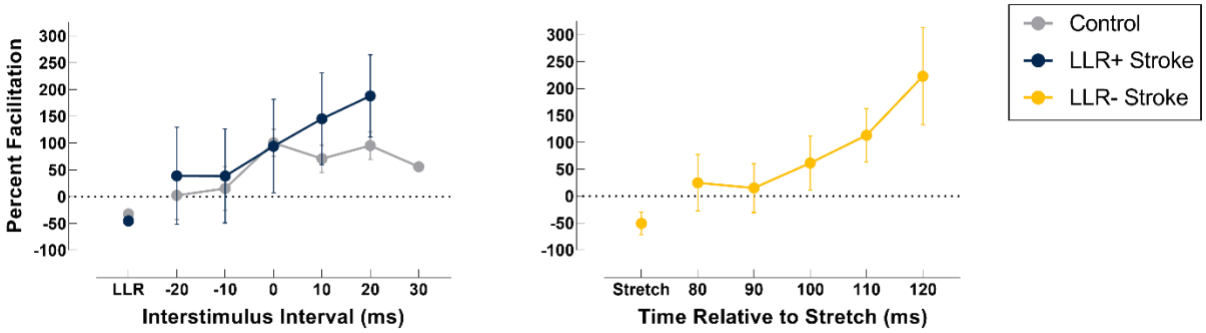

Figure 4.4. All groups achieved facilitation when TMS was timed to coincide with LLRs. The y-axis represents percent facilitation of the evoked response relative to the sum of the separate LLR and MEP responses. The x-axis of the left graph represents time between LLR and MEP arrival, where negative intervals are MEPs arriving before the LLR, 0ms is a simultaneously timed response, and positive intervals indicate MEPs arriving after LLR onset. Controls are shown in grey and LLR+ individuals with chronic stroke are dark blue. The right graph shows LLR- individuals with chronic stroke. Because they do not have LLRs to time to stimulation, the x-axis for this plot shows the interval between stretch and MEP arrival in milliseconds (ms). Values are mean ± standard error.

In the two-way Status*Timing mixed effects ANOVA, there was a significant main effect of TMS Timing relative to LLR latency, $F(5,618)=64.14$, $p<0.001$, $\omega^2=0.33$. Tukey HSD *post-hoc* tests indicate that nearly every pairing was significantly different. The most notable differences were that all TMS conditions were significantly greater than the LLR condition alone. The later time intervals (i.e., LLR+TMS differences of 0, 10, and 20ms in the individuals with measurable LLRs and time points 100, 110, and 120ms after stretch in LLR- individuals) were significantly greater than the early time intervals (i.e., -20, -10ms for LLR+ and 80, 90ms for LLR-). All p-values for the reported differences were less than 0.01. There was no significant main effect of LLR status (i.e., Control/LLR+/LLR-) when

controlling for subject, $F(2,6)=0.134$, $p=0.88$, $\omega^2=0.036$. There was a significant interaction between LLR Status and TMS Timing on facilitation, $F(10,618)=4.35$, $p<0.001$, $\omega^2=0.044$, indicating that each group revealed facilitation at various TMS timings. Individuals with stroke revealed generally greater magnitudes of facilitation in the later time intervals relative to the earlier time intervals than healthy controls.

Figure 4.5 shows the same data as in Figure 4.4, but at an individual subject level. All healthy individuals show some amount of facilitation, but the size of the effect varies. One healthy individual has data ranging in ISIs from -10 to 30 due to a technical error that added a timing delay of 15ms rather than the standard 5ms used in the rest of the sample. In the LLR+ sample, two individuals have responses with the same shape as the control group. One individual, LLR_14, has facilitation at all LLR+TMS intervals. The remaining LLR+ individual, LLR_05, did not reveal facilitation. Despite lacking LLRs, three of the four LLR- individuals reveal patterns of facilitation. The fourth LLR- individual did not have a measurable MEP threshold, even when stimulating at 100% stimulator output.

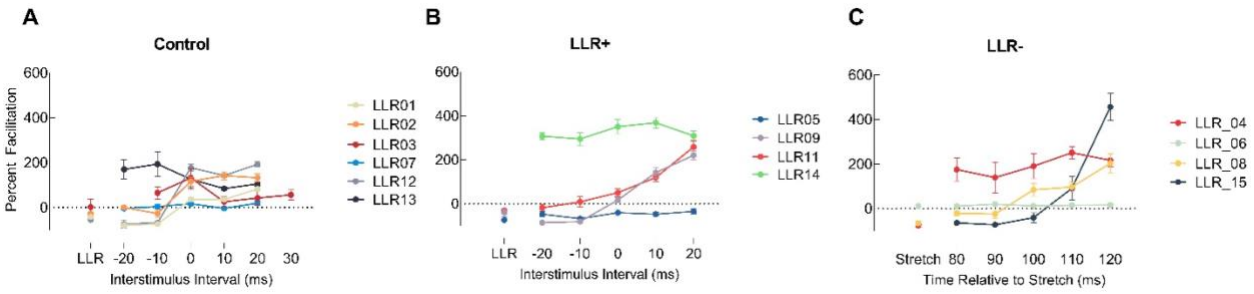

Figure 4.5. Individual subject data show variation in degree of facilitation both within and across groups. Most individuals show some degree of facilitation in the later time intervals, however one LLR+ individual and one LLR- individual do not. Healthy controls are shows in the left plot **(A)**, LLR+ individuals in the center **(B)**, and LLR- individuals on the right **(C)**. Data are mean ± standard error across trials.

Clinical and Functional Tests

Broadly, the LLR- group included the lowest functioning individuals in our study sample (Figure 4.6). The LLR- individuals (*Mdn*=25) showed more impairment on the FMA lower extremity motor sub-score (*Mdn*=33), *T*=15, *p*=0.03. Kruskal-Wallis ANOVA revealed significant differences in SPPB score, *H*(2)=11.2, *p*=0.004, η^2=0.61. Pairwise Wilcoxon rank sum *post-hoc* testing revealed that the healthy control group (*Mdn*=12) was significantly different from the LLR- group (*Mdn*=6.5), with a Bonferroni-corrected p-value of 0.02. There were no differences between the LLR+ group (*Mdn*=12) and either of the other two groups. Kruskal-Wallis ANOVA of DGI score also revealed significant differences, *H*(2)=9.37, *p*=0.009, η^2=0.61. Pairwise Wilcoxon rank sum *post-hoc* tests revealed higher DGI scores in healthy individuals (*Mdn*=24) than LLR- individuals (*Mdn*=14.5), with a Bonferroni-corrected p-value of 0.03. The LLR+ group (*Mdn*=23.5) was not different from either the control or LLR- groups.

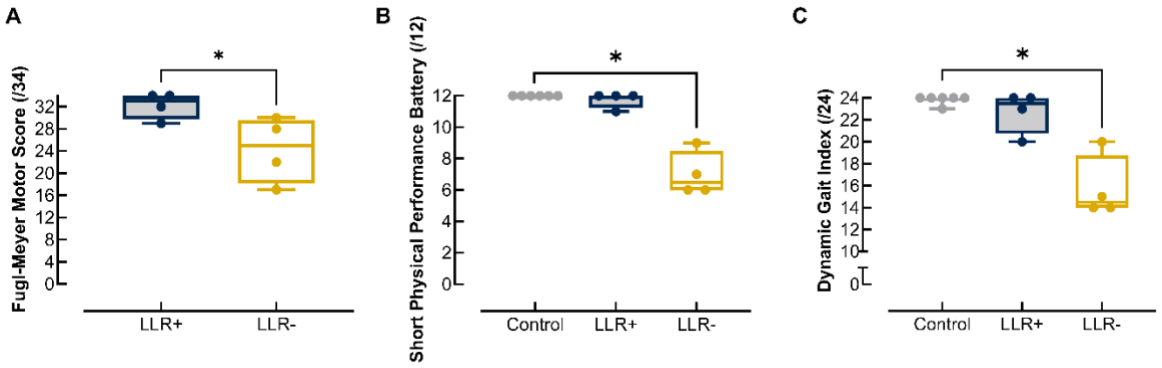

Figure 4.6. LLR- individuals consistently score lower on clinical tests of impairment and function. **A)** Fugl-Meyer Assessment lower extremity motor sub-score shows greater impairment in LLR- individuals than LLR+ individuals. **B)** Short Physical Performance Battery score is significantly lower in LLR- individuals than healthy controls, who all received maximal scores on this functional test. **C)**

Dynamic Gait Index score is also significantly lower in LLR- individuals than healthy individuals. Boxplots show full range of data. * indicates significance at $p<0.05$.

Walking speeds were significantly different across LLR Status (Figure 4.7). Kruskal-Wallis ANOVA revealed differences in SSWS by Status, $H(2)=8.33$, $p=0.015$, $\eta^2=0.53$. Pairwise Wilcoxon rank sum *post-hoc* testing revealed that healthy controls (*Mdn*=1.28m/s) had faster self-selected speeds than LLR- individuals (*Mdn*=0.39m/s), $p_{adj}=0.018$. The LLR+ group (*Mdn*=1.22m/s) was not different from either the control group ($p_{adj}=1.0$) or the LLR- group ($p_{adj}=0.086$). FCWS also varied by LLR Status, $H(2)=9.21$, $p=0.01$, $\eta^2=0.60$. Wilcoxon rank sum *post-hoc* testing revealed that healthy controls (*Mdn*=1.92m/s) had faster fastest comfortable speeds than the LLR- group (*Mdn*=0.47m/s), $p_{adj}=0.018$. The LLR+ group (*Mdn*=1.55m/s) was not different from either the control group ($p_{adj}=1.0$) or the LLR- group ($p_{adj}=0.086$).

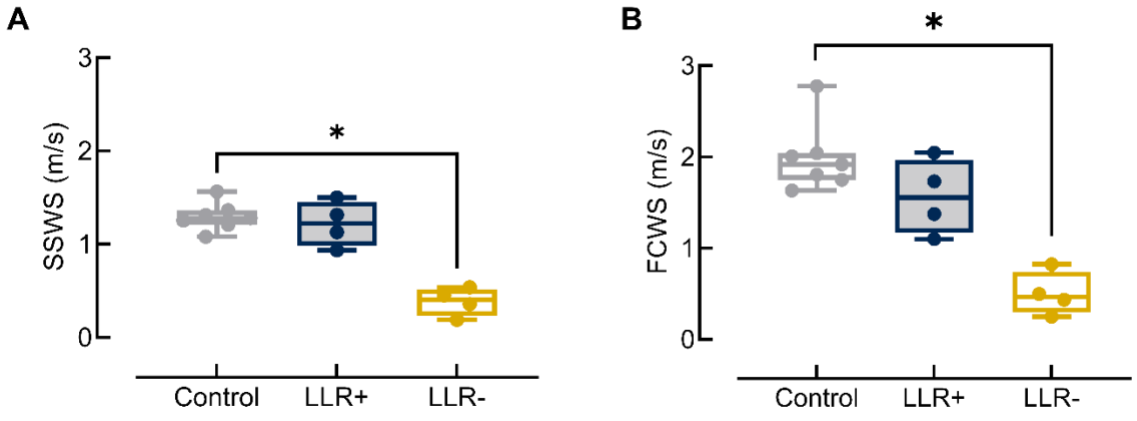

Figure 4.7. LLR- individuals have slower walking speeds than healthy controls or LLR+ individuals. A) Self-selected walking speed (SSWS) is significantly slower in LLR- individuals than healthy controls. **B)** Fastest comfortable walking speed (FCWS) is significantly slower in the LLR- group than either the LLR+ or control groups. LLR+ individuals are not significantly different from healthy

individuals at either speed. Walking speeds were measured overground on an instrumented walkway with assistive device use as needed. Boxplots represent full range of data. * indicates significance at $p<0.05$.

Discussion

The goal of this study was to confirm that the LLRs observed in Chapter 3 had a cortical component; these data convincingly confirm that argument for both healthy adults and LLR+ individuals with chronic stroke. This result is consistent with findings in the upper (51,144) and lower extremities (43,162) in healthy individuals. Our isolated plantarflexion task was designed to be relevant to plantarflexor torque production during the late stance phase of gait, just prior to the foot leaving the ground, because this propulsive phase is known to be important for capacity to increase walking speed after stroke (111). There is also evidence suggesting that LLRs are present if the ankle is rapidly dorsiflexed during the stance phase of gait in healthy individuals (155). The authors suggest that the LLR could serve a protective role in response to ground instability. This stretch speed also appears sufficient for cortical involvement, despite being relatively slow compared to other protocols discussed in the literature, and to maximal angular velocity of the ankle during gait. In addition to what the literature signifies about healthy individuals, our data confirm that the TA LLR involves cortical structures in individuals with stroke, and the lesion and/or secondary corticospinal damage may contribute to loss of LLRs.

Our secondary study aim was to characterize the LLR and its relationship to post-stroke motor function. The LLR itself did not differ in timing or size between healthy individuals and LLR+ individuals. MEP characteristics also did not differ statistically between groups. This suggests that the LLRs we measured in people with chronic stroke

are physiologically the same as those found in healthy age-matched individuals, thus this group of participants has a patent transcortical reflex pathway. It is also promising to note that brain stimulation is not necessary to elicit this potential biomarker, making the LLR more clinically accessible. Additionally, the lack of statistical difference in MEP characteristics suggests that motor dysfunction is not the only contributor to LLR absence. The effect sizes suggest that a larger sample may show differences in MEP latency between controls and the LLR- group. As there was only one individual without MEPs in this study, these results cannot be generalized to MEP absent individuals, though it would be reasonable to hypothesize that a level of CST dysfunction to the extent of MEP absence could contribute to LLR absence in that subset of the post-stroke population. The confirmation that LLR- individuals are clinically lower-functioning in a second sample validates that there is a behavioral consequence of LLR dysfunction, the underlying mechanism of which is still unknown. Taken together, this points toward a model that requires consideration of the sensory and integratory portions of the reflex pathway, as well as the motor portion, to gain understanding regarding the mechanism of LLR absence.

The unexpected finding of this study is observation of MEP facilitation in the later time intervals after stretch in the LLR- group. These time intervals aligned with when the LLR is expected to occur if one were present (100-120ms after stretch). The magnitude of the facilitation is related to the size of the original response being smaller than what was observed in healthy individuals, and thus may be exaggerated, but even when presented in native units is a remarkable amount of facilitation and in line with similar studies in the literature (43,155). It appears that most of these LLR- individuals do retain

some residual neural substrate necessary to elicit a response. There are several potential mechanisms for response absence that can be facilitated in the presence of suprathreshold TMS, including an insufficient afferent signal from stretch alone, impaired sensorimotor integration, and difficulty synchronizing response output. Although this relatively slow stretch was sufficient to produce an LLR in healthy individuals and half of our sample with chronic stroke, increasing the input signal gain by increasing the background contraction and/or stretch speed should increase LLR amplitude (57,58). It remains unclear whether faster, more powerful stretches could elicit LLRs in the individuals we classified here as LLR-, since they do reveal facilitation of evoked responses with the addition of the large motor cortex stimulus provided by suprathreshold TMS. Cortical sensorimotor integration is a unique feature to the transcortical reflex relative to segmental reflexes, providing the opportunity for sensorimotor influences such as vision or perceptual set to modulate the response (59). It is reasonably accepted that sensorimotor integration is known to be impaired in some individuals with chronic stroke (163,164). Finally, motor responses must be highly synchronized to be reliably observed in EMG. Our data suggest it is possible that the motor cortex in these LLR- individuals does produce some level of response, but it is insufficient to produce a synchronized response in the absence of additional excitatory input like the TMS we provided in our experiment. Further research is needed to explore each of these proposed mechanisms in depth.

It is worth noting that this is a small, relatively high-functioning group of individuals with chronic stroke. This was a convenience sample drawn mostly from individuals receiving follow-up care from a comprehensive stroke center. When comparing sample

populations between this study and Chapter 3, this sample is a younger, more diverse group of individuals reasonably matched in chronicity. Lesion etiology and location were also more varied in the current sample, with half of the participants having hemorrhagic or embolic strokes. Many clinical gait studies limit enrollment to ischemic stroke (10). Taken together, it is promising that the main findings of Chapter 3 hold in this more diverse sample, but caution should be taken when generalizing results into the earlier phases after stroke or into non-ambulatory individuals at this time. It is currently unknown whether LLR absence after stroke is part of the initial stroke sequelae or develops over time. Further research is needed to determine both the time course and impact of spontaneous recovery on transcortical reflexes.

Overall, the results of this study provide further evidence that LLR presence or absence may be a promising physiologic marker of lower extremity function following stroke. Once again, we found unambiguous differences among individuals that were functionally relevant in terms of motor impairment, and we showed that our paradigm reliably evokes LLRs. We then confirmed that these LLRs include a cortical component. The transcortical reflex serves as a first line of defense to rapid external perturbations, allowing the ankle joint to stabilize when we encounter challenging situations in our physical environment, particularly during walking (155). Absence of this response is associated with slower, more dysfunctional walking patterns and potentially puts individuals at greater risk of falls. This marker could be a clinically useful indicator of individuals who need targeted rehabilitation of ankle stability and mitigation of fall risk if they wish to become safer community ambulators. It is currently unknown whether the transcortical reflex is mutable after stroke, and if upregulating the reflex would lead to

improvements in walking function, but the finding that this LLR- group appears to have residual substrate is a positive indicator of rehabilitation potential in these individuals. The underlying mechanism of this reflex absence also requires further investigation, especially in the aspects of sensory function and sensorimotor integration.

Chapter 5: Influence of Sensorimotor Function on LLRs

Introduction

Somatosensory deficits are poorly characterized following stroke and are often not the focus of conventional rehabilitation. In one study of 95 individuals in the subacute phase after unilateral hemispheric stroke, at least 67% of individuals had measurable somatosensory deficits (80). Another study of individuals with first ever stroke in the subacute phase that distinguished both between body region and type of somatosensory impairment reported that 63% had impaired proprioception at the ankle and 31% had impaired or absent light touch sensation in the paretic lower extremity (165). Routine clinical examination often involves assessment of light touch and proprioception, and sometimes involves vibration sense (166). Although more than 50% of clinicians agree that somatosensory assessment is relevant to a patient's prognosis, fewer agree that test results are relevant to treatment planning. Somatosensation is important to motor function for providing information that can be used in movement planning and execution, as well as feedback of motor actions (167). Despite this well-accepted role of somatosensation in motor behavior, much knowledge has yet to be gained regarding sensory rehabilitation strategies and their effect on motor recovery (164).

Vibration, light touch, and proprioception all travel along the dorsal column, medial lemniscal tract, synapsing in the thalamus and primary somatosensory cortex (82). However, there are also distinct features present across modalities. Vibration and proprioception are dynamic senses that involve both cutaneous and muscle- or joint-level receptors and afferents (168). Conversely, light touch is a static, cutaneous sensation (82). Various features of each sensation, including receptor type and firing rate, are

encoded and travel within closely localized but modality-specific pathways through the spinal cord, brainstem, thalamus, and into the primary and secondary somatosensory cortices. In patients with stroke, we would not typically expect dysfunction to occur at the peripheral or spinal level, but there are various supraspinal areas where a lesion could impact somatosensory function either directly or indirectly through network changes (169,170). It is advantageous to assess multiple modalities so that mechanisms of sensory dysfunction after stroke can be better understood at both the individual and group levels.

There is a need for better markers of lower extremity dysfunction following stroke, and these markers need to assess both motor and sensory function. The long-latency reflex (LLR) is a candidate marker because, as shown in Chapters 3 and 4, it provides unambiguous differentiation between higher- and lower-functioning individuals (171). As with any oligosynaptic central nervous system reflex, there are sensory, motor, and integratory portions to the LLR (for schematic, see Figure 1.1). In Chapter 4 we showed that corticospinal tract (CST) efficacy, a probe of motor system function, was unlikely to be the sole contributor to LLR absence in some individuals with chronic stroke. Here, we will assess somatosensory function through a series of experiments in individuals with chronic stroke and healthy individuals. We will then further characterize the relationship between LLR status and somatosensory function in subsequent analyses.

The goals of these experiments are to: 1) determine whether vibratory assessment can classify differences between individuals with chronic stroke and age-matched healthy adults; 2) to investigate the relationship between vibratory function and LLR status; and

3) to further characterize LLR absent individuals by assessing light touch and proprioception.

Methods

Subjects

This study is a retrospective analysis of all sensory data collected from lower extremity studies that occurred in our lab between 2014 and 2022. Experiment 1 is our largest sample, with 38 individuals with stroke and 28 healthy controls. Experiment 2 contains the subset of Experiment 1 subjects that had been classified as LLR+ or LLR-, 22 individuals with stroke and 18 healthy controls. Experiment 3 is the further subset of individuals with light touch and proprioception data available, 8 individuals with stroke and 7 healthy age-matched adults. Study demographics for each experiment are provided in Table 5.1. There are individuals represented that appear in more than one experiment within this chapter. Individuals with stroke all had unilateral, supratentorial stroke at least six months prior to study enrollment. Healthy individuals were included if they were free of any orthopedic or neurologic impairment that could affect walking or lower extremity function on the side being studied.

Table 5.1. Demographic information for the three experiments.

	Experiment 1		Experiment 2		Experiment 3	
	Control	Stroke	Control	Stroke	Control	Stroke
Demographics						
n	28	38	18	22	7	8
Sex (m/f)	10/18	30/8	9/9	18/4	3/4	6/2
Race (white/Black/Asian/not stated)	24/2/2/0	31/4/1/2	15/1/2/0	17/3/1/1	7/0/0/0	5/1/1/1
Ethnicity (Hispanic or Latino/NH/not stated)	0/26/2	1/32/5	0/17/1	1/20/1	0/7/0	1/6/1
Age (yrs)	62±11	63±11	59±11	61±13	56±14	56±18
Chronicity* (yrs)	---	6.4±4.5	---	6.5±4.8	---	6.5±3.3
Lesion location (cortical/subcortical/mixed)	---	7/16/15	---	5/8/9	---	2/2/4
Paretic/test leg (r/l)	16/12	21/17	9/9	12/10	2/5	3/5
Self-selected walking speed (m/s)	1.27±0.22	0.77±0.37	1.32±0.17	0.82±0.42	1.3±0.15	0.80±0.49

*Date of stroke is unknown for one participant. Data are presented as mean ± standard deviation when applicable. *n* number of subjects, *m* male, *f* female, *NHL* not Hispanic or Latino, *yrs* years, *r* right, *l* left, *m/s* meters per second

Experimental procedures were approved by either the University of Florida Health Science Center Institutional Review Board (IRB-01) or the University of California, Davis Institutional Review Board, depending on study site. Testing occurred at the Brain Rehabilitation Research Center in the Malcom Randall VA Medical Center in Gainesville, FL or at the Biomechanics, Rehabilitation, and Integrative Neuroscience (BRaIN) Lab in Davis, CA, and spanned 1-3 days for each participant. The MoCA was used to screen for ability to follow three-step commands and provide informed consent. Testing was conducted in accordance with the Declaration of Helsinki.

Instrumentation

Vibratory sensation was assessed using a Bio-Thesiometer (Bio-Medical Instrument Co., Newbury, OH, USA). The handheld device has a 13mm diameter plastic probe that vibrates at 120 Hz, or two times the AC frequency. The amplitude of the vibration is adjusted by increasing the voltage setting on the device, and thresholds are recorded in units of volts. The device manufacturer provides a calibration table for each unit to convert thresholds from volts to microns (μm). The device is portable and easy to use and provides more objectivity than assessing vibratory sensation using a tuning fork.

Light touch sensation was assessed using the Aesthesio® Precise Tactile Sensory Evaluator 20 Piece Kit (DanMic Global, LLC, San Jose, CA, USA). The evaluators are nylon Semmes-Weinstein monofilaments of varying lengths and diameters that are calibrated to bend when specific forces are applied. This kit contains evaluators ranging from 1.65 (0.008g target force) to 6.65 (300g), on a logarithmic scale. Logarithmic increases in force are used because participants sense these logarithmic increases as linear (172).

Proprioception was assessed by a joint position sense protocol built into the Biodex System 4 Pro Isokinetic Dynamometer (Shirley, NY, USA). The Combination Ankle Attachment allows approximately 50 degrees of plantarflexion and 50 degrees of dorsiflexion if the ankle neutral position aligns with the footplate perpendicular to the motion arm. Position matching is measured in degrees, and average position and average error (i.e., |target position – actual position|) are reported by the device in one-degree increments. The experimenters observe that our device appears to conduct the protocol within ±1deg of the intended position throughout testing.

Dynamic isolated plantarflexion was assessed using a Biodex Isokinetic Dynamometer, either a System 3.2 or a System 4 Pro™ (Biodex, Shirley, NY, USA). Surface EMG was collected from the medial gastrocnemius (MG), soleus (SOL), and tibialis anterior (TA) muscles of both legs using Ag/AgCl gel surface electrodes (Conmed Cleartrace^2, Utica, NY, USA). Electrodes were trimmed to accommodate a 20mm interelectrode distance and placed according to SENIAM guidelines (127). EMG was acquired using a Motion Lab Systems MA-420 system with snap-on preamplifiers (Motion Lab Systems, Baton Rouge, LA, USA). Data were streamed at 2000Hz through a Power1401 data acquisition system and experimental control provided by custom written Signal scripts (v6 or v7, Cambridge Electronic Design, Cambridge, England).

Sensory Testing Protocol

Vibratory sensation and light touch sensation were both assessed with the participant lying supine on a plinth and their shoe and sock removed from the test leg. After familiarization with each test, participants were instructed to close their eyes and focus on the testing location, which was announced and palpated by the evaluator each

time a new location was used. Number of personnel and background noise were minimized to allow for participant focus. Both sensory tests were evaluated at the following locations: hallux (dorsomedial aspect of first metatarsal); heel of foot (calcaneal tuberosity, plantar side); medial malleolus; and proximal tibia (2 fingers distal to inferior patellar border). Each location was evaluated three times to ensure consistency. The same experimenter [CLB] served as the primary evaluator for each of the sensory tests across all participants.

For vibratory sensation testing, the Biothesiometer probe was held against each landmark by the primary evaluator with enough pressure to maintain contact. For VPT, a second evaluator adjusted the voltage knob on the device slowly, approximately 2 V/s, until the participant indicated verbally that they could feel the vibration. The device was turned down to 0 V between each trial. After three VPT trials, the primary evaluator kept the probe on the same location but switched to VDT assessment. For VDT, the second evaluator turned the knob to the highest setting (50 V) and slowly worked down at a rate of approximately 2 V/s until the participant indicated they could no longer feel the vibration. Again, the device was turned down to 0 V between each trial. After three VDT trials, the primary evaluator moved to the next testing location.

For light touch sensation assessment, evaluation began with the median normative value published by Plucknette et al. within one of three age groups: 3.61 (0.4 g) for ages 18-34, 4.31 (2 g) for 35-64, and 4.74 (6 g) for 65 and older (103). As indicated in the device instructions, the primary evaluator would touch the landmark with precisely enough force to make the filament start to bend, and then remove the filament smoothly at a rate approximately equal to filament application. A verbal response indicating detection of

sensation in any one of the three attempts corresponded to a positive detection. If participants could sense the starting filament, testing progressed to the next lighter filament in order until the participant could no longer feel filament. The target force of the lightest filament detected was labeled the sensory threshold. For individuals that could not sense the starting filament, we jumped up to the 5.07 (10g) filament, the clinically accepted threshold filament for protective sensation in the foot (102), and continued down to the lightest filament that could be sensed. If participants could not sense the 5.07 filament, we serially increased filament size until a threshold was detected.

We assessed proprioception by evaluating passive joint position sense on a Biodex System 4 Pro dynamometer (Shirley, NY). Participants were seated with approximately 90 degrees (deg) hip flexion and 30 deg knee flexion, and their paretic or test foot strapped to the dynamometer foot plate. The Biodex built-in Passive Position Sense protocol was used, with a starting position at neutral and moving to each of three different target positions: 15 deg plantarflexion, 5 deg plantarflexion, and 5 deg dorsiflexion. The 5 deg positions were selected because 5 deg of dorsiflexion was known to be achievable by most individuals with chronic stroke from previous studies, and the 5 deg plantarflexion target mirrored that achievable target. The 15 deg plantarflexion target was selected to give a target under the typical end range of plantarflexion, but some distance removed from 5 deg. At the start of each trial, participants sat with their eyes closed while the experimenter moved their ankle from the starting position to the target position, held the target position for 10 seconds, then moved back to the starting position. At this point, the experimenter handed the hold/release trigger button to the participant. The participant pressed the hold/release button one time to allow the dynamometer to

move toward the target position at a speed of 2 deg/s, then pressed the hold/release button once more to stop the pedal when they sensed that the target had been reached. This sequence was repeated three times for each target position, with a 10 sec rest break within a target position and a 30 sec break between targets.

Study Variables

LLR presence/absence was recorded for each participant, as analyzed in Chapters 3 and 4. Briefly, a participant was considered LLR present (LLR+) if they had measurable LLRs in at least 50% of trials, and LLR absent (LLR-) otherwise.

The vibration data were converted from Volts (V) to microns (μm) using the device calibration table. Thresholds for VPT and VDT were calculated as the average of the three trials collected within each location. The vibratory threshold (VT) was then calculated as the mean of the VPT and VDT. Data were tested for normality and lognormality using the D'Agostino-Pearson test, and found to be logarithmically distributed. Data analysis was therefore carried out on log-transformed threshold values.

Light touch sensory thresholds were converted from g to μm. Data were assessed for normality and lognormality using the D'Agostino-Pearson test and found to be logarithmically distributed, so thresholds are log-transformed for statistical analyses.

For the proprioception test, we calculated absolute error and joint position. Absolute error is the absolute value of the difference between the achieved position and the target position. We then averaged these values over the three trials conducted for each target. Since this error metric is agnostic to whether the participant over- or undershot the target, we also calculated the average position chosen by the participant across the three trials.

We sought to compare some of these sensory metrics to the most common clinical index of post-stroke impairment, the lower extremity Fugl-Meyer Assessment. Here, we evaluated both the total score (out of 100 possible points) and the sensory sub-score (out of 12). Lower scores indicate more impairment. The sensory sub-score evaluates light touch sensation on the plantar surface of the foot and leg, and proprioception at the hip, knee, ankle, and great toe.

Statistical Analysis

For Experiment 1, we assessed the ability of vibratory thresholds to predict whether an individual was healthy or had experienced a stroke. This was done via logistic regression (173). Due to high correlation between varying threshold types, we computed three models, one for each of VPT, VDT, and VT. Initially, a hierarchical regression was carried out with three candidate models: 1) all main effects (threshold for each of the four locations evaluated); 2) main effects plus all two-way interactions; and 3) main effects with the addition of age as a predictor.

For Experiment 2 the sample size was not large enough for a multivariate logistic regression, so we conducted ANOVAs on vibratory thresholds at each location. All thresholds were significantly non-normal, as evaluated by the Shapiro-Wilk normality test (p's>0.05). The data were then log-transformed using a base 10 logarithm, as is a convention in sensory literature (104,174). The log-transformed data subsequently passed the Shapiro-Wilk normality test. Data were then evaluated for homogeneity of variance using Levene's test, and all values were similar across groups (p's>0.05). Sensory thresholds were compared by LLR Status (Control/LLR+/LLR-) using separate one-way ANOVAs with Tukey's HSD *post-hoc* tests for significant main effects. The p-

values were adjusted for multiple comparisons using a Bonferroni correction. Omega (ω) effect sizes were reported for each ANOVA and standardized mean differences (d) for the *post-hoc* tests. For the Fugl-Meyer sensory score data, the LLR+ and LLR- groups were compared using a Wilcoxon Signed Rank test. All tests were evaluated for significance at α=0.05.

Experiment 3 also used separate one-way ANOVAs to evaluate fine touch thresholds. As with Experiment 2, the data required logarithmic transformation to pass the Shapiro-Wilk normality test. The variance was similar across all groups, as evaluated by Levene's test. One-way ANOVAs with Tukey's HSD *post-hoc* tests were applied, when appropriate. For the proprioception data, three of the datasets did not pass the normality test. Because the error values can be negative, log-transformation was not an option. The data passed Levene's test for homogeneity of variance. Because of the lack of normality, we conducted Kruskal-Wallis ANOVAs for both proprioception error and achieved position. Effect sizes for each comparison are reported using partial eta squared (η^2). All ANOVA p-values were Bonferroni-corrected and significance reported using α=0.05.

Data analysis was conducted using R version 4.1.2 and RStudio version 2021.09.1. R packages used include tidyverse, readxl, psych, rstatix, compute.es, and multcomp (158–161,175,176).

Results

Experiment 1

In Experiment 1, we determined whether vibratory thresholds could predict stroke status. Starting with a hierarchical model with disappearance threshold, Model 1 revealed

$\chi^2(4)=13.50$, $p=0.006$, Model 2 $\chi^2(5)=13.85$, $p=0.017$, and Model 3 $\chi^2(10)=24.82$, $p=0.006$. Since the more complex models did not increase model significance, we chose to move forward with the most interpretable model for all three thresholds (Model 1, main effects only). After running the hierarchical models, we evaluated residuals for outliers. One subject, a healthy control, was determined to be an outlier, with a studentized residual of -3.40 and notably higher sensory thresholds than all other healthy individuals. Re-running the models without the subject resulted in a higher χ^2 and better prediction accuracy, so that subject was removed from subsequent analyses.

Results of the three logistic regression models are shown in Table 5.2. VDT model characteristics include $\chi^2(4)=25.17$, $p<0.001$, and $R^2=0.31$ (Hosmer-Lemeshow), 0.35 (Cox-Snell), 0.46 (Nagelkerke). The heel, medial malleolus, and tibia were all significant predictors in the model. VPT model characteristics include $\chi^2(4)=10.50$, $p=0.03$, and $R^2=0.13$ (Hosmer-Lemeshow), 0.17 (Cox-Snell), 0.23 (Nagelkerke). There were no significant predictors in this model. VT model characteristics include $\chi^2(4)=18.01$, $p=0.001$, and $R^2=0.23$ (Hosmer-Lemeshow), 0.28 (Cox-Snell), 0.37 (Nagelkerke). Only the heel was a significant predictor in this model. VDT appears to be the most sensitive descriptor of differences between healthy individuals and individuals with stroke in this sample.

Table 5.2. Logistic regression results for each of the three threshold models.

	B (SE)	95% CI for odds ratio		
		Lower	Odds Ratio	Upper
Vibration Disappearance Threshold (VDT), n=59				
Intercept	-1.03 (0.64)			
Hallux	0.17 (0.16)	0.86	1.18	1.63
Heel	0.39 (0.15)**	1.15	1.48	2.07
Medial Malleolus	-0.60 (0.21)**	0.34	0.55	0.77
Tibia	0.32 (0.15)*	1.06	1.37	1.91
Vibration Perception Threshold (VPT), n=57				
Intercept	-0.69 (0.49)			
Hallux	0.15 (0.13)	0.91	1.16	1.55
Heel	0.19 (0.14)	0.97	1.21	1.72
Medial Malleolus	-0.11 (0.11)	0.71	0.90	1.10
Tibia	0.09 (0.07)	0.95	1.09	1.29
Vibratory Threshold (VT), n=56				
Intercept	-1.13 (0.63)			
Hallux	0.15 (0.17)	0.83	1.16	1.65
Heel	0.38 (0.18)*	1.08	1.46	2.21
Medial Malleolus	-0.34 (0.18)	0.48	0.71	0.98
Tibia	0.18 (0.13)	0.95	1.20	1.59

*p<0.05, **p <0.01

Experiment 2

Experiment 2 follows from the results of Experiment 1 but looks for an effect of LLR Status (control/LLR+/LLR-) on vibratory thresholds. Figure 5.1 shows all three thresholds and four locations in the 38-subject subset. Because Experiment 1 indicated that the most salient differences between control and stroke occurred in VDT, we performed separate one-way ANOVAs on log-transformed VDTs for the four test locations. At the hallux, despite a large effect size, there was a non-significant effect of LLR status on VDT, $F(2,34)=4.19$, $p_{adj}=0.09$, $\omega=0.15$. There was a significant effect of LLR status on heel VDT, $F(2,33)=5.21$, $p_{adj}=0.04$, $\omega=0.19$. Tukey HSD *post-hoc* tests indicated that the LLR- group had higher thresholds than the control group, $p=0.01$,

d=1.62. The LLR+ group was not significantly different from the control group (*p*=0.15, *d*=0.54) or the LLR- group (*p*=0.34, *d*=-0.59). The medial malleolus VDT did not reveal a significant effect of LLR status, $F(2,33)=1.33$, $p_{adj}=1$, $\omega=0.02$. The proximal tibia VDT was also not different by LLR status, but the effect size is in the medium range, $F(2,33)=2.21$, $p_{adj}=0.5$, $\omega=0.06$.

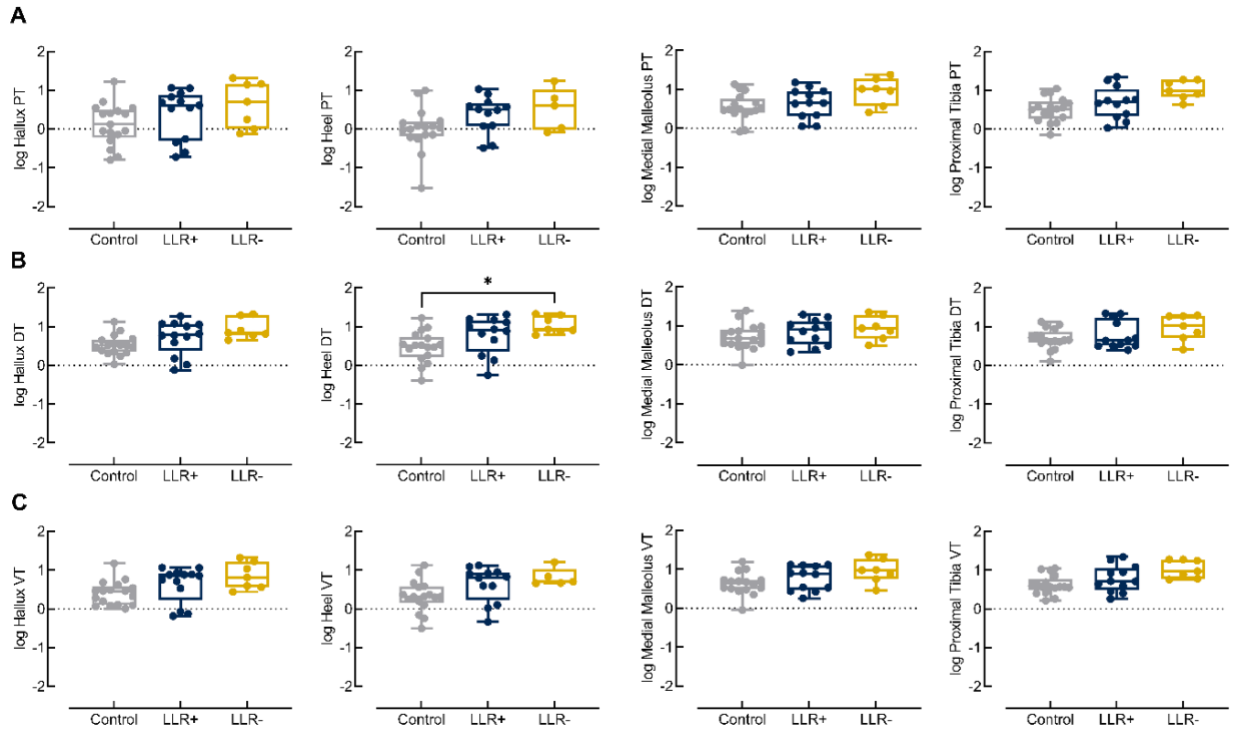

Figure 5.1. Vibratory thresholds vary across threshold type and location. Each row represents a different threshold type: **A)** top, vibration perception threshold (VPT); **B)** middle, vibration disappearance threshold (VDT); **C)** bottom, vibratory threshold (VT), which is the average of VPT and VDT. The four columns represent different testing locations, (from left to right) hallux, heel, medial malleolus, and proximal tibia. Statistical analyses were carried out on row **B)** only, where there is a significant effect of heel VDT on LLR status. Boxplots represent median and range of log-transformed threshold data. * represents difference between groups at $p_{adj}<0.05$.

We sought to characterize the extent to which the Fugl-Meyer sensory sub-score could differentiate between LLR+ and LLR- individuals (Figure 5.2). There is a ceiling effect with this test and most participants received maximal scores, but there were detectable differences between groups in a one-tailed Wilcoxon rank-sum test, W=36, p=0.02, r=0.51. If we look at the three individuals with submaximal scores, two are LLR- and one is LLR+. Additionally, two subjects could not be included in this analysis (open circles in Figure 5.2) and six others had only partial datasets due to vibratory thresholds that were too high to measure with the Biothesiometer. The two excluded subjects had sensory scores of 4 and 11. The individual with a score of 4 had peripheral neuropathy in addition to stroke, so it is not surprising that they had low sensation scores. Because of the large ceiling effect of this test, there is not much agreement between vibratory impairment and Fugl-Meyer sensory score with respect to LLR status.

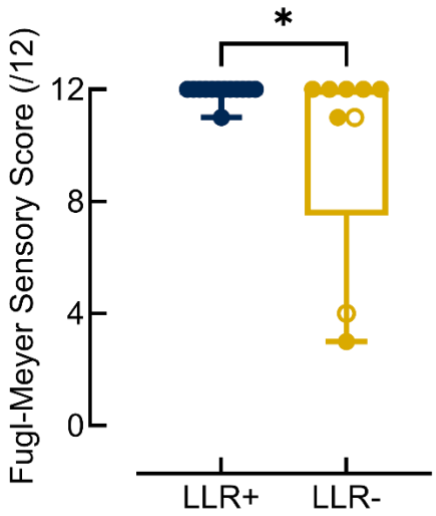

Figure 5.2. Fugl-Meyer sensory score differs by LLR status. LLR- individuals have more sensory impairment, as assessed by the Fugl-Meyer, than LLR+ individuals. The two LLR- individuals indicated by open circles were unable to be included in the vibratory sensation analysis because they had no sensory thresholds as measured by the testing device. Boxplots represent median range of data,

medians for both groups are maximal scores of 12. * represents significance difference between groups at $p<0.05$.

Experiment 3

We performed four separate one-way ANOVAs on log-transformed light touch thresholds assessed using monofilaments (Figure 5.3). For the hallux, there was no effect of LLR status on threshold, $F(2,11)=0.90$, $p_{adj}=1$, $\omega^2=-0.01$. There was also no significant relationship between LLR status and heel touch threshold, $F(2,11)=0.15$, $p_{adj}=1$, $\omega^2=-0.14$. At the medial malleolus there was not a significant effect of LLR status but the effect size for the comparison was large, $F(2,10)=0.2.19$, $p_{adj}=0.65$, $\omega=0.39$. There was a significant effect of LLR status on tibia threshold, $F(2,11)=6.74$, $p_{adj}=0.049$, $\omega^2=0.67$. Tukey HSD *post-hoc* tests revealed that the LLR- group had higher thresholds than the control group, $p<0.01$, $d=2.47$. There were no significant differences between LLR+ and control ($p=0.32$, $d=0.93$) or LLR+ and LLR- ($p=0.17$, $d=-1.43$), but the mean differences are large.

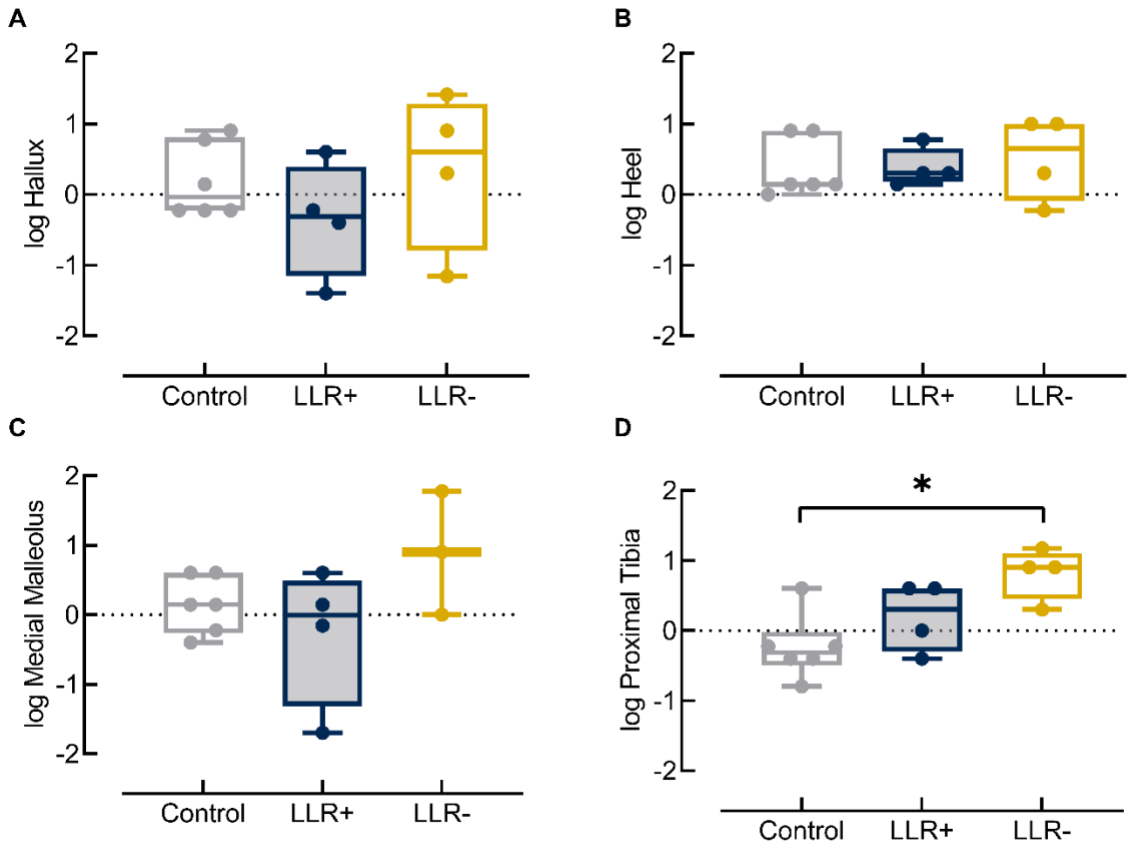

Figure 5.3. Log-transformed light touch perception thresholds. The following locations were evaluated: **A)** hallux, **B)** heel, **C)** medial malleolus, and **D)** proximal tibia. Boxplots represent median and range of data. * indicates significant group differences at p_{adj}<0.05.

For the proprioception tests, we carried out separate Kruskal-Wallis ANOVAs on the error values and joint positions for each of the three target positions (Figure 5.4). For the 15deg plantarflexion target, no one overshot the 15deg position with their estimate, so the distributions of the error and final position are equivalent ($H(2)=1.30$, $p=0.52$, η^2=-0.064). For the 5deg plantarflexion target, neither the error ($H(2)=2.0$, $p=0.37$, η^2=-0.00044) nor the position ($H(2)=0.779$, $p=0.68$, η^2=-0.11) were different across LLR Status. Finally, there were also no significant effects of LLR Status on the 5deg

dorsiflexion target error ($H(2)=1.65$, $p=0.44$, $\eta^2=-0.032$) or position ($H(2)=0.29$, $p=0.86$, $\eta^2=-0.16$). During the experiments we noted that our device would periodically adjust within ±1deg from the target or starting position, and the observed standard errors of achieved positions ranged from 0.5deg for the 5deg targets to 0.9deg for the 15deg target. Thus, we ascribe the lack of differences in these metrics to a lack of sensitivity to detect meaningful differences in the test we selected.

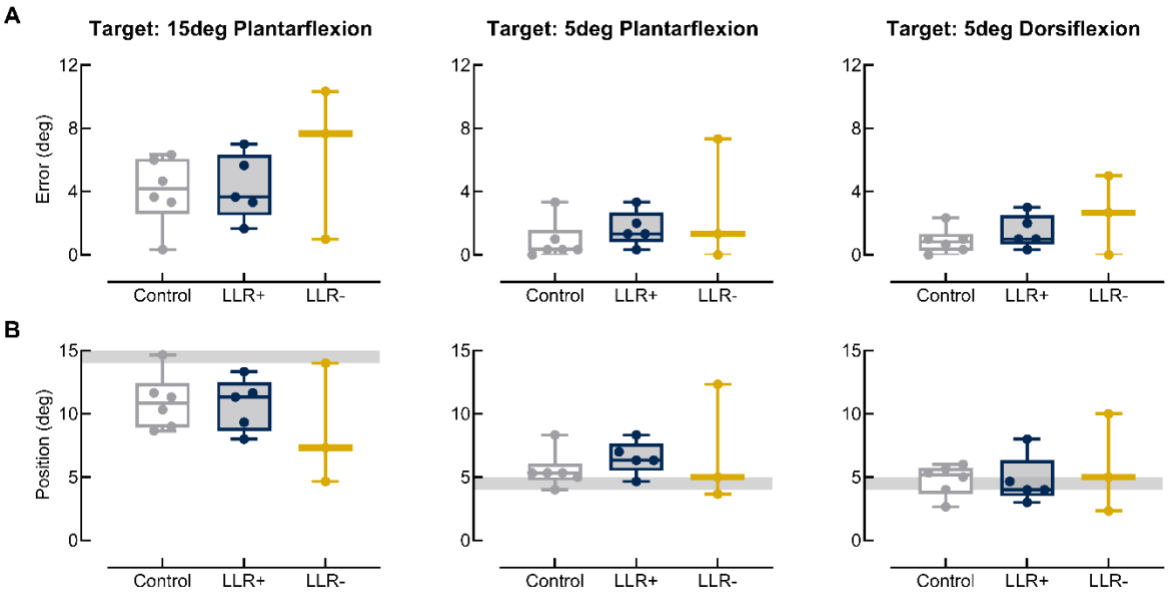

Figure 5.4. Proprioception A) errors and B) average joint angles recorded in an ipsilateral joint position matching task. All tests began at neutral and target angles included 15 degrees of plantarflexion (left column), 5 degrees of plantarflexion (middle column), and 5 degrees of dorsiflexion (right column). The gray shaded area in the bottom plots represent target angles. Boxplots represent median and range of data.

Discussion

In this set of experiments, we characterized vibratory function across a large sample of individuals with chronic stroke. We found that vibration disappearance

threshold appears to be the most effective predictor of differences between healthy individuals and individuals with stroke, but there is great heterogeneity across our sample. We further characterized the relationship between LLR status and thresholds of vibration, light touch, and proprioception. The large variance within the control datasets with these tools, coupled with the heterogeneity that is common across individuals with stroke (16), create a need for large enough samples to detect significant differences in sensation.

A difficulty that we frequently encounter within our work is participant recruitment. Despite stroke's status as the primary cause of long-term disability in adults, the pool of eligible individuals within a defined set of inclusion/exclusion criteria is finite, and the subset of individuals interested in participating in in-person neurophysiologic research is smaller still. However, one potential solution to this problem is combining datasets. Despite our most recent project's small sample of 15 individuals, by adding approximately eight minutes of testing to our lower extremity studies we were able to amass a large enough sample for logistic regression. This requires careful bookkeeping and adequate training of research personnel to be sure that procedures are consistent and data useable, but it is a viable strategy for labs that generally work with small samples. Another worthwhile strategy is collaboration between research groups with access to different populations of individuals. Future work will seek to expand sample size for the fine touch and proprioception measurements.

Vibration disappearance threshold was able to differentiate between control and stroke better than perception threshold. Not only was the VDT model stronger overall, but it had three significant test sites while the VPT model had none. Vibratory threshold fell between the two values, and the significant heel location was likely driven by VDT.

Theoretically, there should be a physiologic difference between perception of a stimulus and loss of a stimulus. When the foot leaves the ground, we lose tactile sensation as the limb swings and until the next contact. Normative data indicate that VDT is more variable than VPT (104). But, as with other types of central nervous system variability, this does not inherently mean the measure is worse, simply different. Most clinical sensory testing in stroke centers around perception. The Fugl-Meyer sensory sub-score measures perception of touch in different body locations and asks participants about the difference between the paretic and non-paretic sides. Monofilaments also assess perception of a touch stimulus. The lack of robust findings and treatment of somatosensory function in the clinical literature calls to question whether perception threshold testing is sufficient to indicate sensory dysfunction, or whether sensory perception itself is impaired after stroke. The disappearance threshold results may suggest that sensory integration, not perception, is impaired in this sample. Future work should focus on the sensory processing necessary to perform complex movements to gain a more complete picture of sensation after stroke.

Since vibration, light touch, and proprioception all travel along the same tract, it is tempting to assess only one or two of these modalities and generalize those findings to overall sensory dysfunction. This is frequent practice in the clinic where sensory impairment is often assessed qualitatively and may or may not extend to all domains of somatosensation (166). Conversely, it is also tempting to ascribe particular modalities within the highly conserved dorsal column, medial lemniscal tract along with particular lesions or distinct deficits (77,169). The varying group-level results in this dataset suggest that multiple sensory modalities should be characterized at multiple locations within the

limb or area of interest. Focusing on foot locations that physically contact the ground during walking would miss the findings of sensory impairment at the medial malleolus and proximal tibia. These two locations are also closely localized to the origin and insertion points of the tibialis anterior muscle under investigation during our LLR presence/absence task.

When assessing mechanisms of the LLR specifically, further work is needed to assess the functional roles of different types of somatosensory feedback. We ascribe monosynaptic stretch reflexes to muscle spindle activity (177), but with oligosynaptic reflexes there is time for integration of multiple afferent signals to influence the reflex output. Results from anesthesia and ischemia studies in the upper limb suggest that LLRs are primarily mediated by muscle afferents, because LLR amplitude does not decrease when cutaneous and joint receptors are blocked (178). One study found increases in LLR amplitude following anesthesia, suggesting that cutaneous afferents may play a role in tonic inhibition or gain control of LLRs in normal circumstances (179). In primates, muscle spindle afferent firing patterns follow similar dynamics to the LLR (180). Despite the possibility that they are not the primary mediator of LLRs, the findings of dysfunctional vibratory and cutaneous sensation in LLR- individuals therefore warrant further study. As mentioned in the results section, no conclusions should be drawn from our proprioception data, the sensory modality that may be most relevant to LLRs, because of the high variability and instrumentation error in healthy control data in our small sample. There are more robust and functionally relevant methods for measuring proprioception, via split-belt treadmill speed matching (181) or belt asymmetry perception (182), and metrics like these

could be utilized in future research to provide an assessment of active proprioception and its role in walking function.

The results of this study add to the body of literature indicating that somatosensory impairment is present in the lower extremities in some individuals with chronic stroke. The pattern of increased sensory thresholds in LLR- individuals relative to healthy controls may relate to LLR absence, the lower functional status of these individuals compared to LLR+ individuals, or both. Although the evidence from these somatosensory tests may not be strong enough to explain LLR absence in its entirety, this contributes knowledge to the complex interaction between transcortical reflexes and a damaged central nervous system. Sensation, coupled with the integration of parallel processes occurring supraspinally, is vital for maintaining balance and producing effective movement (167,183). Impaired somatosensation relates to increased frequency of falls, leading to downstream risks of worsening disability and death (184). Future work in rehabilitation research needs to consider the role of somatosensation if the field aims to increase treatment response rates across the population of individuals with stroke.

Chapter 6: The Relationship Between Sensory Impairment and Gait Performance

Introduction

Hemiparetic gait is typically slow, asymmetric, and metabolically inefficient (185). These impairments are often attributed to poor motor function following stroke, yet the role of impaired sensation is often overlooked. Proprioceptive inputs contribute to coordinated motor output and economical gait patterns (186,187). However, clinical sensory testing fails to consider the complexity of sensory processing and its impact of motor output. Most clinical tests only involve delineation of whether the sensory modality being tested is present, absent, or impaired (81,84). Here, we will discuss vibration sensation because vibration is a dynamic form of touch sensation.

Humans are most sensitive to vibration between 200-250Hz (82). Pacinian corpuscles are the skin's most common vibration receptors, responding to vibration in the 60-400Hz range. Sensory information travels via medium- and large-diameter afferent nerve fibers (168), and vibration thresholds reflect the functional integrity of those fibers (104). These fibers synapse in the ventral posteriolateral (VPL) nucleus of the thalamus and then travel to Area 1 within the primary somatosensory cortex (168). Other sensations that share the dorsal column, medial lemniscal tract (i.e., touch, proprioception) follow similar paths, but modality separation is conserved along the length of the tract (82). In the case of stroke, we would typically expect the receptors and nerve fibers to behave normally, but the cortical and subcortical areas may fail to perceive or appropriately process the sensations.

The most common assessment of vibration sense utilized in the clinic involves placing a vibrating tuning fork onto a body landmark and asking the patient if they can

feel the vibration, or if it feels different from the contralateral side (166). This creates a binary (present/absent) or at best non-specific (present/impaired/absent) result. However, there are portable, relatively inexpensive handheld devices that allow for objective measurement of vibratory thresholds. These devices can be used to quantify both vibration perception (VPT) and disappearance (VDT) thresholds.

Motor impairment following stroke is well documented and, in many cases, contributes to asymmetric gait. This is apparent in both kinematic and kinetic measures of gait, with common deficits including, but not limited to, decreased ankle power in late stance, toe clearance in swing, and knee flexion in both late stance and swing (111,114,188). Each of these measures scale with speed, but slow walking speeds alone cannot account for the differences between people with chronic stroke and healthy older adults. Given that rehabilitation strategies focused on motor recovery and/or compensation have an approximate 50% response rate (10,12,13), it is necessary to consider the role that sensory dysfunction may play in this motor recovery gap.

Unlike the extensive attention that motor deficits receive in lower extremity rehabilitation, sensory dysfunction following stroke remains poorly understood. In the characterizations that do exist, sensory dysfunction is noted but its role in relation to walking is only broadly defined. A study in subacute stroke documented that patients with both motor and sensory deficits improve their walking at a slower rate than those with motor deficits alone, and they often do not achieve independent community ambulation within 30 weeks of their stroke (80). Further, more than 67% of the patients observed in that study had both motor and sensory deficits, with pure sensory deficits being among the rarest of classifications observed. Another study found associations between both

proprioception and sensory integration and level of ambulation at discharge from inpatient rehabilitation (183). These and other studies found patterns of sensory dysfunction with broad, clinical measures of impairment, so we expect to find associations between quantitative assessments of both sensory control and gait biomechanics. These associations will be more informative than previous observations, providing the field some direction as they narrow down more impactful targets for sensorimotor gait rehabilitation.

Here our goal is to investigate the relationship between vibratory thresholds and gait biomechanics following stroke. We hypothesized that participants post-stroke would reveal deficits in vibratory sensation associated with biomechanical gait deficits. These results will inform the extent to which somatosensory rehabilitation is needed as part of walking rehabilitation in stroke.

Methods

Subjects

Twenty-nine individuals with chronic stroke and 21 neurologically healthy individuals participated in this study. Participants were recruited from the north Florida region. Demographic data for the stroke cohort, reported in Table 6.1, illustrate a functionally diverse group of stroke survivors with mild-to-moderate motor impairment. Inclusion criteria for individuals with stroke included presence of no more than two unilateral, hemispheric strokes at least six months prior to enrollment, ability to walk at least 10m with or without an assistive device, and ability to follow three-step commands.

Table 6.1. Participant demographics.

	Control	Stroke
Demographics		
n	21	29
Sex (m/f)	7/14	23/6
Race (white/Black/Asian/not stated)	17/2/2/0	25/3/0/1
Ethnicity (Hispanic or Latino/NH/not stated)	0/19/2	0/25/4
Age (yrs)	64±9	65±9
Chronicity (yrs)	---	6.7±4.8
Lesion location (cortical/subcortical/mixed)	---	6/12/11
Fugl-Meyer LE Motor Score, median (range)	---	27 (12-34)
Paretic/test leg (r/l)	10/11	15/14
Assistive device use (AFO/other device/none)	---	11/3/15
Self-selected walking speed (m/s)	1.26±0.25	0.76±0.35

Age, chronicity, and self-selected walking speed are given as mean ± standard deviation. Fugl-Meyer is given as median (range). All other values provided as counts. *m*, male; *f*, female; *NH*, not Hispanic or Latino; *yrs*, years; *LE*, lower extremity; *r*, right; *l*, left; *AFO*, ankle-foot orthosis.

All procedures described herein were approved by the University of Florida Institutional Review Board (IRB-01) and conducted according to the principles expressed in the Declaration of Helsinki. All participants provided written informed consent prior to participation.

Protocol and Instrumentation

Gait analysis. Reflective markers were placed on anatomical landmarks using a modified Helen Hayes marker set (189). Locations of the anterior superior iliac spines were digitized using a digitizing pointer (C-Motion, Inc., Germantown, MD, USA). Participants were fit with a modified mountain climbing harness (Robertson Harness, Henderson, NV, USA), which provided fall arrest; no substantial body weight support was provided. In contrast to other studies (188,190,191), no handrail support was provided.

Participants post-stroke were asked if they could walk safely on the treadmill without wearing an orthosis. Seven participants used an Aircast® AirSport™ (DJO Global, Vista, CA, USA) to provide mediolateral stability. Two participants required their ankle-foot orthoses to maintain safe walking, and these individuals were excluded from kinematic analyses. We used a digitizing pointer to digitize the locations of the medial and lateral malleoli for both types of braces.

Participants were familiarized with walking on the treadmill prior to data collection. Marker data were recorded by 12 infrared cameras (Vicon MX, Vicon Motion Systems Ltd., Oxford, UK) at 200 Hz as participants walked at their self-selected speed on an instrumented split-belt treadmill (Bertec, Columbus, OH, USA) which measured three-dimensional ground reaction forces (GRF) at 2000 Hz. Data were recorded in 30-60 second trials. Individual subject data were averaged across all valid steps.

Sensory testing. Participants were tested in supine with the sock and shoe removed from the test leg. We tested four lower extremity sites: hallux (dorsomedial aspect of first metatarsophalangeal joint), heel, medial malleolus, and tibial tuberosity. We used two assessors to minimize the variability in the pressure applied concurrently

with the vibratory stimulus. Our primary assessor used a handheld Biothesiometer (Bio-Medical Instrument Co., Newberry, OH, USA) to apply light pressure perpendicular to the skin surface, to assess vibratory sensation using the method of limits (104). Briefly, the stimulus strength was increased from zero to the point where vibratory sensation is first perceived (vibration perception threshold, VPT), and then the stimulus strength is decreased from a supraliminal level to the point where the sensation disappeared (vibration disappearance threshold, VDT). The participant was instructed to verbally alert the assessors when they could first detect, and no longer detect, a vibratory stimulus as the intensity was turned up and down, respectively; a secondary assessor recorded the voltage on the Biothesiometer display at that instant. We recorded three consecutive VPTs and VDTs at each site. We took the average of the three trials for each threshold (104).

Data Analysis

Marker and kinetic data were collected, labeled, and reduced with Vicon Nexus software (Versions 1.8-2.2, Vicon Motion Systems Ltd., Oxford, UK) then processed and low-pass filtered (4^{th} order bidirectional Butterworth; cutoff 6 Hz for marker, 10 Hz for GRF data) using Visual 3D software (Versions 5-6, C-Motion, Germantown, MD, USA) prior to constructing biomechanical models. The resulting kinematic and kinetic data were post-processed with custom Matlab scripts (Version R2015a-2020a, The Mathworks, Natick, MA, USA).

Study Variables

We investigated joint kinematics and joint kinetics at the hip, knee, and ankle during relevant gait phases, particularly at key transition points (for example, see Figure

6.1). Joint kinematics of interest are all sagittal plane angles including peak hip extension, peak hip flexion in swing, peak knee flexion in swing, peak ankle plantarflexion in pre-swing and swing, and peak ankle dorsiflexion in swing. Peak hip extension angle is taken over the full gait cycle but typically occurs late in stance, just prior to toe-off. The joint kinetics are the fundamental sagittal plane powers defined by Winter and others (99). The hip powers include: H1, peak concentric extension from loading response to midstance; H2, peak eccentric flexion from midstance to pre-swing; and H3, peak concentric flexion during pre-swing and early swing. The knee powers include: K1, peak eccentric extension in loading response; K2, peak concentric extension in midstance and terminal stance; K3, peak eccentric extension in early swing; and K4, peak eccentric flexion in late swing. The ankle powers include: A1, peak eccentric plantarflexion in midstance and terminal stance; A2, peak concentric plantarflexion in pre-swing; and A2 slope, the slope of the line connecting A1 and A2. All powers are expressed relative to subject mass, in W/kg, and A2 slope is expressed in W/kg·sec.

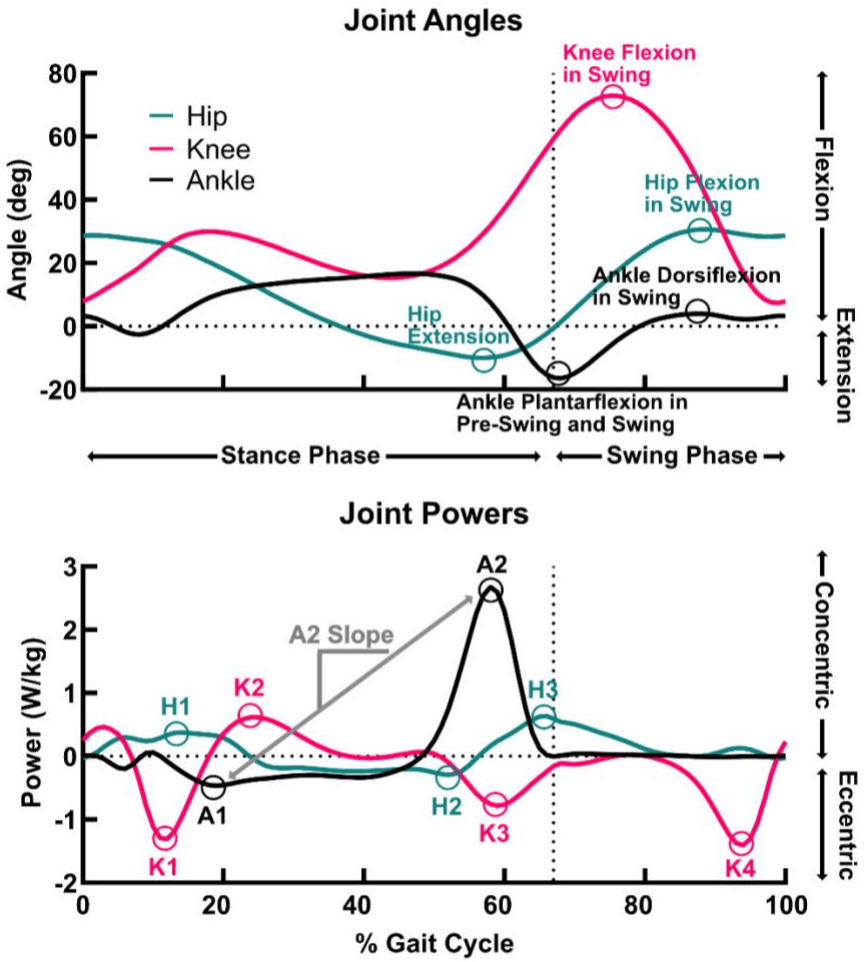

Figure 6.1. Representative healthy subject illustrating kinematic (top) and kinetic (bottom) variables employed in this study. Teal, hip angles and powers; pink, knee angles and powers; black, ankle angles and powers. Dotted line at 67% of gait cycle indicates toe-off.

Statistical Analysis

This is an exploratory analysis of the association between vibration thresholds and kinematic and kinetic measures of gait. We assessed normality across all variables using the Shapiro-Wilk normality test. Within the kinematic variables, peak knee flexion in swing and peak ankle dorsiflexion in swing were not normally distributed (p<0.05), while all other variables were normally distributed. None of the kinetics assessed were normally distributed by the Shapiro-Wilk test. As in Chapter 6, the vibratory thresholds were base

10 log-transformed. After log-transformation, they were normally distributed. Due to the many non-normal variables within our dataset, we conducted a Kendall's tau non-parametric correlation analysis. As this is the first step in our analysis, we used a cutoff of α=0.05 to identify associations to explore further.

Significant correlations were then investigated using linear regression if the variables were normally distributed. The outcome variable was the kinematic or kinetic variable of interest, while the predictors were vibratory threshold and Group (Control vs. Stroke). We also added Treadmill Speed as a predictor because most kinematic and kinetic values are known to scale with walking speed (192,193).

All statistical analyses were performed with R version 4.1.2 and RStudio version 2021.09.1. R packages used include tidyverse, car, and psych (158,160,194).

Results

Joint Kinematics

We performed Kendall's tau non-parametric correlation analyses for every pair of kinematic variables (6) and vibratory thresholds (4 locations x 2 threshold types). The results are provided in Table 6.2, with significant correlations highlighted in gold. Significant correlations include: peak hip extension and hallux PT; peak hip extension and medial malleolus PT; peak hip flexion in swing and hallux DT; peak hip flexion in swing and heel DT; peak knee flexion in swing and hallux DT; peak ankle plantarflexion in pre-swing and swing and hallux DT; peak ankle plantarflexion in pre-swing and swing and medial malleolus DT; peak ankle plantarflexion in pre-swing and swing and tibia DT; and peak ankle dorsiflexion in swing and tibia DT. Significant PT correlations are displayed in Figure 6.2, while significant DT correlations are in Figure 6.3. For the non-parametric peak

knee flexion angle in swing and hallux DT association, we also computed separate Kendall's tau correlations for the control and stroke datasets to look for group-level associations. The stroke group had a negative association between knee flexion angle and hallux DT (τ=-0.333, p=0.017), while the control group had a weak positive association (τ=0.129, p=0.415).

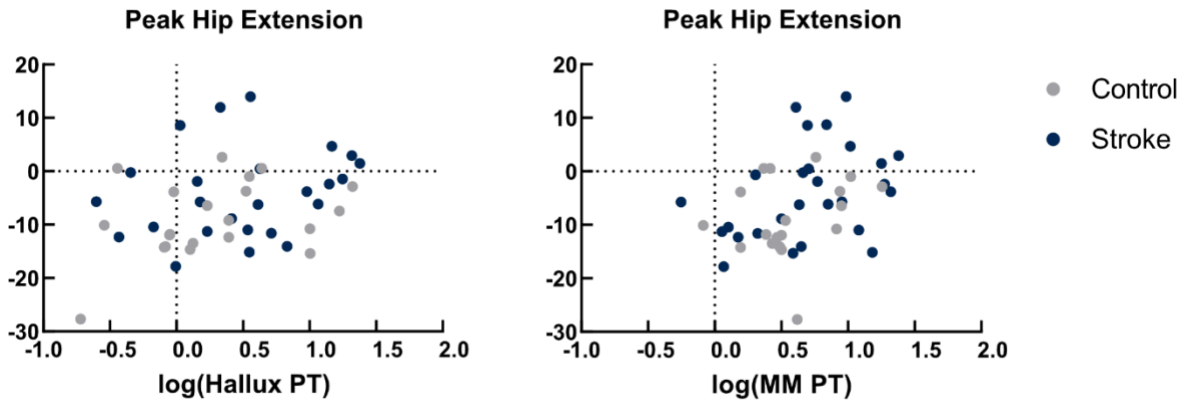

Figure 6.2. Scatterplots of peak hip extension angle vs. hallux perception threshold (PT, left) and medial malleolus perception threshold (right). Control data are shown in gray; stroke data are dark blue.

Table 6.2. Kendall's tau correlation analysis for kinematic variables and vibratory thresholds. The top, bolded numbers within each cell are Kendall's tau sample estimate, while the bottom values are p-values. P-values <0.05 are highlighted in gold for emphasis. *PT,* perception threshold; *DT,* disappearance threshold, *MM,* medial malleolus.

	Hallux PT	Hallux DT	Heel PT	Heel DT	MM PT	MM DT	Tibia PT	Tibia DT
Peak Hip Extension	**0.224** 0.028	**0.197** 0.051	**0.115** 0.271	**0.127** 0.223	**0.249** 0.015	**0.044** 0.660	**0.135** 0.185	**0.062** 0.539
Peak Hip Flexion in Swing	**-0.068** 0.507	**-0.206** 0.041	**-0.065** 0.540	**-0.204** 0.049	**0.030** 0.769	**-0.122** 0.226	**-0.188** 0.066	**-0.156** 0.121
Peak Knee Flexion in Swing	**-0.137** 0.179	**-0.201** 0.047	**-0.099** 0.345	**-0.166** 0.111	**-0.162** 0.114	**-0.130** 0.199	**-0.194** 0.058	**-0.132** 0.190
Peak Ankle Plantarflexion in Pre-Swing and Swing	**0.131** 0.207	**0.207** 0.043	**0.101** 0.339	**0.201** 0.056	**0.130** 0.210	**0.211** 0.039	**0.131** 0.207	**0.285** 0.005
Peak Ankle Dorsiflexion in Swing	**0.139** 0.180	**0.101** 0.325	**0.055** 0.608	**0.091** 0.392	**0.130** 0.210	**0.168** 0.099	**0.080** 0.440	**0.316** 0.002

Table 6.3. Linear regression of significant vibration perception thresholds.

	R^2	*B*	*SE B*	*β*	*p*
Peak Hip Extension vs. Hallux PT	0.372				0.0002*
Constant		2.21	3.57	0.617	0.540
$\log_{10}$(HalluxPT)		2.70	1.81	1.50	0.142
Group		-0.871	2.38	-0.366	0.716
Treadmill Speed		-13.1	3.46	-3.78	0.0005†
Peak Hip Extension vs. MM PT	0.403				<0.0001*
Constant		0.223	3.87	0.058	0.954
$\log_{10}$(MMPT)		5.89	2.58	2.29	0.027†
Group		-1.09	2.38	-0.456	0.651
Treadmill Speed		-13.8	3.48	-3.96	0.0003†

* indicates model significance at p<0.00625. † indicates factor significance at p<0.05.

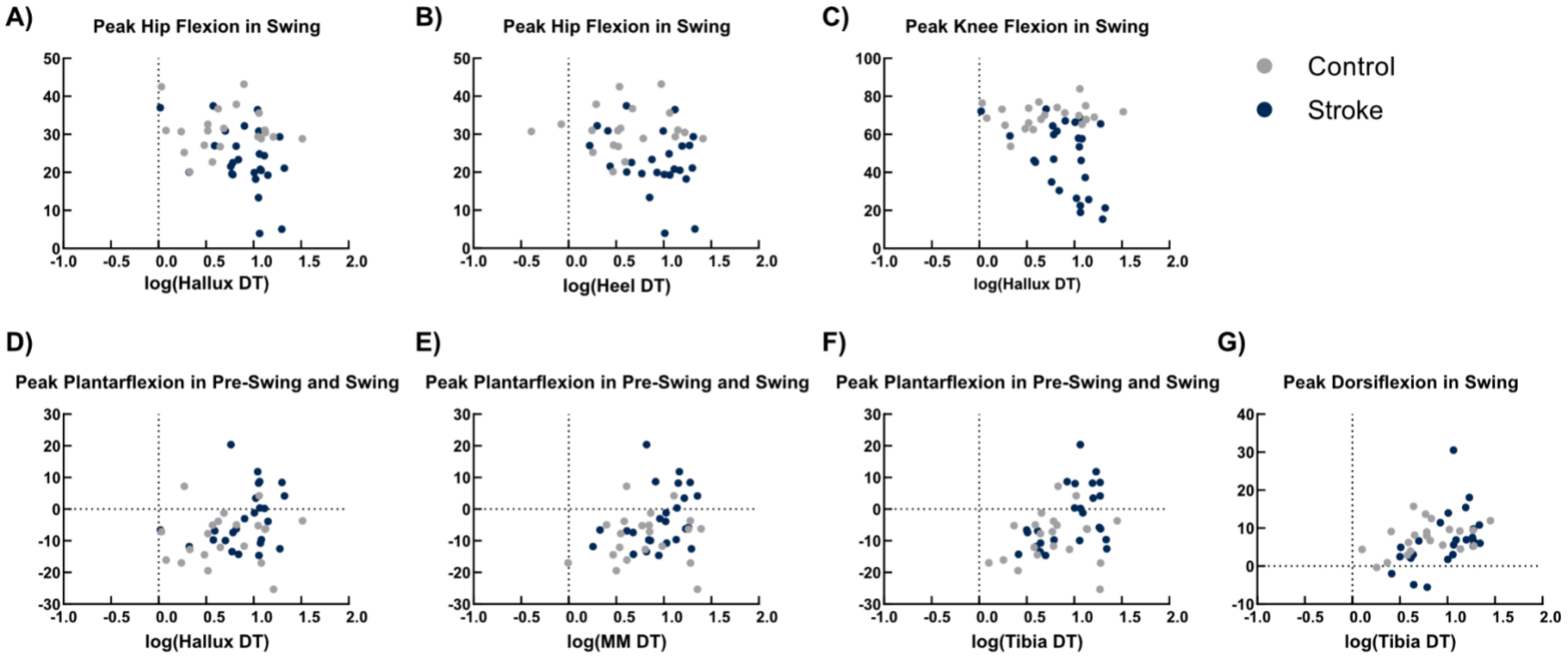

Figure 6.3. Scatterplots of significant associations between disappearance thresholds (DTs) and joint angles. A) Peak hip flexion angle in swing and hallux DT; B) peak hip flexion angle in swing and heel DT; C) peak knee flexion in swing and hallux DT; D) peak ankle plantarflexion in pre-swing and swing and hallux DT; E) peak ankle plantarflexion in pre-swing and swing and medial malleolus (MM) DT; F) peak ankle plantarflexion in pre-swing and swing and proximal tibia DT; and G) peak ankle dorsiflexion angle in swing and proximal tibia DT. Control data are shown in gray; stroke data are dark blue.

Table 6.4. Linear regression of significant vibration disappearance thresholds.

	R^2	*B*	*SE B*	*β*	*p*
Peak Hip Flexion in Swing vs. Hallux DT	0.308				0.001*
Constant		28.18	4.60	6.13	<0.0001†
$\log_{10}$(HalluxDT)		-3.71	3.08	-1.21	0.235
Group		-4.65	2.54	-1.83	0.074
Treadmill Speed		6.11	3.70	1.65	0.106
Peak Hip Flexion in Swing vs. Heel DT	0.308				0.002*
Constant		27.3	4.17	6.53	<0.0001†
$\log_{10}$(HeelDT)		-2.16	2.79	-0.77	0.442
Group		-5.35	2.72	-1.97	0.056
Treadmill Speed		5.73	3.73	1.54	0.132
Peak Plantarflexion in Pre-Swing and Swing vs. Hallux DT	0.400				<0.0001*
Constant		4.68	4.73	0.990	0.328
$\log_{10}$(HalluxDT)		1.95	3.17	0.615	0.542
Group		-1.08	2.63	-0.410	0.684
Treadmill Speed		-16.4	3.81	-4.32	<0.0001†
Peak Plantarflexion in Pre-Swing and Swing vs. MM DT	0.419				<0.0001*
Constant		2.16	4.56	0.47	0.639
$\log_{10}$(MMDT)		4.92	3.28	1.50	0.141
Group		-1.45	2.56	-0.568	0.573
Treadmill Speed		-16.5	3.63	-4.55	<0.0001†
Peak Plantarflexion in Pre-Swing and Swing vs. Tibia DT	0.491				<0.0001*
Constant		0.409	4.42	0.093	0.927
$\log_{10}$(TibiaDT)		8.20	3.18	2.58	0.014†
Group		-1.69	2.47	-0.683	0.498
Treadmill Speed		-17.3	3.56	-4.86	<0.0001†
Peak Dorsiflexion in Swing vs. Tibia DT	0.192				0.0283
Constant		1.70	3.68	0.463	0.646
$\log_{10}$(TibiaDT)		8.25	2.651	3.11	0.003
Group		-2.46	2.06	-1.19	0.239
Treadmill Speed		-0.784	2.96	-0.265	0.793

* indicates model significance at p<0.00625. † indicates factor significance at p<0.05.

Next, we performed linear regressions on the eight significant pairs with normally distributed data. Both perception threshold models were significant (Table 6.3). For peak hip extension vs. hallux PT, treadmill speed was the only significant predictor. For peak

hip extension vs. medial malleolus PT, both the vibratory threshold and the treadmill speed were significant factors. All disappearance threshold models except peak ankle dorsiflexion in swing vs. tibia DT were significant after correcting for multiple comparisons (Table 6.4). Both models for peak hip flexion in swing had only the constant as a significant predictor. Peak plantarflexion in pre-swing and swing for hallux DT and medial malleolus DT had treadmill speed as a significant predictor. Peak plantarflexion in pre-swing and swing vs. tibia DT had both the vibratory threshold and treadmill speed as significant predictors.

Joint Kinetics

As with the kinematic data, we performed Kendall's tau correlation analyses for every pair of kinetic variables (10) and vibratory thresholds (4 x 2). Table 6.5 shows the three significant correlations: H1 and hallux PT; H1 and hallux DT; and H3 and hallux PT. The separate control and stroke correlations were all non-significant and are displayed in Figure 6.2.

Table 6.5. Kendall's tau correlation analysis for kinetic variables and vibratory thresholds. The top, bolded numbers within each cell are Kendall's tau sample estimate, while the bottom values are p-values. P-values <0.05 are highlighted in gold for emphasis. *PT,* perception threshold; *DT,* disappearance threshold, *MM,* medial malleolus.

	Hallux PT	Hallux DT	Heel PT	Heel DT	MM PT	MM DT	Tibia PT	Tibia DT
H1	**-0.217** 0.038	**-0.281** 0.007	**-0.072** 0.500	**-0.065** 0.546	**-0.095** 0.363	**-0.147** 0.156	**-0.113** 0.279	**-0.111** 0.276
H2	**0.109** 0.297	**0.127** 0.218	**0.093** 0.381	**0.045** 0.677	**0.195** 0.063	**-0.025** 0.807	**0.115** 0.270	**-0.047** 0.643
H3	**-0.211** 0.044	**-0.133** 0.197	**-0.055** 0.608	**0.039** 0.724	**-0.108** 0.302	**0.021** 0.837	**-0.056** 0.592	**0.013** 0.902
K1	**-0.005** 0.960	**0.020** 0.845	**0.030** 0.786	**-0.054** 0.617	**0.004** 0.968	**-0.074** 0.475	**-0.060** 0.564	**-0.069** 0.501
K2	**-0.077** 0.460	**-0.081** 0.434	**-0.099** 0.349	**0.003** 0.983	**0.008** 0.935	**0.021** 0.837	**-0.065** 0.537	**0.007** 0.947
K3	**0.022** 0.832	**0.012** 0.907	**0.032** 0.771	**0.019** 0.868	**0.072** 0.491	**0.011** 0.914	**0.033** 0.754	**0.063** 0.538
K4	**0.168** 0.108	**0.131** 0.203	**0.027** 0.802	**0.010** 0.934	**0.059** 0.571	**0.019** 0.853	**0.109** 0.297	**0.053** 0.603
A1	**0.151** 0.148	**0.075** 0.469	**-0.038** 0.725	**-0.056** 0.603	**-0.002** 0.984	**0.005** 0.961	**-0.003** 0.976	**-0.016** 0.872
A2	**-0.065** 0.537	**-0.135** 0.190	**-0.042** 0.695	**0.061** 0.574	**-0.049** 0.642	**-0.074** 0.475	**-0.062** 0.551	**-0.134** 0.188
A2 Slope	**-0.075** 0.473	**-0.081** 0.434	**0.023** 0.833	**0.028** 0.803	**-0.008** 0.935	**-0.015** 0.883	**-0.012** 0.911	**-0.061** 0.551

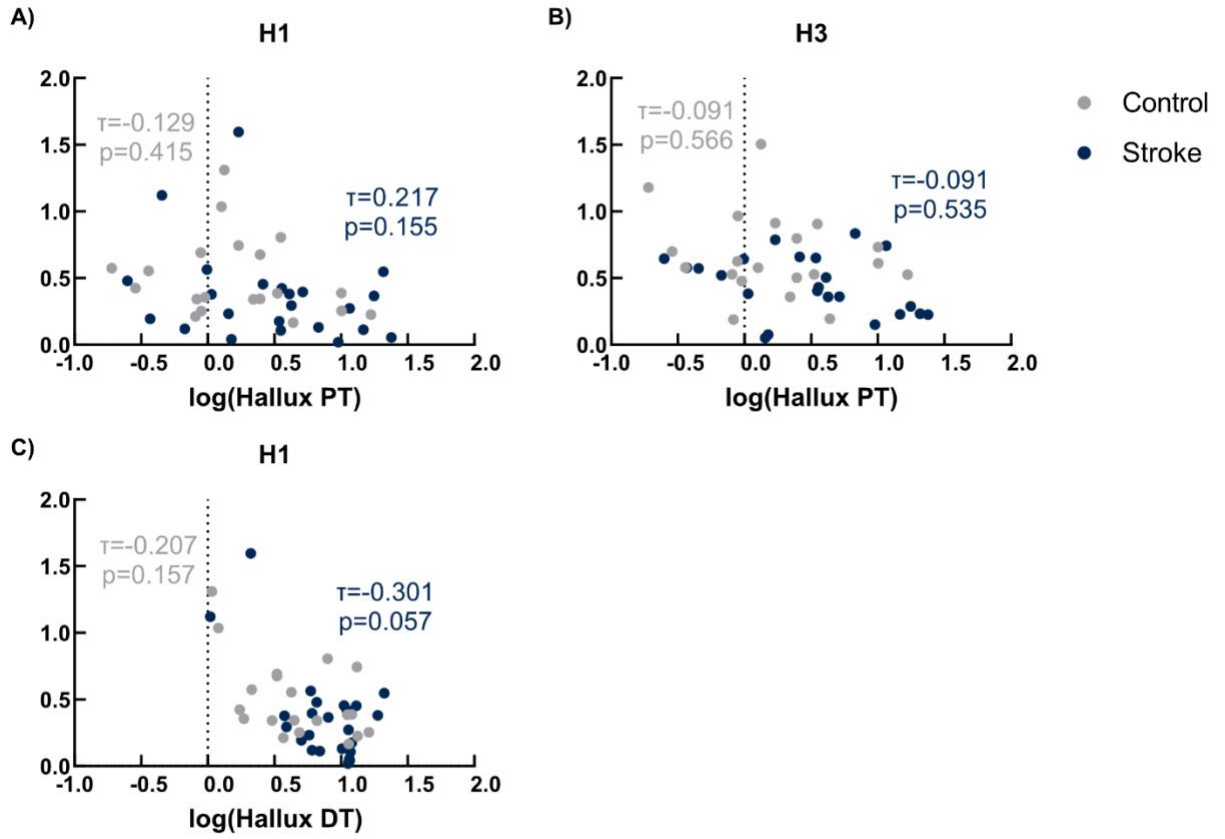

Figure 6.4. Scatterplots of significant associations between joint power by vibratory threshold. A) H1 and hallux perception threshold (PT); B) H3 and hallux PT; and C) H1 and hallux disappearance threshold (DT).

Discussion

Overall, there is an association between kinematic and kinetic measures of gait and vibration sense, most notably present in vibration disappearance thresholds. There were no statistically significant differences between individuals with chronic stroke and the age-matched healthy individuals within this sample. However, there does appear to be a differential pattern in the linear regression findings between stance and swing

variables, where stance variables are dictated mostly by treadmill speed, but swing variables are not. These may relate to different attributes of gait function.

The linear regression results can be interpreted within the frameworks of Gage and Perry, generally known as the functions or parameters of normal gait (195,196). Peak hip extension and peak plantarflexion both relate to Perry's requirement of terminal stance stability (195), which is likely mediated by foot and ankle sensory information and related to gait speed, as is reflected in our data. This is an important moment in the gait cycle for tactile and proprioceptive sensory information in the foot and ankle because we have no visual information to rely on from the trailing limb at this point in the gait cycle. On the other hand, the notable swing models did not have treadmill speed as a significant predictor. Peak hip and knee flexion in swing contribute both swing phase clearance (114,196) and additional propulsion to encourage forward progression (195). Although non-significant, group differences appear in the plots with some individuals with stroke failing to achieve adequate hip and knee flexion and the same individuals having higher VDTs at the foot and ankle. Lastly, peak dorsiflexion in swing is important for foot prepositioning (196), and this is strongly related to VDT at the proximal tibia, closely located to the bulk of the main dorsiflexor muscle. Altogether, it appears that vibratory thresholds relate to important aspects of normal gait, and differential effects are observed between these thresholds and stance and swing variables.

Many studies regarding peripheral neuropathies report values for VPT, but not VDT (106,197). Indeed, those who have investigated both thresholds found VDT to be significantly more variable than VPT (104). Thus, many authors have concluded VPT is sufficiently informative for clinical screening purposes in peripheral neuropathy (198).

However, Goldberg and Lindblom suggested people with cerebral lesions may reveal characteristic differences between VPT and VDT, thus investigation of both values provides more information than VPT alone can provide (104). This suggests that VPT and VDT measure different constructs of vibratory sensation. As variability can be helpful for maintaining safe walking (199,200), perhaps the increased variability in VDT has meaning in complex, coordinated movements like walking. For example, individuals with higher VDTs do not achieve as much plantarflexion as those with lower thresholds, with some individuals never transitioning out of dorsiflexion in this phase. Both higher perception thresholds and less plantarflexion are abnormal, and indicative of sensorimotor dysfunction. Because the foot is in the process of leaving the ground, there is an understandable relationship between joint angles and the ability to process and integrate the loss of sensation into meaningful movement. This common thread of VDT being the most informative threshold in our data from both chapter 5 and this study is noteworthy and requires further investigation into the ability of VDT to assess sensorimotor integration as it applies to walking after stroke.

To our knowledge, this is the first study to investigate the association between vibratory thresholds and gait biomechanics in individuals with stroke. One study in healthy adults found a negative association between hallux VPT and peak pressure under the hallux during both walking and running (201). This comparison and our comparison between hallux vibration thresholds and A2 are somewhat alike, but we did not see a significant association in our dataset. Another study after anterior cruciate ligament reconstruction found associations between knee moments and angles with VPTs at the medial malleolus, lateral malleolus, and first metatarsal, but most of their biomechanical

measures were taken in the frontal plane (202). This study, like ours, did not find an association between knee flexion and VPT in either the ACL reconstructed leg or the contralateral leg. More often studies in the literature measure associations between vibration perception and clinical measures of gait like walking speed or presence of dysfunctional patterns (203–205). Another common thread in the literature involves using vibration as a treatment modality, such as using vibrating insoles along with gait and balance training (206,207). It is important to understand how vibration sense impacts walking in a dysfunctional nervous system, especially if vibration is being utilized as a treatment tool.

We also noted a ceiling effect of the Biothesiometer in our data. This phenomenon is well-documented in the diabetic population when the severity of peripheral neuropathy exceeds what can be detected by vibration of 50V (106,208). Diabetes is a common comorbidity for people with stroke, though we did not explicitly include or exclude participants with peripheral neuropathy. Fifteen individuals had at least one VPT greater than 50V/25.5μm, five had more than one, and one could not sense vibration at any of the locations tested. These individuals had to be excluded from statistical analysis for the location(s) that could not be evaluated. A VPT exceeding 50 V indicated these individuals presented with greater than moderate sensory loss (106), thus illustrating the need for evaluating at multiple locations within an individual.

This exploratory study indicates that vibratory thresholds may be associated with certain aspects of gait biomechanics. We are, to the best of our knowledge, the first group to report these associations in individuals with stroke. Although there were no significant differences between the individuals with stroke and healthy individuals included in this

sample, there are notable findings that apply to both individuals with stroke and healthy older adults within this study. Our differential findings in stance and swing highlight the importance of sensory function to multiple requirements for an effective gait pattern. These data underscore the point that it is important to consider sensory function, in addition to motor function, as a rehabilitation target for individuals with gait dysfunction following stroke.

Chapter 7: Conclusions and Future Directions

The overall goal of this dissertation was to provide proof-of-concept for use of the LLR to characterize walking impairment and recovery potential after stroke. We accomplished this by probing the LLR in the tibialis anterior as well as sensory, motor, and walking function to characterize the functional status of LLR present or absent individuals with chronic stroke, as well as the state of these measures in age-matched healthy individuals. Chapters 3-6 of this dissertation describe individual analyses of LLRs and sensorimotor function post-stroke.

Chapter 3 presents the dynamic tibialis anterior muscle stretch paradigm under study and the initial finding of LLR absence in a subgroup of individuals with chronic stroke. Importantly, LLR presence or absence was an unambiguous pattern visible in unfiltered EMG of plantarflexor stretches in the individuals we studied. LLR- individuals are clinically lower-functioning and walk slower than healthy individuals (171). On the other hand, LLR+ individuals may walk slower, but are otherwise not physiologically distinct from age-matched healthy individuals. The initial experiment that produced these data was not designed to measure LLRs in the tibialis anterior, thus was unable to confirm whether the task was measuring a transcortical reflex. The clinical implication of transcortical reflex impairment would be a loss of the first line of defense in response to a hazard or environmental stimulus (123,132), which could result in increased fall risk or altered walking patterns.

Chapter 4 was designed specifically to target the LLR and validate the findings from Chapter 3 in an independent sample. We augmented the LLR with TMS to confirm whether there was a cortical contribution to TA LLRs in LLR+ individuals with stroke. We

confirmed that LLR presence or absence is a common phenomenon in individuals with chronic stroke, and again showed that LLR- individuals were the lowest-functioning individuals represented in this sample. Further, the addition of TMS timed at intervals that coincided with the LLR confirmed the cortical contribution of this response in LLR+ individuals with stroke. Interestingly, most of the individuals with stroke had facilitation of evoked responses when TMS was delivered at the average LLR latency, even in the case of LLR- individuals. One LLR- individual did not show a facilitation with TMS, creating more of a continuum of response to include individuals that lack the necessary corticospinal substrate for normal motor function. Investigation of the characteristics of the LLR and TMS responses did not provide a clear mechanism for LLR absence, leading to additional questions regarding the relative importance of the sensory portion of the transcortical reflex pathway.

Chapter 5 investigated the role of sensory function in LLR presence or absence, through measuring multiple modalities of somatosensation. We employed clinical assessments of vibration, light touch, and proprioception to characterize the individuals' sensory function, because the literature lacks thorough characterization of sensation after stroke. We first assessed whether vibratory thresholds could predict differences between healthy individuals and individuals with chronic stroke. The vibration disappearance threshold was the most successful method for detecting differences between groups. We then assessed disappearance thresholds across individuals by LLR status and found that the disappearance threshold at the heel may differentiate the groups the best. In assessing fine touch, thresholds at the proximal tibia were higher in the LLR- group than healthy individuals. However, our sample was too small to draw definitive conclusions

about the mechanisms of somatosensory dysfunction and its relationship to the LLR. Larger samples and more sensitive measures, especially for proprioception, are needed to further characterize the role that somatosensory dysfunction plays in LLRs, and in walking function in general.

Chapter 6 continued the theme of sensory function following stroke, this time from a walking biomechanics perspective. We conducted an exploratory analysis of the association between vibratory thresholds and kinematic and kinetic measures of walking. There were some key findings that align with our understanding of the functions of normal gait, and a differential relationship between model predictors in stance and swing. In agreement with our findings from chapter 5, vibration disappearance thresholds were more frequently associated with joint kinematics and kinetics than vibration perception thresholds. These findings can be used to inform future study of the role of dysfunctional sensation in walking function after stroke. In particular, there is a need to gain greater understanding of the relationship between VDT, sensorimotor integration, and its impact on complex movement like walking.

Future Directions

Chronicity

All individuals studied in this dissertation were in the chronic phase (>6 months) of stroke recovery. While most of these individuals possessed the substrate necessary to produce motor evoked responses, not all of them revealed LLRs. As a result, we cannot know whether LLR absence is a consequence of the stroke itself or a phenomenon that develops during the acute or subacute phases of recovery. Future experiments should

assess individuals very early after stroke and follow them over time to determine the time-course of LLR absence and its relationship to early functional recovery.

Relationship between LLRs and CST Integrity

LLRs likely require a corticospinal tract that is at least partially intact. All the individuals in these studies were capable of walking at least 10 meters, with or without an assistive device. This precludes us from generalizing our results to the most impaired individuals with stroke. Other studies that have no minimum motor function requirements find that there are a reasonable proportion of individuals in which a magnetic stimulator cannot evoke consistent responses at the highest setting (209). Across this dissertation, our samples contain only three individuals that the field would classify as, “MEP negative,” and although they were among the lowest functioning individuals we studied, they still possess the ability to walk. This is an insufficient number of individuals to generalize to this subset of the post-stroke population. It would stand to reason that MEP negative individuals would also be LLR-, as the three were in our studies, because there is too much neural damage to produce a long-latency reflex response. But it would prove difficult to study LLRs in the lowest-functioning individuals after stroke, because our method of generating LLRs requires active contraction at the joint being stretched (144).

Sensorimotor Integration

Throughout this work, neither corticospinal tract integrity nor sensory function alone were adequate predictors of LLR status. The remaining portion of the transcortical loop is one we were not able to probe in this set of experiments: sensorimotor integration. Sensorimotor integration in this context is the cortical process of using sensory information to assist motor output (210). This is theorized to occur in many ways including

connections from sensory cortices to motor cortices (211), thalamocortical projections (212), and through higher-order processing throughout the brain. It is not enough for an individual to be able to detect a sensation, it is important that the sensory information be utilized to effect behavior. There are multiple methods for assessing sensorimotor integration that we could utilize in future experiments. Short-latency afferent inhibition (SAI) is a technique that targets direct projections from S1 to M1 (213), and probing the relationship between LLRs and SAI could provide insight on the role of those projections as well as the role of LLR absence in sensorimotor integration in general (163).

It would also be interesting to explore the interplay between vibration disappearance thresholds, sensorimotor integration, and walking dysfunction. The loss of a sensory signal provides afferent feedback during the gait cycle, and it appears to be important to integrate that sensation into the motor plan moving forward. Further experiments that could disentangle the complex phenomena of losing the sensation of ground contact and its impact on subsequent steps would provide insight into how we integrate feedback during walking. Anesthesia of the sole of the foot would block sensory feedback but still allow for safe gait and investigation of the role of sensory information during the stance-to-swing transition. Höhne et al. gave participants lidocaine injections to the sole of the foot and showed that vibratory sensation was impaired and gait altered compared to pre-injection walking (214). However, the authors measured VPTs and not VDTs and focused their biomechanical analyses solely on the stance phase, not showing data for the full stance-to-swing transition to provide sufficient comparison to our findings from Chapter 6. Alternatively, incorporating sensory feedback changes into learning certain gait pattern modifications, such as an intervention where participants learn to

modulate hip extension and ankle plantarflexion angles, could also provide insights into how both healthy individuals and individuals with stroke integrate information regarding the loss of ground contact at this point in the gait cycle.

Mutability of the LLR and Response to Treatment

As was mentioned in Chapter 1, good biomarkers can predict mutability of a response and/or capacity for recovery. It would be a natural next step to determine whether the LLR is mutable, and ideally responsive to sensorimotor rehabilitation. Modalities that facilitate motor output, particularly paired associative stimulation (PAS), should be able to upregulate the LLR in addition to MEP amplitude. This is because PAS likely works through the same transcortical pathway as the LLR (212). The finding that evoked responses were facilitated in the presence of both single pulse TMS and stretch in the LLR- group is particularly promising that an intervention like PAS may be able to reveal LLRs through inducing plasticity in the transcortical pathway. It would also be informative to measure LLR amplitude and timing before and after rehabilitations such as strength training or gait training, to determine whether any aspect of the LLR can predict which individuals respond to treatment. These types of studies will also provide predictive probabilities and the further validation that is needed to determine whether the LLR is a clinically viable biomarker.

Clinical and Translational Research Impact

This dissertation builds upon a body of literature from both animal and human studies that investigate control of the sensorimotor system. My goal was to bring the important aspects of basic neurophysiology together with known behavioral impairments

in human health. As fascinating as neurophysiology itself can be, it is of the utmost importance to drive these physiologic questions from a place of understanding of human behavior, and vice versa. As we move forward and investigate the LLR response to motor rehabilitation, we move closer to translating these research findings into clinical practice. The appeal of the LLR as a biomarker is that it does not require investigative technology such as a magnetic stimulator. Although we are not yet ready to move into clinical trials where LLR status informs treatment selection, it is my goal to determine the feasibility of this path forward so that we can work to improve therapeutic response rates and get more people back to higher levels of walking function.

Chapter 8: References

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